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ORIGINAL PAPER

Isolation, structural analysis, and expression characteristics of the maize TIFY gene family

Zhongbao Zhang[1,2] · **Xianglong Li**[1,2] · **Rong Yu**[3] · **Meng Han**[1,2,4] · **Zhongyi Wu**[1,2,4]

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Abstract TIFY, previously known as ZIM, comprises a plant-specific family annotated as transcription factors that might play important roles in stress response. Despite TIFY proteins have been reported in *Arabidopsis* and rice, a comprehensive and systematic survey of *ZmTIFY* genes has not yet been conducted. To investigate the functions of *ZmTIFY* genes in this family, we isolated and characterized 30 *ZmTIFY* (1 *TIFY*, 3 *ZML*, and 26 *JAZ*) genes in an analysis of the maize (*Zea mays* L.) genome in this study. The 30 *ZmTIFY* genes were distributed over eight chromosomes. Multiple alignment and motif display results indicated that all *ZmTIFY* proteins share two conserved TIFY and Jas domains. Phylogenetic analysis revealed that the *ZmTIFY* family could be divided into two groups. Putative *cis*-elements, involved in abiotic stress response, phytohormones, pollen grain, and seed development, were detected in the promoters of maize *TIFY* genes. Microarray data showed that the *ZmTIFY* genes had tissue-specific expression patterns in various maize developmental stages and in response to biotic and abiotic stresses. The results indicated that *ZmTIFY4*, *5*, *8*, *26*, and *28* were induced, while *ZmTIFY16, 13, 24, 27, 18,* and *30* were suppressed, by drought stress in the maize inbred lines Han21 and Ye478. *ZmTIFY1*, *19,* and *28* were upregulated after infection by three pathogens, whereas *ZmTIFY4*, *13*, *21*, *23*, *24*, and *26* were suppressed. These results indicate that the *ZmTIFY* family may have vital roles in response to abiotic and biotic stresses. The data presented in this work provide vital clues for further investigating the functions of the genes in the *ZmTIFY* family.

Keywords Maize · Abiotic and biotic stress · *JAZ* · ZIM · Expression behavior · *Cis*-elements

Abbreviations

ABA	Abscisic acid
CCT	CONSTANS, CO-like, TOC1
JA	Jasmonic acid
JAZ	JASMONATE ZIM-DOMAIN
PPD	PEAPOD
ZIM	Zinc-finger protein expressed in inflorescence meristem

Introduction

TIFY family, previously known as ZIM (zinc-finger protein expressed in inflorescence meristem; Nishii et al. 2000), is one of the most vital transcription factor families in higher plants and plays important roles in the abiotic and biotic

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✉ Zhongyi Wu
zwu22@126.com

Zhongbao Zhang
happyzzb@126.com

[1] Beijing Agro-Biotechnology Research Center, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China

[2] Beijing Key Laboratory of Agricultural Genetic Resources and Biotechnology, Beijing 100097, China

[3] College of Life Science, Capital Normal University, Beijing 100048, China

[4] College of Agriculture, Hubei Collaborative Innovation Center for Grain Industry, Yangtze University, Jingzhou 434023, Hubei, China

stress response (Zhang et al. 2012; Aparicio et al. 2013). The TIFY family is named for a conserved core motif TIF [F/Y] XG, located within a TIFY domain about 28 amino acids in length. This family of TFs can be divided into four subfamilies: TIFY, PPD, JAZ, and ZML, according to their distinct domain structures (Vanholme et al. 2007; Bai et al. 2011).

All TIFY proteins contain a TIFY domain. In addition, the ZML subfamily, including ZIM and ZIM-like (ZML) TFs, contains C2C2-GATA and CCT domains (CONSTANS, CO-like, TOC1). The PPD and JAZ subfamilies lack GATA and CCT domains (Chung et al. 2009). In addition to the TIFY domain, the JAZ subfamily also contains a conserved, C-terminus sequence Jas motif. This motif contains the characteristic sequence SLX2FX2KRX2RX5PY, consisting of approximately 27 amino acids, and is similar in sequence to the CCT domain (Staswick et al. 2008; Chung et al. 2009). PPD proteins, including a unique N-terminal PPD domain and TIFY subfamily, contain only the TIFY domain (Staswick et al. 2008; Zhang et al. 2012).

Jasmonate (JA) is a pivotal regulator of plant responses to both biotic and abiotic stresses, such as wounding, pathogen infection, drought, and salinity stress (Devoto et al. 2005; Wasternack 2007). JAZ proteins are targets of the $\mathrm{SCF}^{\mathrm{COI1}}$ complex and function as negative regulators in the JA signaling pathway (Zhang et al. 2012).

