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Research Article

Comprehensive identification and expression analysis of the *TIFY* gene family in cucumber

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Abstract

The TIFY family, a plant-specific gene family with the conserved motif of TIF[F/Y]XG, plays important roles in plant growth, development and abiotic stress response. This family encodes four subfamilies of proteins, including ZIM-like (ZML), TIFY, PPD and JASMONATE ZIM-domain (JAZ). In this study, 17 *TIFY* family genes were identified in cucumber through genome-wide analysis, including one *PPD*, two *TIFYs*, four *ZMLs*, and 10 *JAZs*. Phylogenetic analysis revealed that TIFY proteins from cucumber and other plant species can be divided into seven groups, which were designated as TIFY, JAZ I–IV, ZML and PPD. An analysis of conserved domain distribution demonstrated that there are four other domains (Jas, CCT, PPD and GATA domains) in CsTIFY proteins. Tissue expression profiling of the *CsTIFY* genes revealed that some of them displayed development- and tissue-specific expression patterns. Expression analysis based on transcriptome data and qRT-PCR revealed that the expression levels of some cucumber *TIFY* genes were altered under multiple abiotic stresses. In addition, several *CsJAZ* genes were downregulated in cucumber plants under root-knot nematode (RKN) infection, suggesting that they negatively affect the resistance response of cucumber to RKN. Our findings lay a foundation for further functional studies of the *TIFY* family genes in cucumber.

Keywords: abiotic stress; cucumber; gene expression; root-knot nematode (RKN); *TIFY* gene family

Introduction

Extreme temperature (high or low temperature), high salinity, drought, heavy metals and ultraviolet rays are unfavourable and even fatal environmental factors for plant growth and development. However, plants can adapt to a certain range of environmental changes through some complex regulatory networks, where transcription factors (TFs) play key roles by activating or inhibiting the target genes at mRNA transcription level (Zhou *et al.*, 2020). TIFY is a plant-specific TF previously named as ZIM (Zinc-finger protein expressed in inflorescence meristem), whose core motif is TIF[F/Y]XG ("X" represents any amino acid) (Vanholme *et al.*, 2007). The TIFY family can be divided into four phylogenetic subfamilies with diverse domain

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architectures: TIFY, JAZ (jasmonate ZIM domain), ZML (ZIM/ZIM-like), and PPD (PEAPOD) (Bai *et al.*, 2011; Yang *et al.*, 2019). Among them, the TIFY subfamily harbours only the TIFY domain, while other three subfamilies contain additional domains besides the TIFY domain. For example, the JAZ subfamily harbours the Jas domain (also known as the CCT_2 domain) with a characteristic SLX2FX2KRX2RX5PY at the C-terminal region; the PPD subfamily has an N-terminal PPD domain and a divergent Jas domain lacking conserved PY (proline-tyrosine) in the C-terminus, while the ZML subfamily bears the C2C2-GATA zinc finger DNA binding domain and CCT domain (Chung *et al.*, 2009; Huang *et al.*, 2016; Yang *et al.*, 2019; Zhang *et al.*, 2020; He *et al.*, 2020).

In recent years, many genes of the TIFY family have been cloned and functionally verified in some plant species, revealing their important roles in different biological processes and hormone responses. Particularly, the JAZ subfamily genes have recently been extensively studied for their orchestrating functions in the jasmonic acid (JA) signalling pathway. JAZs act as transcriptional repressors of JA responsive genes by binding to TFs, such as MYC and MYB family. In the presence of JA, JA receptor Coronatine insensitive 1 (COI1) can target JAZs, and subsequently cause SCF^{COI1}-mediated 26S proteasome degradation of JAZs, release JAZ-interacting TFs and enable their interaction with other cofactors, finally resulting in the activation of JA-related physiological features (Xie *et al.*, 1998; Thines *et al.*, 2007; Chini *et al.*, 2016). For instance, rice OsJAZ1/OsTIFY3 mediates the degradation of OsJAZ1 via OsCOI1b during spikelet development through JA signalling, meanwhile it interacts with OsMYC2 to repress the activation of an E-class gene *OsMADS1* to regulate the spikelet development (Cai *et al.*, 2014). *OsJAZ9/OsTIFY11a* was reported to participate in K deficiency, water-deficit and salt stress tolerance by influencing the JA level and JA response (Wu *et al.*, 2015; Singh *et al.*, 2020; Singh *et al.*, 2021). In *Arabidopsis*, two R2R3-MYB TFs, MYB21 and MYB24, are the direct targets of JAZs involved in controlling JA-regulated stamen development (Song *et al.*, 2011). Many JAZs also function in the intricate crosstalk of JA with other hormones, such as abscisic acid (ABA) (Fu *et al.*, 2017; Liu *et al.*, 2019), gibberellins (GAs) (Um *et al.*, 2018; Zhou *et al.*, 2015b), and salicylic acid (SA) (de Torres Zabala *et al.*, 2016; Zhang *et al.*, 2019). In addition, a number of other *TIFY* family genes have been functionally characterized for their important roles in plant growth, development and biotic and abiotic stresses. For example, AtTIFY4a/AtPPD1 and AtTIFY4b/AtPPD2 were involved in the regulation of leaf and silique development (White, 2006; Gonzalez *et al.*, 2015), and AtPPD2 can interact with LIKE HETEROCHROMATIN PROTEIN1 (LHP1) to regulate lateral organ growth (Zhu *et al.*, 2020). Overexpression of rice TIFY11 subfamily genes could promote plant growth (increases in caryopsis number, grain weight and plant height) by desensitizing plants to JA (Hakata *et al.*, 2017). Overexpression of *Triticum Durum TdTIFY11a* in *Arabidopsis* showed enhanced tolerance to salt stress (Ebel *et al.*, 2018). Overexpression of *VvTIFY9* from *Vitis vinifera* in *Arabidopsis* resulted in enhanced resistance to powdery mildew disease with an increase in SA content, suggesting the important role of *VvTIFY9* in SA-mediated powdery mildew resistance (Yu *et al.*, 2019).

In recent years, the *TIFY* family genes have been identified at the genome-wide level in many plant species, such as Moso Bamboo (*Phyllostachys edulis*) (Huang *et al.*, 2016), tomato (*Solanum lycopersicum*) (Chini *et al.*, 2017; Heidari *et al.*, 2021), Chinese sand pear (*Pyrus pyrifolia*) (Ma *et al.*, 2018), watermelon (*Citrullus lanatus*) (Yang *et al.*, 2019), tea plants (*Camellia sinensis*) (Zhang *et al.*, 2020), wheat (*Triticum aestivum*) (Ebel *et al.*, 2018; Singh and Mukhopadhyay, 2021), and maize (*Zea mays*) (Sun *et al.*, 2021). Previous studies have clearly demonstrated that the *TIFY* family members are involved in the growth, development, external stimulation response and hormone transduction of plants. It is therefore necessary to study the *TIFY* gene family in cucumber, a major vegetable which is easily affected by various environmental stresses in the natural environment. In this study, bioinformatics methods were employed to analyze the chromosome position, phylogenetic relationship, conserved motifs, gene structure and expression profiles of

the cucumber *TIFY* family genes. Our findings provide a theoretical basis for further studying the biological functions of *TIFY* genes in the growth and development of cucumber.

Materials and Methods

Identification and sequence analysis of TIFY family members in cucumber

Three methods were used to identify all members of the *TIFY* family in cucumber. Firstly, the keyword "TIFY" was used as a query to search the cucumber database (http://cucurbitgenomics.org/organism/2). Secondly, the *Arabidopsis* TIFY family members were downloaded from the TAIR database (https://www.arabidopsis.org/index.jsp) and used as the query sequences for an online BLASTP search against the cucumber genome database, with an E value of e-5. Thirdly, the Hidden Markov Model (HMM) profile for TIFY (Pfam ID: PF06200) was downloaded from the Pfam database (http://pfam.xfam.org/), and searched in the cucumber genome database by using the HMMER3.0 software. After removal of the redundant sequences, all acquired protein sequences were examined for the presence of a TIFY domain using the NCBI (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi), SMART (http://smart.embl-heidelberg.de/) and Interpro (http://www.ebi.ac.uk/interpro/). The online website ExPASy (https://web.expasy.org/protparam/) was used to analyse the physical and chemical properties of the cucumber TIFY family members, including the molecular weight (MW), isoelectric point (pI) and GRAVY values. In addition, the subcellular localization of cucumber TIFY proteins was predicted by the online tool Plant-mPLoc server (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/).

Phylogenetic and conserved motif composition analysis

The protein sequences of TIFYs from different plants were aligned with the online multiple sequence alignment tool MAFFT (https://www.ebi.ac.uk/Tools/msa/mafft/) (Madeira *et al.*, 2019), and then the alignment results were used to create a phylogenetic tree with the MEGA 7.0 software by neighbor-joining (NJ) method, with the bootstrap analysis with 1000 replications, pairwise deletion and a Poisson model. To investigate the conserved motifs of TIFY members from cucumber, the complete amino acid sequences of TIFY proteins were analyzed using the online MEME tool (Bailey *et al.*, 2009). The parameters for the analysis were as follows: maximum number of motifs, 8; minimum motif width, 6; and maximum motif width, 50.

