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 CstJAZ 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
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 SLX2FX2KRX2RX5Pγ 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 physiological 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/OsTIFY1a 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 Tritium 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://cucurbitchromosomes.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/bwprsb/bwprsb.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 analysed 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://cucurbitchromosomes.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 (Yanget 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 TBlustools 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* ind 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^{ΔΔC_{T}} 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 indicated 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>
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<th>CDS (bp)</th>
<th>Length (aa)</th>
<th>MW (KD)</th>
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**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 (Yanget al., 2019).
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 CstJAZ4 and CstJAZ10 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 CstJAZ2 and CstJAZ9 contained only one intron. In addition, CstPPD1 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 (CsPPDI, 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, 26, 36, 48 and 77 TIFY family genes were identified in watermelon (Yang et al., 2019), pigeonpea (Sirhindhi et al., 2016), Arabidopsis (Bai et al., 2011; Thirueult 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.
(Zhanget 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 JAZ1–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 GzML 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/CsJAZ2, 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 cattkins (Wang et al., 2017). In addition, some cucumber TIFY genes displayed higher expression levels in vegetative organs such as root and tendrils (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 OsTIFY1a resulted in higher tolerance to salt and mannanitol 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 Phycomitrella 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* (Wanget 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; Yanget 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 JAZI 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.

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