Recent discoveries have shown that TIFY genes play a vital role in JA signaling and the response to various abiotic stresses. In *Arabidopsis thaliana* L., ZIM (At3g17860) is induced by NaCl treatment (Jiang and Deyholos 2006) and several TIFY genes are highly upregulated by ozone treatment (Rao et al. 2000) and wounding (Major and Constabel 2006). Most *OsTIFY* genes are responsive to at least one abiotic stress, including drought, salinity, and low temperature (Ye et al. 2009). *GsTIFY10* is induced by salinity and bicarbonate stress, and the plant hormone JA (Zhu et al. 2011). Some of the grape TIFY genes (*VvTIFY*) are regulated by osmotic, cold, drought, salinity, JA, and abscisic (ABA) treatments (Zhang et al. 2012). The *PvTIFY* genes might play a vital role in regulating bean (*Phaseolus vulgaris* L.) adaptation to phosphorus (P)-starvation, mediated by JA signaling (Aparicio et al., 2013).

These studies in plants indicated that TIFY family genes might be vital not only for plant development but also for the response and adaptation to stresses, but a comprehensive survey of ZmTIFY proteins has not yet been conducted. To investigate the functions of *ZmTIFY* genes in this family, transcript levels of the genes were measured in various maize tissues and seedling leaves under various abiotic and biotic stresses. The results presented herein provide a reference for future studies of TIFY family genes in maize.

Materials and methods

Isolation of TIFY family genes in maize

Sequences of 18 *Arabidopsis* and 20 rice (*Oryza sativa* L.) TIFY proteins were obtained from the TAIR (http://www.arabidopsis.org) and TIGR (http://rice.plantbiology.msu.edu) databases (Ye et al. 2009). To acquire all maize *TIFY* genes, BLASTP searches were carried out in the Gramene database (http://www.gramene.org/Multi/blastview) with the *Arabidopsis* and rice TIFY proteins as queries. All homologous protein sequences of the predicted TIFY family members were downloaded if satisfied with $E < 10^{-10}$ (Zhang et al. 2014). The full-length cDNA sequences of maize *ZmTIFY* genes were downloaded from the Gramene database.

Structural analysis of maize *TIFY* genes

Information on all *ZmTIFY* genes, including ORF length, chromosomal location, full-length cDNA and DNA sequences, were obtained from the B73 maize-sequencing database (http://www.ensembl.gramene.org/Zea_mays/Info/Index). Exon and intron structures of *ZmTIFY* genes were visualized using GSDS (http://www.gsds.cbi.pku.edu.cn/; Guo et al. 2007).

Motif display and phylogenetic analysis of ZmTIFY proteins

The multiple expectation maximization for motif elicitation (MEME) utility software (Bailey et al. 2009) was used to display domains of ZmTIFY proteins. The matrix for phylogenetic analysis included 18, 20, and 30 *TIFY* genes from *Arabidopsis*, rice, and maize, respectively. Amino acid sequences of all proteins were aligned using ClustalX2.0 (Larkin et al. 2007) and the unrooted phylogenetic tree was generated using the MEGA4 software, with the neighbor-joining method and bootstrap values from 1000 replicates at each branch (Tamura et al. 2007).

Promoter regions analysis of *ZmTIFY* genes

To explore *cis*-elements in promoter sequences of *ZmTIFY* genes, 2000 bp of B73 genomic DNA upstream of the initiation codon (ATG) were downloaded from the National Center for Biotechnology Information. The promoter structure was predicted using Promoter 2.0 (http://www.cbs.dtu.dk/services/Promoter/) and PLACE (Plant *cis*-acting regulatory DNA elements, with more than 6 bp; http://www.dna.affrc.go.jp/PLACE/) software (Higo et al. 1999).

Table 1 *ZmTIFY* genes

Gene name	Chr[a]	Protein ID	ORF length	Protein length	Synonym[b]	TIFY motif
TIFY1	1	GRMZM2G343157_P01	495	164	*ZmJAZ1*	TILYGGR
TIFY2	1	223945149	588	195	NO	TIFYGGK
TIFY3	1	226500580	612	203	NO	TIFYGGK
TIFY4	1	GRMZM2G445634_P01	546	181	*ZmJAZ2*	TIFYGGR
TIFY5	1	GRMZM2G110131_P01	648	215	*ZmTIFY*	TIFYGGK
TIFY6	1	GRMZM2G117513_P01	687	228	*ZmJAZ3*	TIFYGGK
TIFY7	1	226507368	348	115	NO	TIFYGGK
TIFY8	1	GRMZM2G024680_P01	651	216	*ZmJAZ4*	TIFYQGQ
TIFY9	1	GRMZM2G145412_P01	549	182	*ZmJAZ5*	TIVYGGR
TIFY10	1	GRMZM2G145458_P01	489	162	*ZmJAZ6*	TISYGGR
TIFY11	1	GRMZM2G382794_P01	531	176	*ZmJAZ7*	TIFYGGK
TIFY12	2	GRMZM2G086920_P01	651	216	*ZmJAZ8*	TIFYGGS
TIFY13	2	GRMZM2G145407_P01	543	180	*ZmJAZ9*	TVFYGGA
TIFY14	2	GRMZM2G171830_P01	405	134	*ZmJAZ10*	TIFYDGR
TIFY15	2	GRMZM2G005954_P01	684	227	*ZmJAZ11*	TIFYGGR
TIFY16	2	GRMZM2G101769_P01	714	237	*ZmJAZ12*	TIFYGGK
TIFY17	4	GRMZM2G151519_P01	1281	426	*ZmJAZ13*	TIFYNGS
TIFY18	5	GRMZM2G065896_P01	837	278	*ZmZML1*	TLVYQGD
TIFY19	5	GRMZM2G064775_P01	657	218	*ZmJAZ14*	TIFYQGQ
TIFY20	5	GRMZM2G173596_P01	483	160	*ZmJAZ15*	IIVYGGR
TIFY21	5	GRMZM2G058479_P01	1074	357	*ZmZML2*	TLSFQGE
TIFY22	6	GRMZM2G338829_P01	333	110	*ZmJAZ16*	TIFYGGK
TIFY23	6	GRMZM2G080509_P01	1077	358	*ZmZML3*	TLSFQGE
TIFY24	7	GRMZM2G126507_P01	1215	404	*ZmJAZ17*	TIFYAGS
TIFY25	7	GRMZM2G116614_P01	657	218	*ZmJAZ18*	TIFYGGK
TIFY26	7	GRMZM2G066020_P01	804	267	*ZmJAZ19*	TIFYGGT
TIFY27	7	GRMZM2G089736_P01	702	233	*ZmJAZ20*	TIFYGGK
TIFY28	9	GRMZM2G036351_P01	519	172	*ZmJAZ21*	TIFYGGR
TIFY29	9	GRMZM2G036288_P01	552	183	*ZmJAZ22*	TIFYGGR
TIFY30	10	GRMZM2G143402_P01	624	207	*ZmJAZ23*	TIFYGGS