Chromosomal location, gene duplication and exon-intron structure analysis

Based on the chromosomal position information provided by the cucumber database (http://cucurbitgenomics.org/organism/2), a chromosomal location image for the cucumber *TIFY* genes was drawn using the MapInspect software. Gene duplication events, including tandem and segmental duplications, were identified by MCScanX based on our previous study (Yang *et al.*, 2019). For gene structure analysis, the coding sequence (CDS) and corresponding genomic DNA (gDNA) sequences were extracted by the TBtools software, and then the exon-intron structure of cucumber *TIFY* genes was displayed with the GSDS tool (http://gsds.gao-lab.org/).

Expression analysis of TIFY family genes in cucumber based on RNA-seq data

Genome-wide RNA-seq data of different tissues and organs such as male and female flowers, leaves, ovaries, roots, stems and tendrils were obtained from the NCBI database (PRJNA80169). The expression data of cucumber *TIFY* family genes under different abiotic stresses including heat (0, 3 and 6 h after 42 °C treatment) and salt (75 mM NaCl and the control with water) were obtained from NCBI under the accession numbers of PRJNA634519 and PRJNA437579), respectively (Chen *et al.*, 2020). To analyze the expression of the cucumber *TIFY* genes in response to biotic stresses, RNA-seq data including cucumber plants inoculated

with root-knot nematode (RKN, *Meloidogyne incognita*) (accession number: SRP125669) (Wang *et al.*, 2018) at different days post inoculation (dpi) were retrieved from NCBI. The transcripts per kilobase million (TPM) values for *CsTIFY* genes were calculated and then demonstrated by the TBtools software (Zhou *et al.*, 2021). A threshold of |log2 (fold change)| > 1 was used to evaluate the significance of differentially expressed genes.

Plant materials and treatments

The cucumber plants ('Chinese long' inbred line 9930) used in this study were planted in a greenhouse under a photoperiod of 16/8 h (day/night) at 18-24 °C. Two-week-old seedlings were treated with cold (4 °C), salt (200 mM NaCl) and drought (10% PEG-6000, w/v) stress as previously described (Zhou *et al.*, 2020). The leaf tissues were sampled with three biological triplicates at 0, 6, 12 and 24 h after each treatment. All samples were placed in liquid nitrogen and kept at −80 °C prior to RNA isolation.

RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from the samples with the RNA prep Pure Plant Kit (TransGen, China), and about 3 μg RNA for each sample was used for first-strand cDNA synthesis with the Superscript III RNase H-Reverse Transcriptase kit. Quantitative real time PCR (qRT-PCR) was performed in an optical 96-well plate according to our previous study on LightCycler LC480 II Real-Time PCR System by using TB Green Premix Ex TaqII Kit (TaKaRa, China) (Zhou *et al.*, 2020). The $2^{-\Delta\Delta Ct}$ method was used to analyze the relative expression levels of the target genes by using the *CsAct3* gene as an internal control. The primer names and sequences for qRT-PCR are listed in Table S1.

Results

Genome-wide identification of TIFY family genes in cucumber

After removal of redundant sequences and structural domain analysis, a total of 17 *TIFY* genes were identified in the cucumber genome, which were designated as *CsPPD1*, *CsTIFY1*–*CsTIFY2*, *CsZML1*–*CsZML4* and *CsJAZ1*–*CsJAZ10* based on their order on the chromosomes and characteristics of conserved domains (Table 1).

The gDNA lengths of cucumber *TIFY* family genes ranged from 750 bp (*CsJAZ2*) to 7287 bp (*CsZML1*), and the lengths of encoded proteins varied from 129 (CsJAZ6) to 450 (CsTIFY1) amino acids. The MWs of the cucumber TIFY proteins ranged from 14.74 (CsJAZ9) to 48.13 (CsZML3) kDa, and correspondingly the pIs ranged from 4.86 (CsZML1) to 10.42 (CsJAZ6) (Table 1). In addition, the GRAVY values varied from −0.871 (CsZML4) to −0.250 (CsJAZ1), suggesting that cucumber TIFY proteins are hydrophilic. Prediction by Plant-mPLoc indicted that all members of the cucumber TIFY proteins are located in the nucleus (Table 1).

Phylogenetic analysis of the TIFY genes among five species

To investigate the phylogenetic relationships of *TIFY* genes among different plants, a phylogenetic tree was constructed using the TIFY protein sequences from *Arabidopsis*, rice, *Brachypodium distachyon*, *Brassica oleracea* and cucumber. All TIFY proteins of five species were classified into seven groups in the phylogenetic tree, which were named as TIFY, JAZ I–IV, ZML and PPD (Figure 1). The JAZ proteins were further divided into four groups (JAZ I–IV), and JAZs from all these plants could be found in JAZ-I, JAZ-II and JAZ-IV, while the JAZ-III group only comprised JAZs from *Arabidopsis*, *B. oleracea* and cucumber (Figure 1).

Table 1. Identification and characterization of *TIFY* family genes in cucumber

Gene	Locus (V2)	Chromosome	Chromosomal position	gDNA (bp)	CDS (bp)	Protein					
						Length (aa)	MW (KDa)	pI	GRAVY	Subcellular prediction	TIFY motif
CsJAZ1	Csa1G042920.1	Chr1	4573626-4577405	3780	1023	340	36.62	9.20	-0.250	Nucleus	TIFYAG
CsJAZ2	Csa1G435720.1	Chr1	15995968-15996717	750	453	150	16.93	7.21	-0.767	Nucleus	TIFYNG
CsJAZ3	Csa1G597690.1	Chr1	22692966-22694494	1529	696	231	25.03	9.98	-0.455	Nucleus	TIFYAG
CsJAZ4	Csa3G645940.1	Chr3	25350295-25351576	1282	630	209	22.87	8.94	-0.631	Nucleus	TIFYDG
CsJAZ5	Csa4G009880.1	Chr4	1470022-1476285	6264	603	200	22.49	6.98	-0.553	Nucleus	TIFYNE
CsJAZ6	Csa4G062400.1	Chr4	4986428-4991013	4586	390	129	14.86	10.42	-0.425	Nucleus	MVFYNG
CsJAZ7	Csa5G628650.1	Chr5	25425307-25429296	3990	1146	381	39.67	9.30	-0.293	Nucleus	TIFYGG
CsJAZ8	Csa6G091930.1	Chr6	6249153-6251831	2679	555	184	20.31	9.41	-0.317	Nucleus	TIFYNG
CsJAZ9	Csa6G523460.1	Chr6	28126619-28128465	1847	399	132	14.74	9.50	-0.805	Nucleus	TIFYNG
CsJAZ10	Csa7G448810.1	Chr7	18408423-18411214	2792	888	295	32.09	9.31	-0.758	Nucleus	TIFYAG
CsZML1	Csa2G370420.1	Chr2	18236800-18244086	7287	1059	352	38.58	4.86	-0.706	Nucleus	TLSFEG
CsZML2	Csa2G370430.1	Chr2	18245818-18251412	5595	855	284	30.70	6.32	-0.630	Nucleus	TLSFRG
CsZML3	Csa7G064580.1	Chr7	3845510-3853491	7982	1335	444	48.13	6.15	-0.405	Nucleus	TLSFRG
CsZML4	Csa7G447800.1	Chr7	18027186-18032948	5763	912	303	33.95	6.39	-0.871	Nucleus	TLSYQG
CsTIFY1	Csa2G379290.1	Chr2	19295221-19299018	3798	1353	450	47.43	9.05	-0.607	Nucleus	TIFYGG
CsTIFY2	Csa3G878900.1	Chr3	36976253-36980341	4089	1203	400	42.77	7.77	-0.636	Nucleus	TIFYGG
CsPPD1	Csa2G222060.1	Chr2	10711729-10716520	4792	1011	336	36.97	6.70	-0.751	Nucleus	TIFYCG