[a] Chromosome number in which the gene resides

[b] The gene names assigned by Bai et al. (2011)

Microarray data collection and analyses of expression profiles

The spatial and temporal specific expression behaviors of *ZmTIFY* genes were analyzed in a set of maize transcriptome data at PLEXdb (http://www.plexdb.org). The microarray data, from genome-wide gene expression analysis of the maize inbred line B73 (GSE27004) and transcriptomic analysis of induced maize senescence, was provided by Dr. Shawn Kaeppler of the University of Wisconsin (Sekhon et al. 2011, 2012). The Affymetrix GeneChip array data of gene expression during infection with three fungi and drought treatment were downloaded from GEO with the accession numbers GSE31188, GSE19501, GSE29747, and GSE16567 (Zheng et al. 2010; Ghareeb et al. 2011; Voll et al. 2011). The data were analyzed using the GeneSpring 12.5 software. A heat map was used to show the number of *ZmTIFY* genes, and the map was generated using the Multi-Experiment viewer (MeV, version 4.8.1) software. The data were adjusted by median centering of genes. The data were clustered by a complete linkage clustering method, using a Euclidean distance metric (Zhang et al. 2014).

Results

Identification of *TIFY* family genes in maize

Thirty *ZmTIFY* genes were identified in the maize genome (1 *TIFY*, 3 *ZML*, and 26 *JAZ*; Table 1). The maize *TIFY* genes were named *ZmTIFY*1 to 30, according to their order in chromosomes 1–10. The length of ZmTIFY proteins

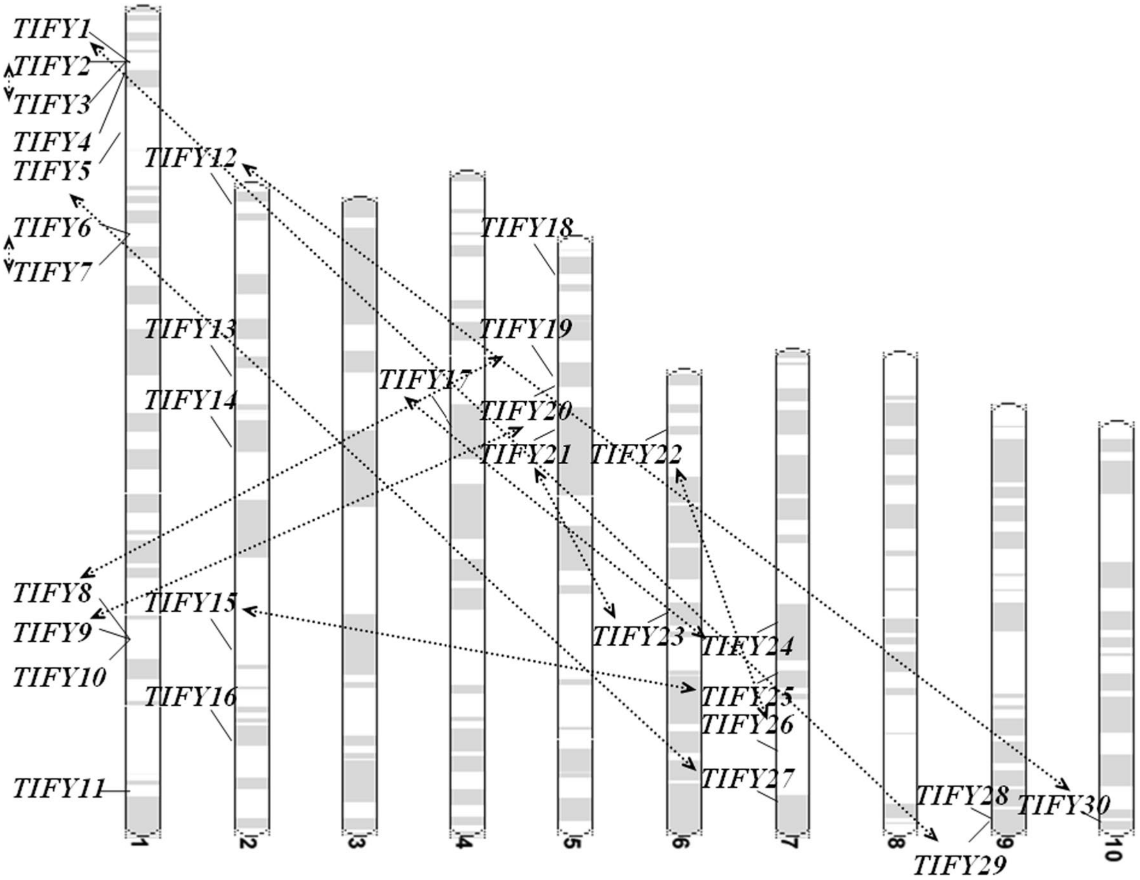

Fig. 1 Chromosomal localization of *ZmTIFY* genes. Segmental duplicates, including *ZmTIFY1*/*ZmTIFY29*, *ZmTIFY2*/*ZmTIFY3*, *ZmTIFY5*/*ZmTIFY27*, *ZmTIFY6*/*ZmTIFY7*, *ZmTIFY8*/*ZmTIFY19*, *ZmTIFY9*/*ZmTIFY20*, *ZmTIFY12*/*ZmTIFY30*, *ZmTIFY15*/*ZmTIFY25*, *ZmTIFY17*/*ZmTIFY24* *ZmTIFY21*/*ZmTIFY23*, and *ZmTIFY22*/*ZmTIFY26*

ranged from 110 amino acids (aa) to 426 aa (Table 1). BLAST analysis against the Pfam and InterPro databases showed that all of the genes included a TIFY domain (accession no. PF06200) and a Jas domain (accession no. PF09425).

The 30 *ZmTIFY* genes were distributed on eight maize chromosomes: 11 genes on chromosome 1, 5 on chromosome 2, 4 on chromosomes 5 and 7, 2 on chromosomes 6 and 9, and 1 on chromosomes 4 and 10 (Fig. 1). Chromosome 1 harbored two *ZmTIFY* gene clusters, *ZmTIFY1, 2, 3, 4* and *ZmTIFY8, 9, 10*.

The full-length cDNA sequences were compared with the corresponding genomic DNA sequences to determine the numbers and positions of exons and introns within each *ZmTIFY* gene using GSDS (http://www.gsds.cbi.pku.edu.cn/chinese.php; Guo et al. 2007). Most *ZmTIFY* genes had two to eight exons, except for *ZmTIFY1, 2, 4, 9, 10, 20, 28,* and *29,* which had one exon (Fig. 2). Based on phylogenetic results (Supplementary Fig. 1), 11 paralogs were identified in *ZmTIFY* genes: *ZmTIFY1*/*ZmTIFY29*, *ZmTIFY2*/*ZmTIFY3*, *ZmTIFY5*/*ZmTIFY27*, *ZmTIFY6*/*ZmTIFY7*, *ZmTIFY8*/*ZmTIFY19*, *ZmTIFY9*/*ZmTIFY20*, *ZmTIFY12*/*ZmTIFY30*, *ZmTIFY15*/*ZmTIFY25*, *ZmTIFY17*/*ZmTIFY24* *ZmTIFY21*/*ZmTIFY23*, and *ZmTIFY22*/*ZmTIFY26* (Fig. 1).

Motif analysis and protein architecture

To identify the conserved domain distribution in TIFY proteins, the MEME web server was employed to analyze the sequences of *Arabidopsis*, rice, sorghum (*Sorghum bicolor* L.), and maize proteins. Three putative conserved domains were detected in the *ZmTIFY* family, including the TIFY domain (accession no. PF06200), Jas domain (accession no. PF09425,) and GATA zinc finger (accession no. PF00320) (Fig. 3). The TIFY domain generally contained 36 amino acids, with a highly conserved pattern of TIFYXG, T [L/I] SFXG, SILYKG, and SNGSEG. In addition, all of the ZmTIFY proteins included a TIFY domain and a Jas domain, with a conserved motif SLX2FX2KRX2RX5PY (Staswick 2008). Moreover, three maize TIFY proteins (ZmTIFY18, 21, 23) contained an additional GATA zinc finger, which was a marker for group II in the phylogenetic tree.