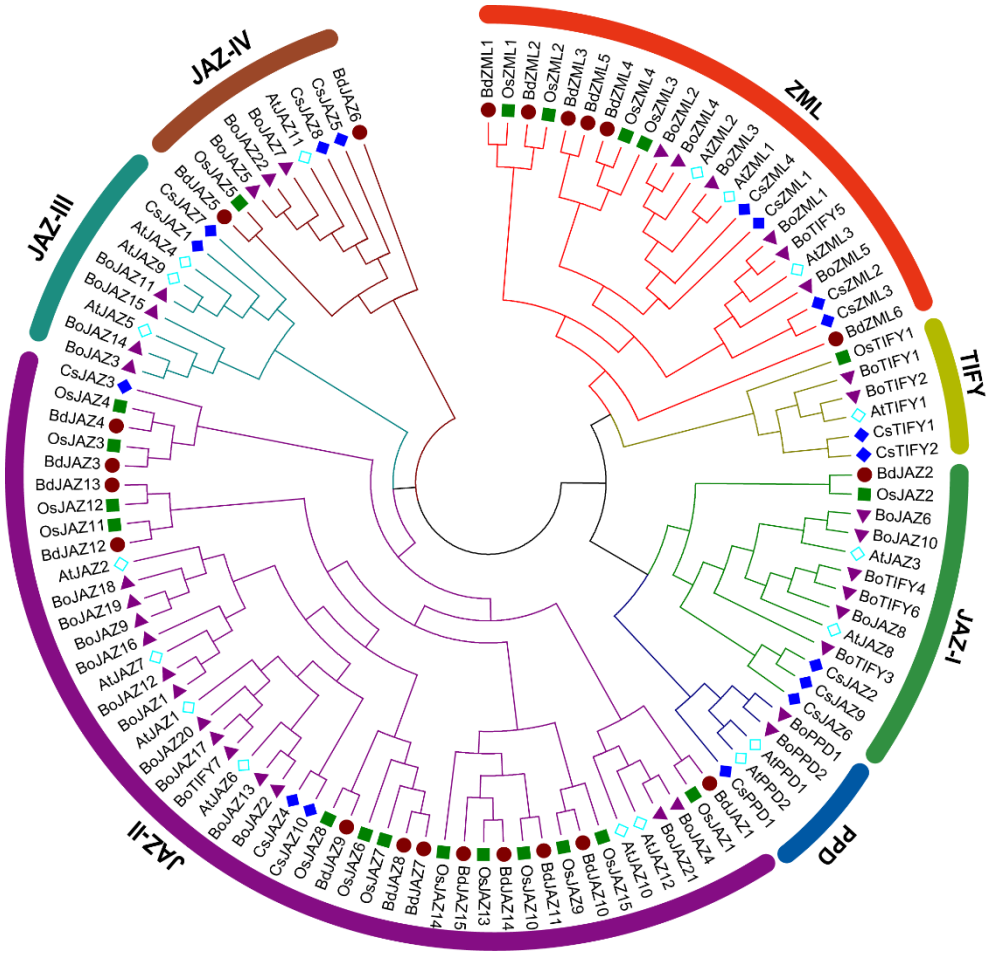

Figure 1. Phylogenetic tree of TIFY proteins from *Arabidopsis*, rice, *Brachypodium distachyon*, *Brassica oleracea* and cucumber

The phylogenetic tree was constructed using the neighbor-joining method as implemented in MEGA7.0 from TIFY protein sequence alignment obtained through online MAFFT tool. At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Bd, *Brachypodium distachyon*; Bo, *Brassica oleracea*; Cs, *Cucumis sativus*.

Conserved motifs and gene structures of CsTIFY members

Of the 17 CsTIFY proteins, CsTIFY1 and CsTIFY2 contained only the TIFY domain, while the remaining members contained other conserved domains, and the positions of these domains in the sequence were relatively fixed (Figure 2). For example, proteins from the JAZ subfamily had the Jas domain at the C-terminal region, while CsZMLs contained the CCT and C2C2-GATA zinc-finger domain. In addition, CsPPD1 contained an N-terminal PPD domain and a C-terminal modified Jas motif domain (Figure 2). Moreover, members of the JAZ, TIFY and PPD subfamilies possessed the typical conserved TIFY domain (TIFYXG), except for CsJAZ5 and CsJAZ6, which carried variations in the conserved sequences as TIFYNE and MVFYNG, respectively (Table 1; Figure S1). However, the characteristic sequence of the TIFY domain of CsZMLs was TLS(F/Y)XG, which is in accordance with the results in other plants, such as rice (Ye *et al.*, 2009), *B. distachyon* (Zhang *et al.*, 2015a), tomato (Chini *et al.*, 2017), pear (Ma *et al.*, 2018), and watermelon (Yang *et al.*, 2019).

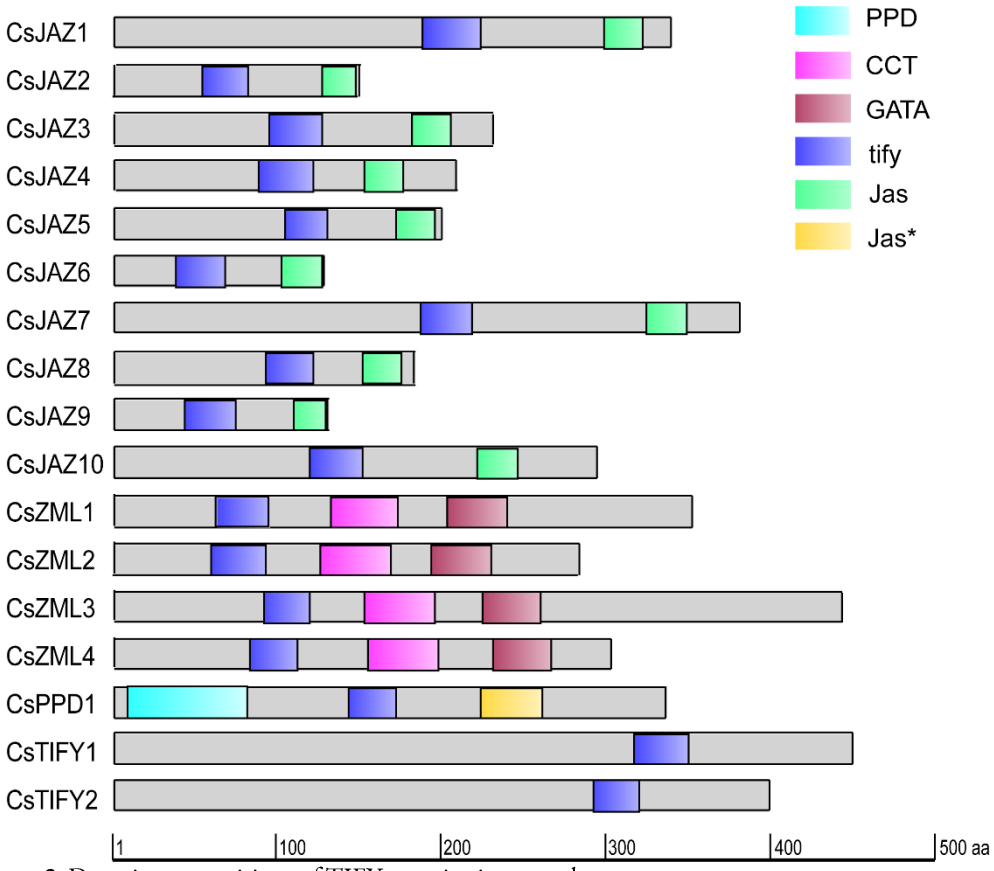

Figure 2. Domain compositions of TIFY proteins in cucumber
Tify, TIFY domain; CCT, CONSTANS, CO-like, and TOC1 (CCT) domain; GATA, a C2C2-GATA zinc-finger DNA-binding domain; PPD, PEAPOD domain; Jas, Jas domain; Jas*, Jas-like domain.

To further assess the conserved motif composition of the CsTIFY proteins, a total of eight conserved motifs were confirmed using the MEME tool. Motifs 1 and 4 constituted the TIFY domain, which was present in all CsTIFY proteins (Figure 3B). Motifs 2 and 5 composed the CCT domain. Furthermore, motif 2 was also present at the C-terminus of all JAZ subfamily protein sequences. Motif 3 represents the C2C2-GATA zinc-finger DNA-binding domain, which only exists at the C-terminal region of the ZML subfamily (Figure 3A, 3B). Motif 6 was found in all members of the TIFY subfamily, as well as in some members of the JAZ subfamily, while motif 7 was specific to CsJAZ4 and CsJAZ10 of the JAZ subfamily (Figure 3A, 3B).

We also examined the gene structure of *CsTIFY* genes. As shown in Figure 3C, the ZML subfamily had the largest number of introns, ranging from six to ten. The number of introns in the JAZ subfamily varied from one to six, among which *CsJAZ2* and *CsJAZ9* contained only one intron. In addition, *CsPPD1* contained eight introns, and both of *CsTIFY1* and *CsTIFY2* harboured five introns (Figure 3C).

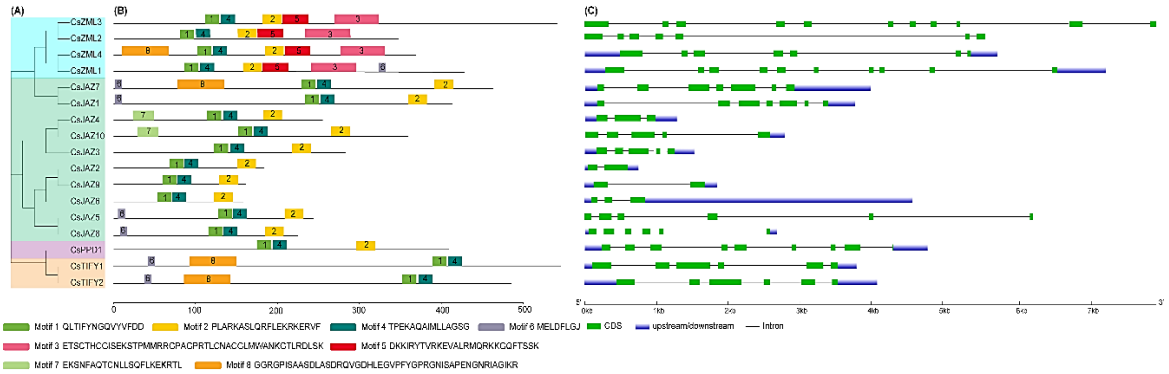

Figure 3. Analysis of phylogenetic relationship, conserved motifs and gene structure of TIFY gene family in cucumber

(A) Phylogenetic tree of *TIFY* genes in cucumber. (B) Conserved motif composition of cucumber TIFY proteins identified with the MEME tool. A summary of the distribution of conserved motifs is shown at the bottom. (C) Gene structures of cucumber *TIFY* genes. The CDS, upstream/downstream, and intron are shown as blue boxes, green boxes and black lines, respectively.