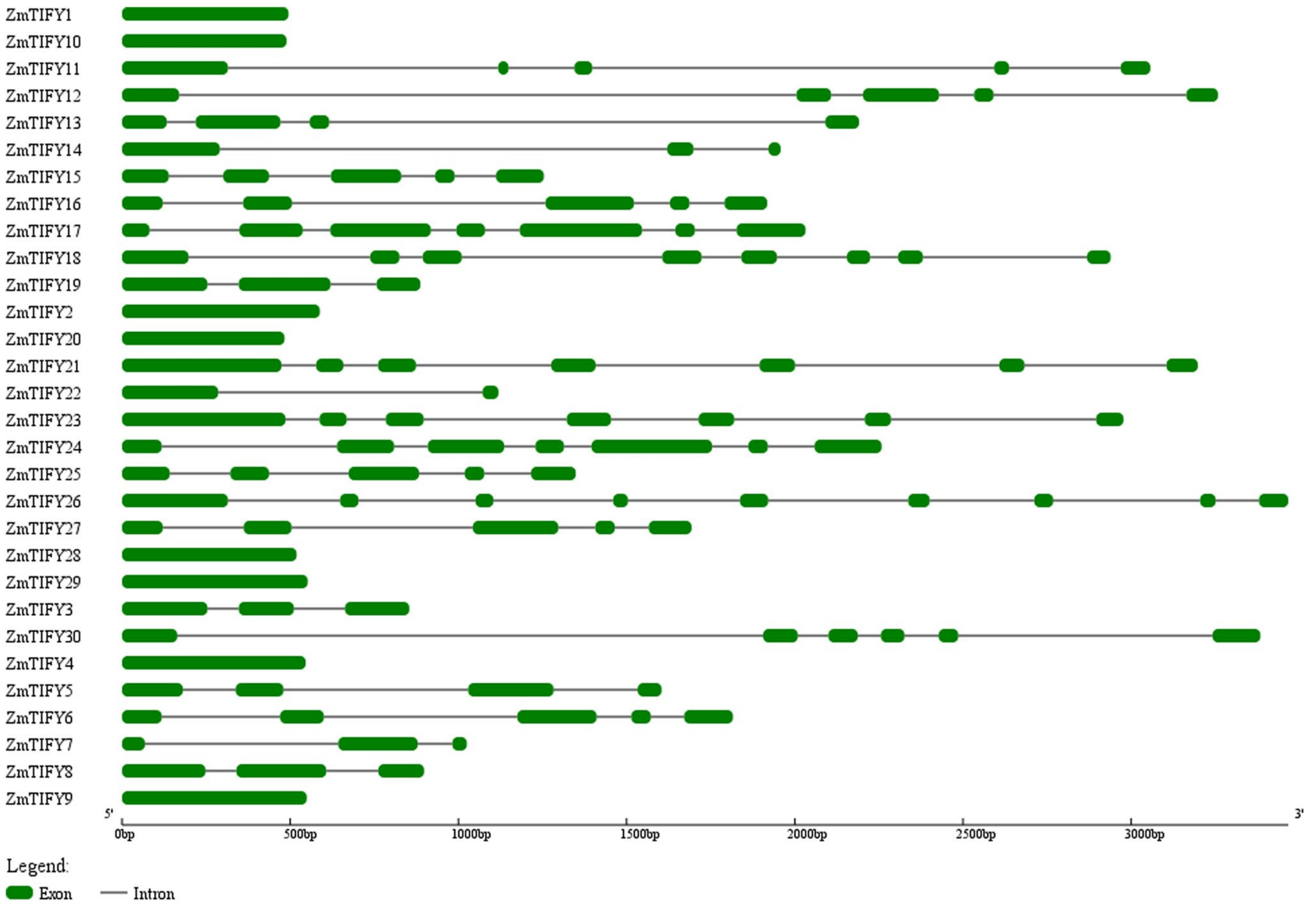

Fig. 2 Gene structure of the *ZmTIFY* gene family. Exons and introns are indicated by *filled boxes* and *single lines*, respectively

Phylogenetic analysis

To investigate the evolutionary pattern and phylogenic relationships among TIFY in maize (30 genes), rice (20 genes), and *Arabidopsis* (18 genes), the predicted amino acid sequences of *TIFY* genes were aligned by ClustalX (1.83) and a phylogenetic tree was constructed using the neighbor-joining (NJ) method by MEGA4 (Fig. 4). All of the proteins fell into two major groups (I and II), with the exceptions of TIFY8 and OsTIFY8, which contained only the TIFY domain. Three proteins contained the TIFY, GATA zinc-finger, and Jas domains (ZmTIFY18, 21, 23) and were clustered together in group I. Proteins that contained TIFY and Jas domains, but not GATA zinc-finger domains, constituted the second major group (II). This group contained all JAZ proteins of *Arabidopsis*, rice, and putative maize JAZ homologs (ZmTIFY1-17, 19, 20, 22, 23-30).

Cis-element analysis

By searching the PLACE database, promoter regions (2-kb range B73 genomic DNA sequences upstream of the translation start site) of maize *TIFY* genes were analyzed. In this study, a series of *cis*-elements involved in the abiotic stress response, phytohormone, pollen-specific and quantitative (Zhang et al. 2014), seed development and germination were identified. The *cis*-elements included the ABRE (ACGTG), DRE (GCCGCC), LTRE (CCGAC; Haberer et al. 2006), LTRE (CCGAC; Hamilton et al. 1998) and SEF (RTTTTTR; Allen et al. 1989) motifs (Supplementary Table 1). In silico sequence analysis showed that 30 annexin genes contain at least three of the five putative *cis*-elements. *Cis*-elements play an essential function in the regulation of gene expression by controlling promoter efficiency. Studies of *cis*-elements could provide vital information for further research into the functions of the *ZmTIFY* gene family.

Expression profiles of *ZmTIFY* family genes in various tissues and organs

To identify the spatial and temporal expression patterns of *ZmTIFY* genes, we used microarray data of the gene expression levels of 60 tissues from varying developmental

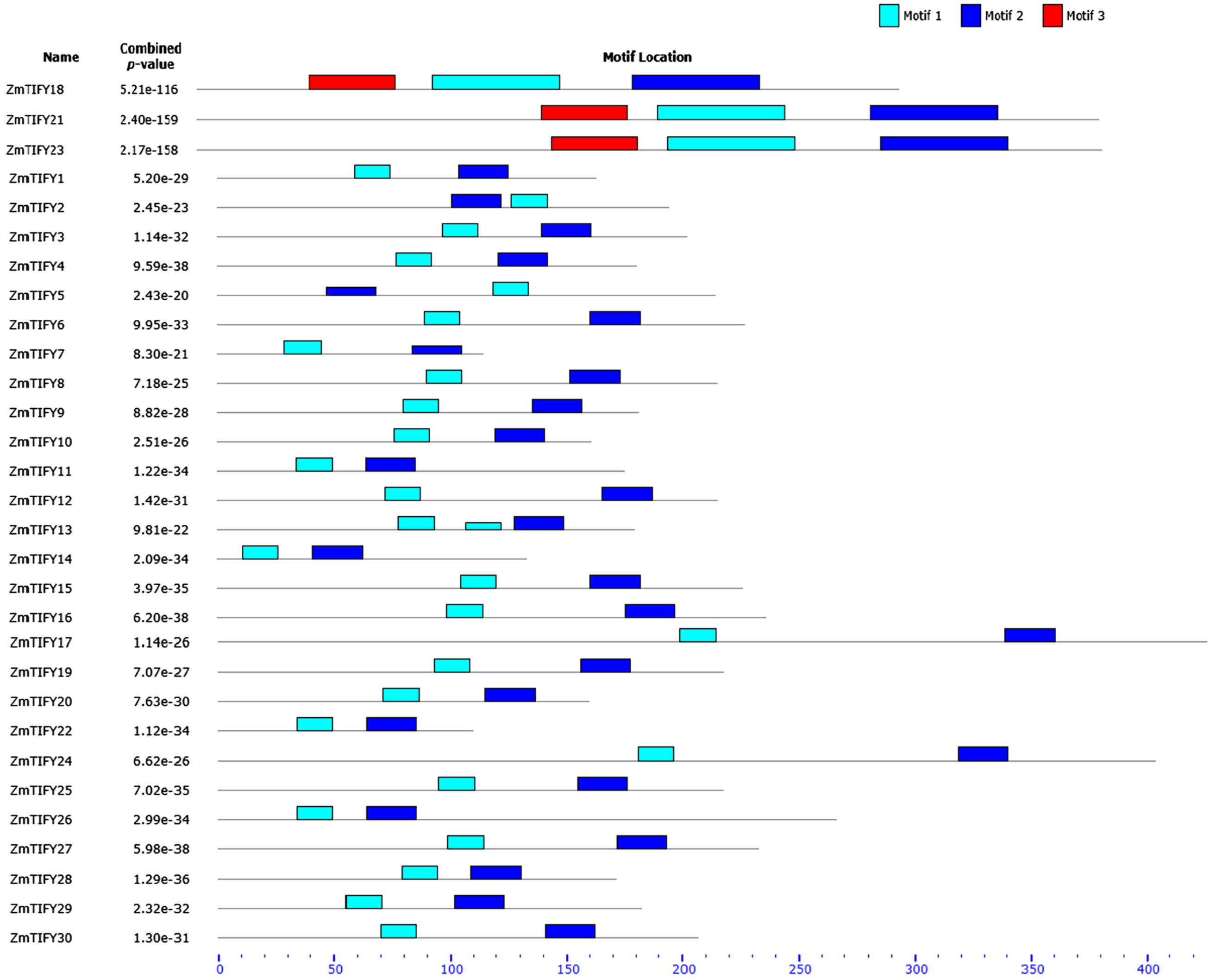

Fig. 3 Putative motif distribution in *ZmTIFY* proteins. Domains of TIFY proteins were investigated using the MEME web server. *Motif 1*: TIFY (PF06200); *Motif 2*: Jas (PF09425); *Motif 3*: GATA zinc finger (PF00320)