Chromosomal location and gene duplication of the CsTIFY genes

To determine the distribution of *CsTIFY* genes on cucumber chromosomes, a chromosome map was drawn. As shown in Figure 4, *CsTIFY* genes were distributed on all cucumber chromosomes. Amongst them, chromosome 2 possessed the largest number of genes (*CsPPD1*, *CsZML1*, *CsZML2* and *CsTIFY1*). Chromosomes 1 and 7 had three genes each; chromosomes 3, 4 and 6 each contained two genes, and chromosome 5 only had one gene. *CsZML1* and *CsZML2* were located on the same chromosome with a very small interval (Figure 4). In the duplication analysis of *CsTIFY* genes, one tandem duplication event (*CsZML1*/*CsZML2*) and four segmental duplication events (*CsJAZ2*/*CsJAZ9*, *CsJAZ3*/*CsJAZ10*, *CsJAZ4*/*CsJAZ10* and *CsTIFY1*/*CsTIFY2*) were identified, respectively (Figure 4).

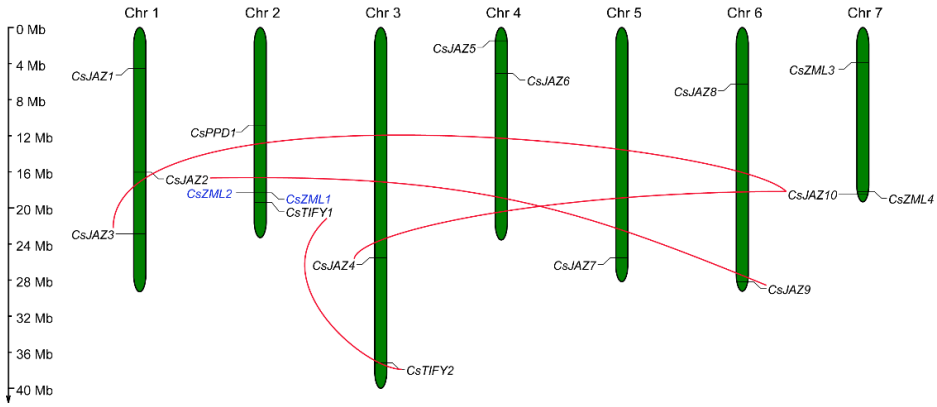

Figure 4. Chromosomal distribution and duplication analysis of the *TIFY* genes

The chromosome numbers are shown at the top of each chromosome. Scale represents a 4 Mb chromosomal distance. Segmental duplication genes are linked by red lines, and tandem duplication genes are marked with blue.

Expression analysis of CsTIFY genes in cucumber tissues

The RNA-seq data from different tissues of cucumber were employed to analyze the tissue expression patterns of *CsTIFY* genes. As shown in Figure 5, the *CsTIFY* genes were expressed in all tested tissues in this study. Amongst them, *CsJAZ1*, *CsJAZ2*, *CsJAZ3*, *CsJAZ6*, *CsJAZ7*, *CsJAZ8*, *CsJAZ9* and *CsJAZ10* exhibited the highest levels in male and/or female flowers (Figure 5). The expression levels of *CsJAZ4* and *CsZML4* were relatively higher in root and tendril, respectively. In addition, *CsJAZ2*, *CsJAZ3*, *CsJAZ4*, *CsJAZ5*, *CsJAZ10*,

CsZML1 and *CsTIFY2* showed different expression levels in unexpanded ovary, unfertilized ovary and fertilized ovary, indicating that they may play an important role in ovary development of cucumber (Figure 5).

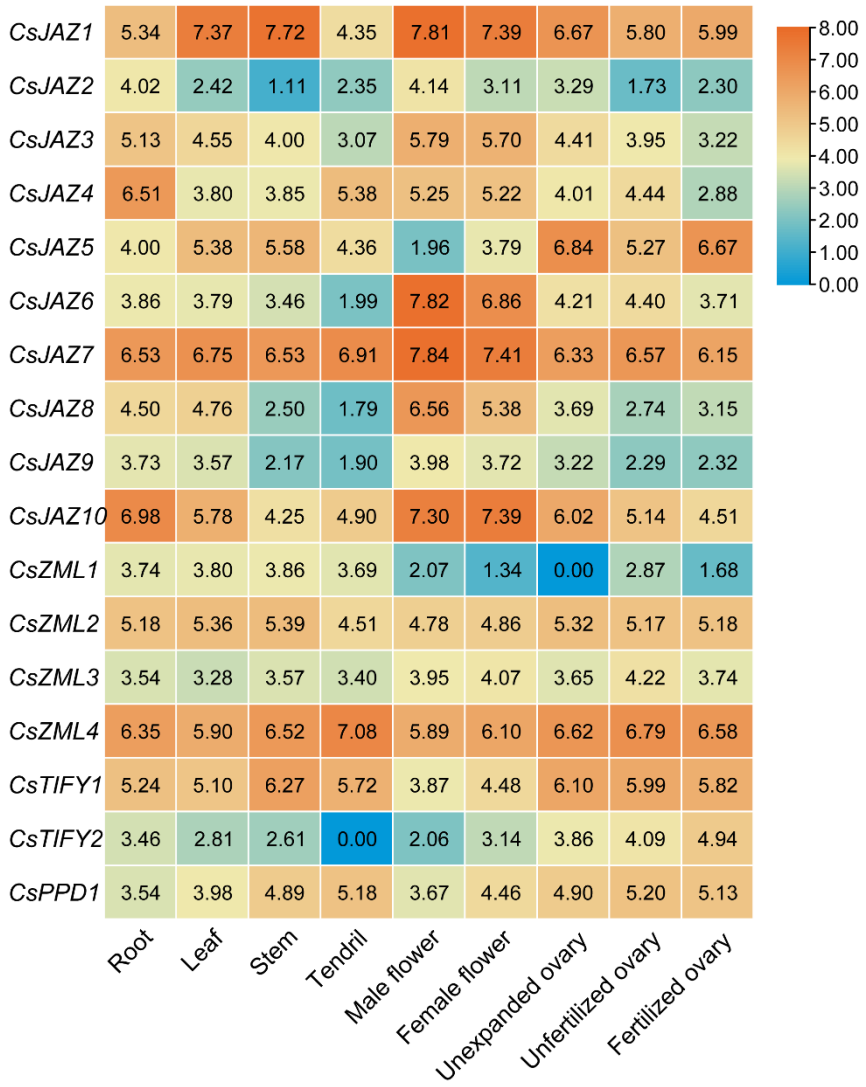

Figure 5. Expression patterns of the *CsTIFY* genes in cucumber tissues. The data in the boxes indicate the log2 of TPM values

Response of CsTIFY genes to different abiotic stress treatments

To determine whether the *CsTIFY* genes are involved in abiotic stress response, we analyzed the expression patterns of *CsTIFY* genes under heat and salt stresses according to the public RNA-seq data. As shown in Figure 6A, a total of 10 genes (*CsJAZ2*, *CsJAZ3*, *CsJAZ4*, *CsJAZ5*, *CsJAZ6*, *CsJAZ7*, *CsJAZ9*, *CsTIFY1*, *CsTIFY2*, and *CsPPD1*) were observably induced, while only *CsZML3* was repressed by heat stress. For the salt stress treatment, the transcription of nearly all *CsTIFY* genes was dramatically increased, except for *CsTIFY1* and *CsPPD1* (Figure 6B). These results demonstrated that the *CsTIFY* genes are associated with temperature and salt stress in cucumber.

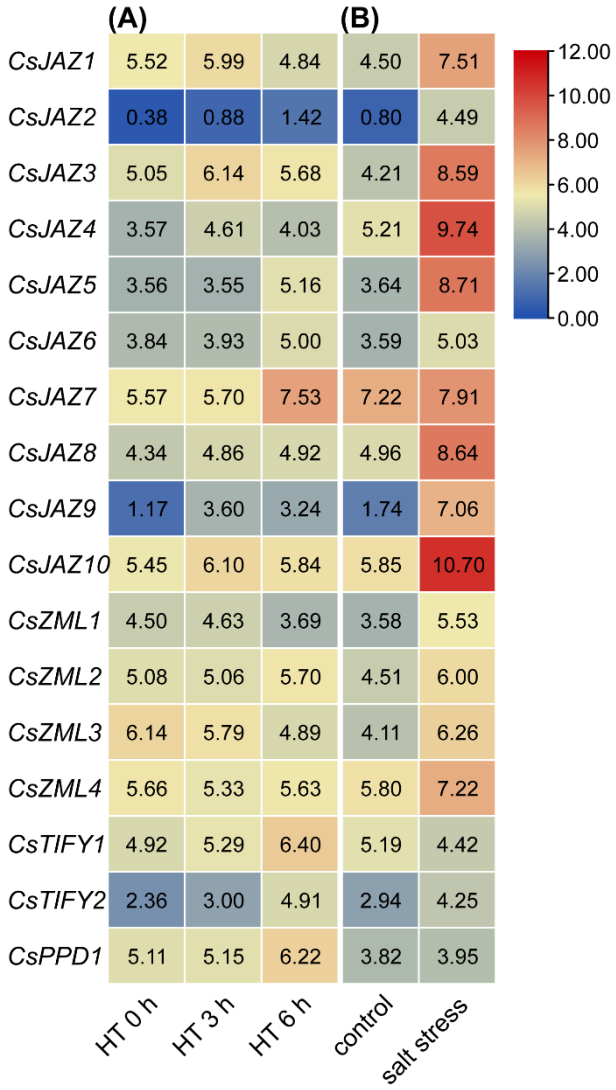

Figure 6. Expression levels of *CsTIFY* genes in response to heat (A) and salt (B) stresses based on RNA-seq expression data

The data in the boxes indicate the log2 of TPM values. (A) HT, heat stress. HT 0 h / HT 3 h / HT 6 h: 0, 3, and 6 h after 42 °C treatment. (B) Seedlings of a salt-sensitive cucumber cv. 'Jinchun No. 2' were subjected to 75 mM NaCl stress for 24 h, using 0 mM NaCl as control.