stages of maize (Sekhon et al. 2011). The heat map indicated that all of the 22 detected genes were involved in various biological processes, and expressed in the majority of tissues, but their individual expression levels were diverse (Fig. 5). *ZmTIFY8* and *19* had higher expression levels in reproductive organs, such as whole seed (DAP), embryo (DAP), endosperm (DAP), and pericarp. The *ZmTIFY15* and *25* transcript levels had higher expression levels mainly in vegetative organs such as seedlings, various leaves, stem, SAM, and outer husk. *ZmTIFY4*, *9*, *14*, *20*, *26,* and *28* had higher expression levels in both vegetative and reproductive organs, such as the primary root, seedling, leaves, whole seed (DAP), embryo (DAP), endosperm (DAP), and pericarp. *ZmTIFY1, 10, 12, 13, 17, 24, 27, and 29* were detected at higher levels in vegetative and early reproductive organs such as seedlings, various leaves, stem, shoot tip, immature tassel, and 2 DAP whole seeds. *ZmTIFY6, 21, 23,* and *30* were expressed at lower levels in vegetative reproductive organs (Fig. 5).

Expression profiles of *ZmTIFY* genes under abiotic and biotic stresses

Many plant gene families are involved in both stress and development responses. To determine whether *ZmTIFY* genes were responsive to drought stress, GEO data GSE16567 (Zheng et al. 2010) was analyzed using the GeneSpring 12.5 software. The results indicated that *ZmTIFY*4, 5, 8, 26, and 28 were induced, while *ZmTIFY16, 13, 24, 27, 18,* and *30* were suppressed, in the maize inbred lines Han21 and Ye478. *ZmTIFY*4, 5, 8, 26, and 28 were induced and reached the highest expression levels in response to severe drought stress (53.1 % of Han21 and 41.4 % of Ye478 leaves; Zheng et al. 2010). *ZmTIFY20, 21,*

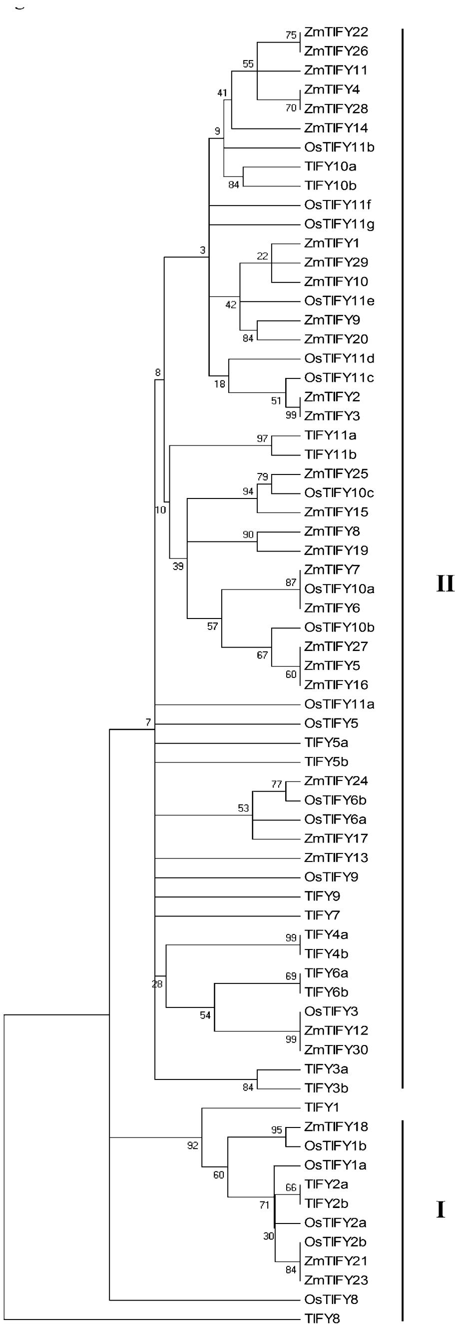

Fig. 4 Phylogenetic tree for *Zea mays*, *Arabidopsis*, and *Oryza sativa TIFY*. The joint unrooted tree was generated using MEGA4 by the neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch (TIFY8 and OsTIFY8 contains TIFY domain only)

and *23* were induced in Han21, while *ZmTIFY12, 15, 19, and 25* were induced in Ye478. *ZmTIFY1, 15,* and *25* were suppressed only in Han21. *ZmTIFY6, 9, 10,* and *17* were not induced in either of the two inbred lines (Fig. 6).

To investigate the *ZmTIFY* (23 genes were detected) genes involved in biotic stresses, we identified differentially expressed genes using microarray data. Data from three experiments involving infection by the pathogens *F. moniliforme*, *Sphacelotheca reiliana,* and *Colletotrichum graminicola* were analyzed. As shown in Fig. 7, *ZmTIFY1*, *ZmTIFY19,* and *ZmTIFY28* were upregulated after infection by the three pathogens, while *ZmTIF*Y4, 13, 21, 23, 24, and *26* were suppressed. *ZmTIFY5, 17, 18,* and *20* were suppressed, while *ZmTIFY 6* and *16* were upregulated by *F. moniliforme*. *ZmTIFY8, 12, 15, 18,* and *25* were induced, while *ZmTIFY 5, 17,* and *20* were suppressed by *S. reiliana*. *ZmTIFY*3 was upregulated, while *ZmTIFY 9, 10,* and *25* were suppressed, by *C. graminicola.*