We finally evaluated the expression patterns of six selected *CsTIFY* genes (two JAZs, two ZMLs, one TIFY, and one PPD) in leaves under cold, drought and salt treatments by qRT-PCR. Under cold treatment, all the tested *CsTIFY* genes were significantly down-regulated at all the time points (Figure 7A). Under drought treatment, all the six selected cucumber *TIFY* genes displayed increasing expression levels, which reached the peak at 12 h or 24 h (Figure 7B). Under salt treatment, the six *CsTIFY* genes exhibited similar expression profiles as under drought treatment, with *CsZML1* being the mostly responsive gene (Figure 7C). These results suggested that the *CsTIFY* genes are involved in response to various abiotic stresses.

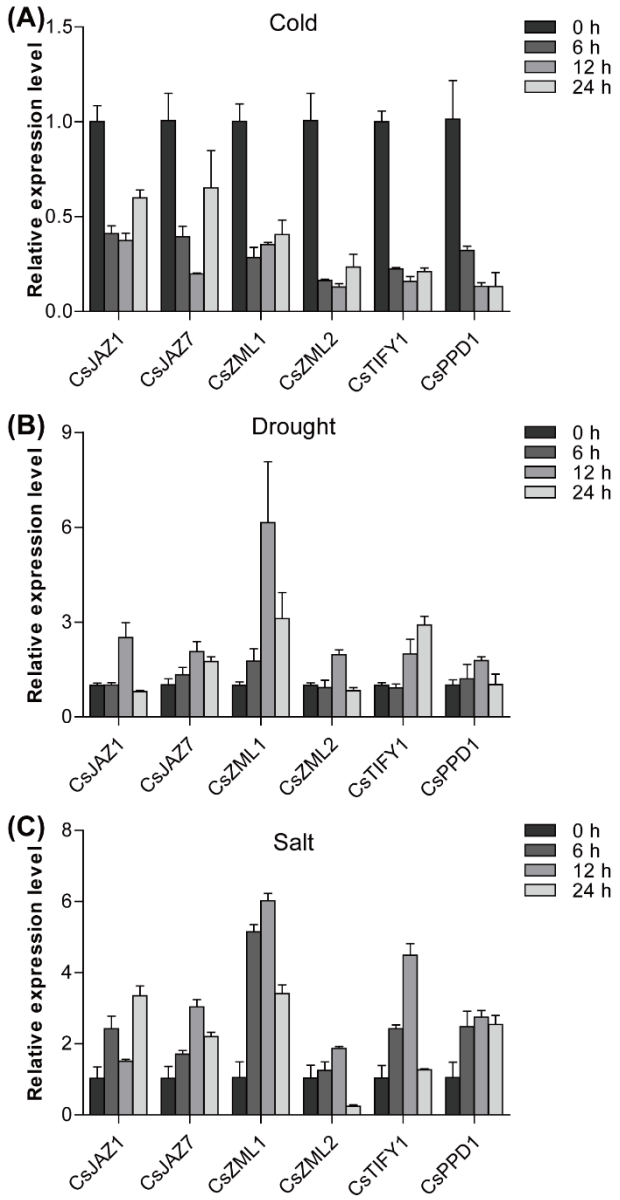

Figure 7. qRT-PCR analysis of six selected cucumber *TIFY* genes after 0, 6, 12 and 24 h treatment of various abiotic stresses including cold (A), drought (B) and salt (C)

Relative expression levels of the selected cucumber *TIFY* genes were analysed at 6, 12, and 24 h of treatments compared with their values at 0 h, which were normalized to 1.0.

Expression analysis of CsTIFY genes in response to RKN infection

To investigate the possible functions of *CsTIFY* genes in response to biotic stress, we determined the expression of *CsTIFY* genes in RKN-susceptible (CC3) and RKN-resistant (IL10–1) cucumber plants under RKN infection. As shown in Figure 7, *CsJAZ1*, *CsJAZ2*, *CsJAZ3*, *CsJAZ4*, *CsJAZ5*, *CsJAZ8* and *CsJAZ10* were significantly down-regulated in CC3 under RKN infection, while the expression of other genes was not altered by RKN infection. In resistant cucumber line IL10–1, only *CsJAZ1*, *CsJAZ2*, *CsJAZ3*, *CsJAZ4*, *CsJAZ8* and *CsJAZ10* were significantly suppressed by RKN infection (Figure 8). These results indicated that these genes may play negative roles in the response of cucumber to RKN infection.

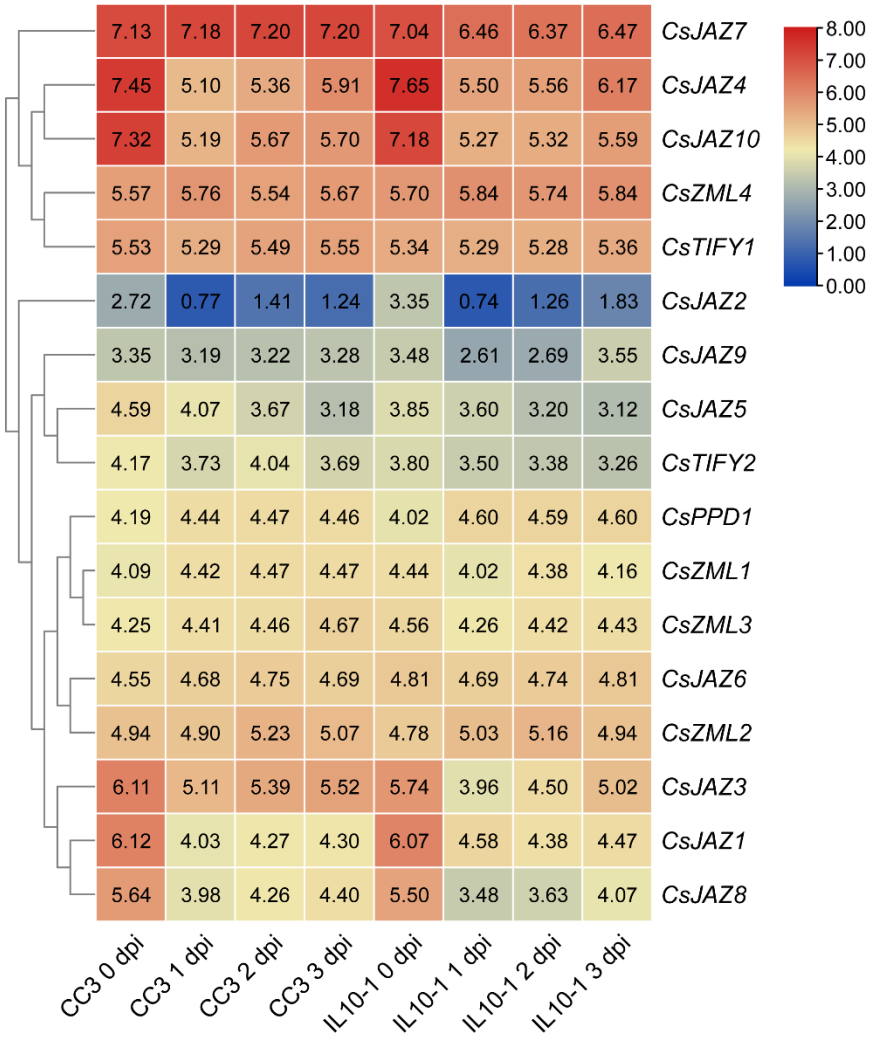

Figure 8. Expression levels of *CsTIFY* genes in response to RKN based on RNA-seq expression data
CC3 and IL10–1 are RKN-susceptible and RKN-resistant cucumber plants, respectively. The data in the boxes indicate the log2 of TPM values.

Discussion

In this study, we identified 17 *TIFY* genes in the cucumber database based on sequence analysis, including 10 *JAZ* genes (*CsJAZ1–CsJAZ10*), four *ZML* genes (*CsZML1–CsZML4*), two *TIFY* genes (*CsTIFY1* and *CsTIFY2*), and only one *PPD* gene (*CsPPD1*) (Table 1). The largest and smallest number of genes were found in the JAZ subfamily and PPD subfamily in cucumber, respectively. In previous reports, 15, 18, 19, 20, 21, 21, 26, 36, 48 and 77 *TIFY* family genes were identified in watermelon (Yang *et al.*, 2019), pigeonpea (Sirhindi *et al.*, 2016), *Arabidopsis* (Bai *et al.*, 2011; Thireault *et al.*, 2015), rice (Ye *et al.*, 2009), *B. distachyon* (Zhang *et al.*, 2015a), pear (Ma *et al.*, 2018), tomato (Heidari *et al.*, 2021), *Brassica rapa* (Saha *et al.*, 2016), maize (Sun *et al.*, 2021; Heidari *et al.*, 2021), and *B. napus* (He *et al.*, 2020), respectively. Similarly, the JAZ subfamily has the largest number of genes, followed by the ZML, TIFY and PPD subfamilies. The TIFY and PPD subfamilies have a very small number of genes, and even no genes in some plant species. For example, the PPD subfamily is generally absent in monocots (Bai *et al.*, 2011; Huang *et al.*, 2016; Heidari *et al.*, 2021), and the TIFY subfamily is also absent in some plants, such as *B. distachyon* (Zhang *et al.*, 2015a) and *C. sinensis*

(Zhang *et al.*, 2020). In addition, a total of two and seven *CsTIFY* genes constitute one and four pairs of tandem and segmental duplication events, respectively (Figure 4). Therefore, it can be speculated that the discrepancies of *TIFY* family genes among different plant species may be attributed to duplication events and differences in genome size.

The phylogenetic analysis revealed that the TIFY proteins of five species could be classified into TIFY, JAZ, ZML and PPD subfamilies (Figure 1), and the JAZ subfamily could be further divided into JAZ I–IV as previously described (Huang *et al.*, 2016; Li *et al.*, 2021). Domain analysis demonstrated that most CsTIFY proteins carry a common characteristic conserved TIFY domain (TIF[F/Y]XG), with a variable pattern of TIFYNE, MVFYNG and TLS(F/Y)XG for some members (Table 1; Figure S1), implying the possible diverse roles of the CsTIFY proteins. The identification of conserved motifs with the MEME tool also verified the presence of the specific domains, including TIFY, CCT, Jas, PPD and C2C2-GATA zinc-finger DNA-binding domain (Figures 2 and 3B). However, gain or loss of certain motifs was found for several duplicated genes, such as *CsZML1*/*CsZML2* (motif 6) and *CsJAZ3*/*CsJAZ10* (motif 7) (Figures 3B and 4), implying that these genes may have discrepant functions. Gene structure analysis revealed that all the 17 *CsTIFY* genes have introns, and the genes in the JAZ subfamily tend to have fewer introns, while the *CsZML* genes usually have longer gene length and more introns than other subfamily genes (Figure 3A and 3C). Similar results have also been observed in other plants, such as watermelon (Yang *et al.*, 2019) and *Populus trichocarpa* (Wang *et al.*, 2020). Moreover, the closely related genes have similar gene structures with the same intron numbers and CDS lengths, such as *CsJAZ1*/*CsJAZ7*, *CsJAZ5*/*CsJAZ8*, *CsJAZ3*/*CsJAZ10*, and *CsTIFY1*/*CsTIFY2*, but a pair of segmental duplication genes (*CsJAZ4*/*CsJAZ10*) have different gene structures with different intron numbers, suggesting the functional diversity of cucumber *TIFY* genes.

As have been described in numerous previous studies, *TIFY* family genes are constitutively expressed but with preferential expression in specific tissues (Zhang *et al.*, 2015b; Wang *et al.*, 2017; Yang *et al.*, 2019). In this study, some *JAZ* genes were found to be predominantly expressed in reproductive organs such as flowers and ovaries, indicating their key roles in flower and ovary development of cucumber (Figure 5). Similarly, *B. rapa* JAZ subfamily genes were found to be highly expressed in flower buds (Saha *et al.*, 2016), and *C. sinensis JAZ2* and *JAZ7* also exhibited higher transcription levels in flowers than in other organs (Zhang *et al.*, 2020). Among the 12 *P. trichocarpa JAZ* genes, six showed abundant expression levels in catkins (Wang *et al.*, 2017). In addition, some cucumber *TIFY* genes displayed higher expression levels in vegetative organs such as root and tendril (Figure 5). In *C. sinensis*, the majority of *JAZ* genes had higher expression in roots than in leaves and stems (Shen *et al.*, 2020). Most *JAZ* genes from *B. napus* and *B. oleracea* were also found to be highly expressed in roots (He *et al.*, 2020).

A number of studies have shown that the *TIFY* family genes are involved in the regulation of response to various abiotic stresses in plants. In this study, a total of 10 and 15 cucumber *TIFY* genes were up-regulated under heat and salt stress treatments, respectively (Figure 6), indicating that they play key roles in response to abiotic stresses. Besides, qRT-PCR results revealed that all six selected cucumber *TIFY* genes exhibited different degrees of increase in expression under drought and salt stress conditions, suggesting their positive roles in response to these two stresses (Figure 7B, 7C). Similarly, many *OsTIFY* genes were upregulated under both drought and salt stress, and overexpression of *OsTIFY11a* resulted in higher tolerance to salt and mannitol stress in transgenic rice plants (Ye *et al.*, 2009). Another rice *TIFY* gene *OsJAZ1* participates in drought resistance by regulating the JA and ABA signaling pathways (Fu *et al.*, 2017). In apple, six *TIFY* genes were upregulated by both drought and salt treatments (Li *et al.*, 2015), and transgenic *Arabidopsis* plants overexpressing apple *MdJAZ2* displayed higher tolerance to salt and drought stress with a decrease in JA sensitivity (An *et al.*, 2017). Overexpression of *PnJAZ1* in *Arabidopsis* and *Physcomitrella* could also promote the tolerance to salt and osmotic stresses (Liu *et al.*, 2019). However, all of the tested *CsTIFY* genes were

downregulated under cold stress (Figure 7A). Although most *TIFY* genes were observed to be upregulated by cold stress in *P. edulis* (Huang *et al.*, 2016), *B. rapa* (Saha *et al.*, 2016) and *C. sinensis* (Shen *et al.*, 2020), there are also some *TIFY* genes displaying downregulated expression in response to cold treatment in different plants such as *P. trichocarpa* (Wang *et al.*, 2017; Wang *et al.*, 2020).

JA has been confirmed to play a crucial regulatory role in plant defense against RKN infection in various plants (Nahar *et al.*, 2011; Yang *et al.*, 2018; Bali *et al.*, 2018; Zhou *et al.*, 2015a). In watermelon, JA is involved in red light-induced defense against RKN with remarkable increases in the expression of some JA biosynthesis and signaling pathway genes (Yang *et al.*, 2018; Guang *et al.*, 2021). In this study, *CsJAZ1*, *CsJAZ2*, *CsJAZ3*, *CsJAZ4*, *CsJAZ8* and *CsJAZ10* were significantly suppressed by RKN infection in both RKN-susceptible and RKN-resistant cucumber plants (Figure 8). Similar findings were also reported in other plants. In sweet potato, the expression of JA-repressor *JAZ1* was downregulated in RKN-resistant plants upon RKN infection, whereas that of the genes involved in JA biosynthesis (*LOX1*) and JA signaling (*MYC2* and *MYC4*) was obviously increased (Lee *et al.*, 2019). *Arabidopsis* MYC2, MYC3, MYC4 and MYC5 were found to act as targets of JAZ repressors and play redundant roles in JA-regulated plant growth and defense responses (Song *et al.*, 2017; Cheng *et al.*, 2011; Fernández-Calvo *et al.*, 2011; Niu *et al.*, 2011). In a recent study, many *CsMYB* genes were upregulated at certain time points after RKN infection in both RKN-resistant and RKN-susceptible cucumber plants (Cheng *et al.*, 2020). Since JAZs are repressors of the JA signaling pathway, the RKN-repressed *CsJAZ* genes may be involved in JA-mediated RKN resistance in cucumber by regulating the TFs that interact with JAZs, such as MYBs and MYCs.

Conclusions

In this work, a comprehensive analysis of the *TIFY* family genes was carried out in cucumber, resulting in the identification of 17 *TIFY* genes (including one *PPD* gene, two *TIFY* genes, four *ZML* genes and 10 *JAZ* genes). Their evolutionary relationships, conserved motifs, gene structures, chromosomal locations, gene duplication events and tissue expression patterns were also investigated. All CsTIFY proteins possess the conserved TIFY domain (TIF[F/Y]XG), or with a variable pattern of TIFYNE, MVFYNG and TLS(F/Y)XG. The closely related TIFY members have similar conserved motifs and gene structures. Expression analysis revealed that some *CsTIFY* genes are preferentially expressed in specific tissues and responsive to multiple abiotic stresses, as well as to RKN infection. Our findings provide valuable information for revealing the function of the *TIFY* family genes in cucumber.

Authors' Contributions

Conceptualization: SL and YC; Data curation: JH, YC, LX, ZH and SL; Formal analysis: JH, YZ and SL; Funding acquisition: YZ and SL; Investigation: JH, YC, LX, ZH and SL; Methodology: SL, JH and YZ; Validation: YZ and SL; Writing - original draft: YZ and SL; Writing - review and editing: YZ and SL. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

An XH, Hao YJ, Li EM, Xu K, Cheng CG (2017). Functional identification of apple MdJAZ2 in Arabidopsis with reduced JA-sensitivity and increased stress tolerance. Plant Cell Reports 36(2):255-265. *https://doi.org/10.1007/s00299-016-2077-9*

Bai Y, Meng Y, Huang D, Qi Y, Chen M (2011). Origin and evolutionary analysis of the plant-specific TIFY transcription factor family. Genomics 98(2):128-136. *https://doi.org/10.1016/j.ygeno.2011.05.002*

Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, ... Noble WS (2009). MEME SUITE: tools for motif discovery and searching. Nucleic Acids Research 37:W202-208. *https://doi.org/10.1093/nar/gkp335*

Bali S, Kaur P, Sharma A, Ohri P, Bhardwaj R, Alyemeni MN, ... Ahmad P (2018). Jasmonic acid-induced tolerance to root-knot nematodes in tomato plants through altered photosynthetic and antioxidative defense mechanisms. Protoplasma 255(2):471-484. *https://doi.org/10.1007/s00709-017-1160-6*

Cai Q, Yuan Z, Chen M, Yin C, Luo Z, Zhao X, ... Zhang D (2014). Jasmonic acid regulates spikelet development in rice. Nature Communication 5:3476. *https://doi.org/10.1038/ncomms4476*

Chen C, Chen X, Han J, Lu W, Ren Z (2020). Genome-wide analysis of the *WRKY* gene family in the cucumber genome and transcriptome-wide identification of WRKY transcription factors that respond to biotic and abiotic stresses. BMC Plant Biology 20(1):443. *https://doi.org/10.1186/s12870-020-02625-8*

Cheng C, Li Q, Wang X, Li Y, Qian C, Li J, ... Chen J (2020). Identification and expression analysis of the *CsMYB* gene family in root knot nematode-resistant and susceptible cucumbers. Frontiers in Genetics 11:550677. *https://doi.org/10.3389/fgene.2020.550677*

Cheng Z, Sun L, Qi T, Zhang B, Peng W, Liu Y, Xie D (2011). The bHLH transcription factor MYC3 interacts with the Jasmonate ZIM-domain proteins to mediate jasmonate response in *Arabidopsis*. Molecular Plant 4(2):279-288. *https://doi.org/10.1093/mp/ssq073*

Chini A, Ben-Romdhane W, Hassairi A, Aboul-Soud MAM (2017). Identification of TIFY/JAZ family genes in Solanum lycopersicum and their regulation in response to abiotic stresses. PLoS One 12(6):e0177381. *https://doi.org/10.1371/journal.pone.0177381*

Chini A, Gimenez-Ibanez S, Goossens A, Solano R (2016). Redundancy and specificity in jasmonate signalling. Current Opinion in Plant Biology 33:147-156. *https://doi.org/10.1016/j.pbi.2016.07.005*

Chung HS, Niu Y, Browse J, Howe GA (2009). Top hits in contemporary JAZ: an update on jasmonate signaling. Phytochemistry 70(13-14):1547-1559. *https://doi.org/10.1016/j.phytochem.2009.08.022*

de Torres Zabala M, Zhai B, Jayaraman S, Eleftheriadou G, Winsbury R, Yang R, ... Grant M (2016). Novel JAZ co-operativity and unexpected JA dynamics underpin *Arabidopsis* defence responses to *Pseudomonas syringae* infection. New Phytologist 209(3):1120-1134. *https://doi.org/10.1111/nph.13683*

Ebel C, BenFeki A, Hanin M, Solano R, Chini A (2018). Characterization of wheat (*Triticum aestivum*) *TIFY* family and role of *Triticum durum* TdTIFY11a in salt stress tolerance. PLoS One 13(7):e0200566. *https://doi.org/10.1371/journal.pone.0200566*

Fernández-Calvo P, Chini A, Fernández-Barbero G, Chico JM, Gimenez-Ibanez S, Geerinck J, ... Solano R (2011). The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. Plant Cell 23(2):701-715. *https://doi.org/10.1105/tpc.110.080788*

Fu J, Wu H, Ma S, Xiang D, Liu R, Xiong L (2017). OsJAZ1 attenuates drought resistance by Regulating JA and ABA signaling in rice. Frontiers in Plant Science 8:2108. *https://doi.org/10.3389/fpls.2017.02108*

Gonzalez N, Pauwels L, Baekelandt A, De Milde L, Van Leene J, Besbrugge N, ... Inzé D (2015). A repressor protein complex regulates leaf growth in *Arabidopsis*. Plant Cell 27(8):2273-2287. *https://doi.org/10.1105/tpc.15.00006*

Guang Y, Luo S, Ahammed GJ, Xiao X, Li J, Zhou Y, Yang Y (2021). The *OPR* gene family in watermelon: Genome-wide identification and expression profiling under hormone treatments and root-knot nematode infection. Plant Biology (Stuttg) 23(1):80-88. *https://doi.org/10.1111/plb.13225*

Hakata M, Muramatsu M, Nakamura H, Hara N, Kishimoto M, Iida-Okada K, ... Ichikawa H (2017). Overexpression of *TIFY* genes promotes plant growth in rice through jasmonate signaling. Bioscience, Biotechnology, and Biochemistry 81(5):906-913. *https://doi.org/10.1080/09168451.2016.1274638*

He X, Kang Y, Li W, Liu W, Xie P, Liao L, ... Hua W (2020). Genome-wide identification and functional analysis of the TIFY gene family in the response to multiple stresses in *Brassica napus* L. BMC Genomics 21(1):736. *https://doi.org/10.1186/s12864-020-07128-2*

Heidari P, Faraji S, Ahmadizadeh M, Ahmar S, Mora-Poblete F (2021). New insights into structure and function of *TIFY* genes in *Zea mays* and *Solanum lycopersicum*: A genome-wide comprehensive analysis. Frontiers in Genetics 12:657970. *https://doi.org/10.3389/fgene.2021.657970*

Huang Z, Jin SH, Guo HD, Zhong XJ, He J, Li X, ... Chen QB (2016). Genome-wide identification and characterization of TIFY family genes in Moso Bamboo (*Phyllostachys edulis*) and expression profiling analysis under dehydration and cold stresses. PeerJ 4:e2620. *https://doi.org/10.7717/peerj.2620*

Lee IH, Shim D, Jeong JC, Sung YW, Nam KJ, Yang JW, ... Kim YH (2019). Transcriptome analysis of root-knot nematode (*Meloidogyne incognita*)-resistant and susceptible sweetpotato cultivars. Planta 249(2):431-444. *https://doi.org/10.1007/s00425-018-3001-z*

Li L, Liu Y, Huang Y, Li B, Ma W, Wang D, ... Wang Z (2021). Genome-wide identification of the TIFY family in *Salvia miltiorrhiza* reveals that SmJAZ3 interacts with SmWD40-170, a relevant protein that modulates secondary metabolism and development. Frontiers in Plant Science 12:630424. *https://doi.org/10.3389/fpls.2021.630424*

Li X, Yin X, Wang H, Li J, Guo C, Gao H, ... Wang X (2015). Genome-wide identification and analysis of the apple (*Malus* × *domestica* Borkh.) TIFY gene family. Tree Genetics & Genomes 11(1):808. *https://doi.org/10.1007/s11295-014-0808-z*

Liu S, Zhang P, Li C, Xia G (2019). The moss jasmonate ZIM-domain protein PnJAZ1 confers salinity tolerance via crosstalk with the abscisic acid signalling pathway. Plant Science 280:1-11. *https://doi.org/10.1016/j.plantsci.2018.11.004*

Ma Y, Shu S, Bai S, Tao R, Qian M, Teng Y (2018). Genome-wide survey and analysis of the *TIFY* gene family and its potential role in anthocyanin synthesis in Chinese sand pear (*Pyrus pyrifolia*). Tree Genetics & Genomes 14:25. *https://doi.org/10.1007/s11295-018-1240-6*

Madeira F, Park YM, Lee J, Buso N, Gur T, Madhusoodanan N, ... Lopez R (2019). The EMBL-EBI search and sequence analysis tools APIs in 2019. Nucleic Acids Research 47(W1):W636-w641. *https://doi.org/10.1093/nar/gkz268*

Nahar K, Kyndt T, De Vleesschauwer D, Höfte M, Gheysen G (2011). The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice. Plant Physiology 157(1):305-316. *https://doi.org/10.1104/pp.111.177576*

Niu Y, Figueroa P, Browse J (2011). Characterization of JAZ-interacting bHLH transcription factors that regulate jasmonate responses in *Arabidopsis*. Journal of Experimental Botany 62(6):2143-2154. *https://doi.org/10.1093/jxb/erq408*

Saha G, Park JI, Kayum MA, Nou IS (2016). A genome-wide analysis reveals stress and hormone responsive patterns of TIFY family genes in *Brassica rapa*. Frontiers in Plant Science 7:936. *https://doi.org/10.3389/fpls.2016.00936*

Shen J, Zou Z, Xing H, Duan Y, Zhu X, Ma Y, ... Fang W (2020). Genome-wide analysis reveals stress and hormone responsive patterns of JAZ family genes in *Camellia Sinensis*. International Journal of Molecular Sciences 21(7):2433. *https://doi.org/10.3390/ijms21072433*

Singh AP, Mani B, Giri J (2021). OsJAZ9 is involved in water-deficit stress tolerance by regulating leaf width and stomatal density in rice. Plant Physiology and Biochemistry 162:161-170. *https://doi.org/10.1016/j.plaphy.2021.02.042*

Singh AP, Pandey BK, Mehra P, Heitz T, Giri J (2020). OsJAZ9 overexpression modulates jasmonic acid biosynthesis and potassium deficiency responses in rice. Plant Molecular Biology 104(4-5):397-410. *https://doi.org/10.1007/s11103-020-01047-2*

Singh P, Mukhopadhyay K (2021). Comprehensive molecular dissection of TIFY transcription factors reveal their dynamic responses to biotic and abiotic stress in wheat (*Triticum aestivum* L.). Scientific Reports 11(1):9739. *https://doi.org/10.1038/s41598-021-87722-w*

Sirhindi G, Sharma P, Arya P, Goel P, Kumar G, Acharya V, Singh AK (2016). Genome-wide characterization and expression profiling of *TIFY* gene family in pigeon pea (*Cajanus cajan* (L.) Millsp.) under copper stress. Journal of Plant Biochemistry and Biotechnology 25(3):301-310. *https://doi.org/10.1007/s13562-015-0342-6*

Song S, Huang H, Wang J, Liu B, Qi T, Xie D (2017). MYC5 is involved in jasmonate-regulated plant growth, leaf senescence and defense responses. Plant and Cell Physiology 58(10):1752-1763. *https://doi.org/10.1093/pcp/pcx112*

Song S, Qi T, Huang H, Ren Q, Wu D, Chang C, ... Xie D (2011). The Jasmonate-ZIM domain proteins interact with the R2R3-MYB transcription factors MYB21 and MYB24 to affect Jasmonate-regulated stamen development in *Arabidopsis*. Plant Cell 23(3):1000-1013. *https://doi.org/10.1105/tpc.111.083089*

Sun P, Shi Y, Valerio AGO, Borrego EJ, Luo Q, Qin J, ... Yan Y (2021). An updated census of the maize *TIFY* family. PLoS One 16(2):e0247271. *https://doi.org/10.1371/journal.pone.0247271*

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, ... Browse J (2007). JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. Nature 448(7154):661-665. *https://doi.org/10.1038/nature05960*

Thireault C, Shyu C, Yoshida Y, St Aubin B, Campos ML, Howe GA (2015). Repression of jasmonate signaling by a non-TIFY JAZ protein in *Arabidopsis*. Plant Journal 82(4):669-679. *https://doi.org/10.1111/tpj.12841*

Um TY, Lee HY, Lee S, Chang SH, Chung PJ, Oh KB, ... Choi YD (2018). Jasmonate ZIM-domain protein 9 interacts with slender rice 1 to mediate the antagonistic interaction between jasmonic and gibberellic acid signals in rice. Frontiers in Plant Science 9:1866. *https://doi.org/10.3389/fpls.2018.01866*

Vanholme B, Grunewald W, Bateman A, Kohchi T, Gheysen G (2007). The tify family previously known as ZIM. Trends in Plant Science 12(6):239-244. *https://doi.org/10.1016/j.tplants.2007.04.004*

Wang H, Leng X, Xu X, Li C (2020). Comprehensive analysis of the *TIFY* gene family and its expression profiles under phytohormone treatment and abiotic stresses in roots of *Populus trichocarpa*. Forests 11(3):315. *https://doi.org/10.3390/f11030315*

Wang X, Cheng C, Zhang K, Tian Z, Xu J, Yang S, ... Chen JF (2018). Comparative transcriptomics reveals suppressed expression of genes related to auxin and the cell cycle contributes to the resistance of cucumber against *Meloidogyne incognita*. BMC Genomics 19(1):583. *https://doi.org/10.1186/s12864-018-4979-0*

Wang Y, Pan F, Chen D, Chu W, Liu H, Xiang Y (2017). Genome-wide identification and analysis of the *Populus trichocarpa* TIFY gene family. Plant Physiology and Biochemistry 115:360-371. *https://doi.org/10.1016/j.plaphy.2017.04.015*

White DW (2006). *PEAPOD* regulates lamina size and curvature in *Arabidopsis*. Proceedings of the National Academy of Sciences of the United States of America 103(35):13238-13243. *https://doi.org/10.1073/pnas.0604349103*

Wu H, Ye H, Yao R, Zhang T, Xiong L (2015). OsJAZ9 acts as a transcriptional regulator in jasmonate signaling and modulates salt stress tolerance in rice. Plant Science 232:1-12. *https://doi.org/10.1016/j.plantsci.2014.12.010*

Xie DX, Feys BF, James S, Nieto-Rostro M, Turner JG (1998). COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science 280(5366):1091-1094. *https://doi.org/10.1126/science.280.5366.1091*

Yang Y, Ahammed GJ, Wan C, Liu H, Chen R, Zhou Y (2019). Comprehensive analysis of TIFY transcription factors and their expression profiles under jasmonic acid and abiotic stresses in watermelon. International Journal of Genomics 2019:6813086. *https://doi.org/10.1155/2019/6813086*

Yang YX, Wu C, Ahammed GJ, Wu C, Yang Z, Wan C, Chen J (2018). Red light-induced systemic resistance against root-knot nematode is mediated by a coordinated regulation of salicylic acid, jasmonic acid and redox signaling in watermelon. Frontiers in Plant Science 9:899. *https://doi.org/10.3389/fpls.2018.00899*

Ye H, Du H, Tang N, Li X, Xiong L (2009). Identification and expression profiling analysis of TIFY family genes involved in stress and phytohormone responses in rice. Plant Molecular Biology 71(3):291-305. *https://doi.org/10.1007/s11103-009-9524-8*

Yu Y, Wan Y, Jiao Z, Bian L, Yu K, Zhang G, Guo D (2019). Functional characterization of resistance to powdery mildew of *VvTIFY9* from *Vitis vinifera*. International Journal of Molecular Sciences 20(17):4286. *https://doi.org/10.3390/ijms20174286*

Zhang G, Yan X, Zhang S, Zhu Y, Zhang X, Qiao H, ... Wang X (2019). The jasmonate-ZIM domain gene *VqJAZ4* from the Chinese wild grape *Vitis quinquangularis* improves resistance to powdery mildew in *Arabidopsis thaliana*. Plant Physiology and Biochemistry 143:329-339. *https://doi.org/10.1016/j.plaphy.2019.09.018*

Zhang L, You J, Chan Z (2015a). Identification and characterization of TIFY family genes in *Brachypodium distachyon*. Journal of Plant Research 128(6):995-1005. *https://doi.org/10.1007/s10265-015-0755-2*

Zhang X, Ran W, Zhang J, Ye M, Lin S, Li X, ... Sun X (2020). Genome-wide identification of the Tify gene family and their expression profiles in response to biotic and abiotic stresses in tea plants (*Camellia sinensis*). International Journal of Molecular Sciences 21(21):8316. *https://doi.org/10.3390/ijms21218316*

Zhang Z, Li X, Yu R, Han M, Wu Z (2015b). Isolation, structural analysis, and expression characteristics of the maize *TIFY* gene family. Molecular Genetics and Genomics 290(5):1849-1858. *https://doi.org/10.1007/s00438-015-1042-6*

Zhou J, Jia F, Shao S, Zhang H, Li G, Xia X, ... Shi K (2015a). Involvement of nitric oxide in the jasmonate-dependent basal defense against root-knot nematode in tomato plants. Frontiers in Plant Science 6:193. *https://doi.org/10.3389/fpls.2015.00193*

Zhou X, Yan S, Sun C, Li S, Li J, Xu M, ... Wang L (2015b). A maize jasmonate Zim-domain protein, ZmJAZ14, associates with the JA, ABA, and GA signaling pathways in transgenic *Arabidopsis*. PLoS One 10(3):e0121824. *https://doi.org/10.1371/journal.pone.0121824*

Zhou Y, Li G, Zhang L, Xu J, Hu L, Jiang L, Liu S (2020). Comprehensive genomic analysis and expression profiling of the BTB and TAZ (*BT*) genes in cucumber (*Cucumis sativus* L.). Czech Journal of Genetics and Plant Breeding 56(1):15-23. *https://doi.org/10.17221/34/2019-CJGPB*

Zhou Y, Ouyang L, Zhou D, Cai Y, He H (2021). Superoxide dismutase family genes in watermelon and their responses to different abiotic stresses. Frontiers of Agricultural Science and Engineering 8(4):645-658. *https://doi.org/10.1007/s00122-021-03857-4*

Zhu Y, Luo X, Liu X, Wu W, Cui X, He Y, Huang J (2020). *Arabidopsis* PEAPODs function with LIKE HETEROCHROMATIN PROTEIN1 to regulate lateral organ growth. Journal of Integrative Plant Biology 62(6):812-831. *https://doi.org/10.1111/jipb.12841*