Discussion

TIFY is a large, plant-specific, TF gene family. In this study, 30 genes belonging to the *TIFY* family in maize were identified. Among the more extensively analyzed plant *TIFY* families, *Arabidopsis* harbored 18 *TIFY* genes, at least 20 *TIFY* genes were expressed in rice (Ye et al. 2009), and least 19 *TIFY* genes in sorghum (http://www.gramene.org/Multi/blastview; Bai et al. 2011). The 30 *ZmTIFY* genes were distributed on eight maize chromosomes and 11 paralogs were identified in these genes. According to the B73 maize genome annotation results, *ZmTIFY2*/*ZmTIFY3* or *ZmTIFY6*/*ZmTIFY7* was located in one tandem duplicate. While segmental duplications might contribute to other nine paralogous genes. The genome structure of the *ZmTIFY* genes showed that most contained one to seven introns, with the exceptions of *ZmTIFY1, 2, 4, 9, 10, 20, 28, and 29*, which lacked introns (Fig. 2). Three genes lack introns and the other members have one to seven introns (Ye et al. 2009). The *Arabidopsis TIFY* genes have one to eight introns (Bai et al. 2011). All of the 30 proteins (1 *TIFY*, 3 *ZML*, and 26 *JAZ*) contained two putative conserved domains, including TIFY and Jas. Three of the thirty proteins—ZmTIFY18 (ZmZML1), ZmTIFY21 (ZmZML2), and ZmTIFY23 (ZmZML3)—contained an additional GATA zinc finger, which functioned as a transcriptional activator and a DNA-binding domain (Merika and Orkin 1993; Shikata et al. 2003).

There is increasing evidence that some gene families play vital roles both in development and the stress response (Qin et al. 2009). The members of the TIFY gene family in rice and *Arabidopsis* showed tissue-specific expression patterns and functions. Most of the *OsTIFY* genes were expressed at very high levels in leaves. *OsTIFY3* showed a constitutively high expression level in vegetative organs (Ye et al. 2009). In *Arabidopsis*, *AtTIFY1*/*ZIM* (AT4G24470) was highly expressed in reproductive and vegetative organs, such as the inflorescence meristem (Nishii et al. 2000), petiole and hypocotyl elongation (Shikata et al.

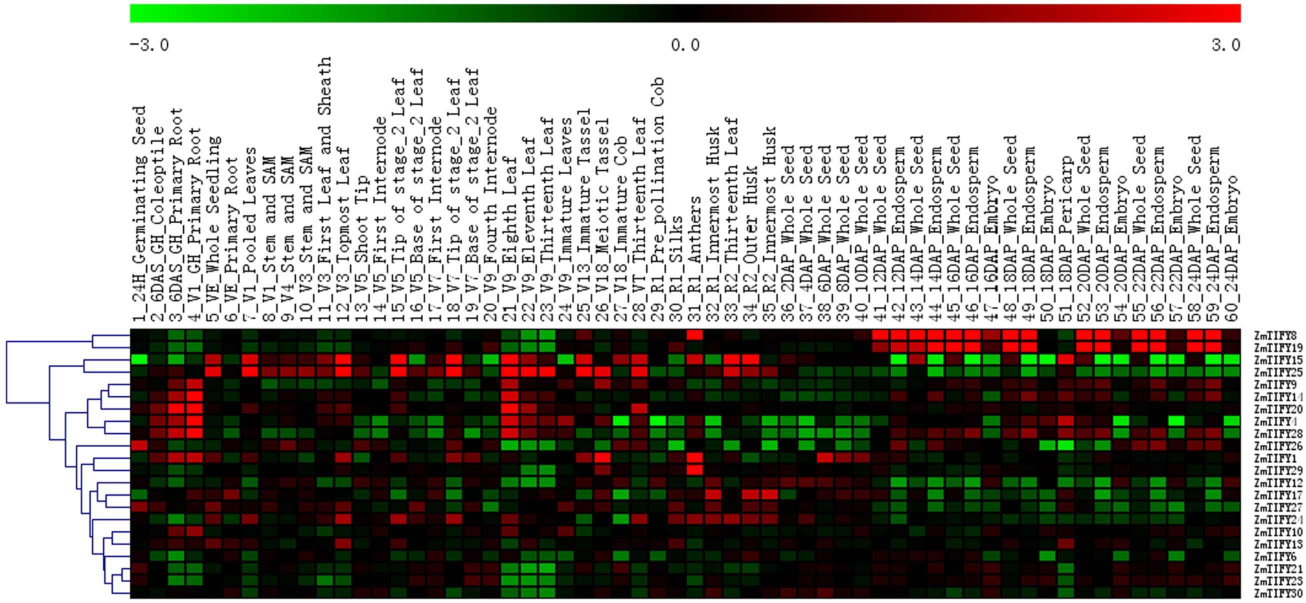

Fig. 5 Organ-specific expression patterns of *ZmTIFY* genes determined using microarray data. Log2 ratios of expression were used to produce this heat map. *Red* indicates higher expression, while *green* signifies lower expression in 60 tissues (color figure online)

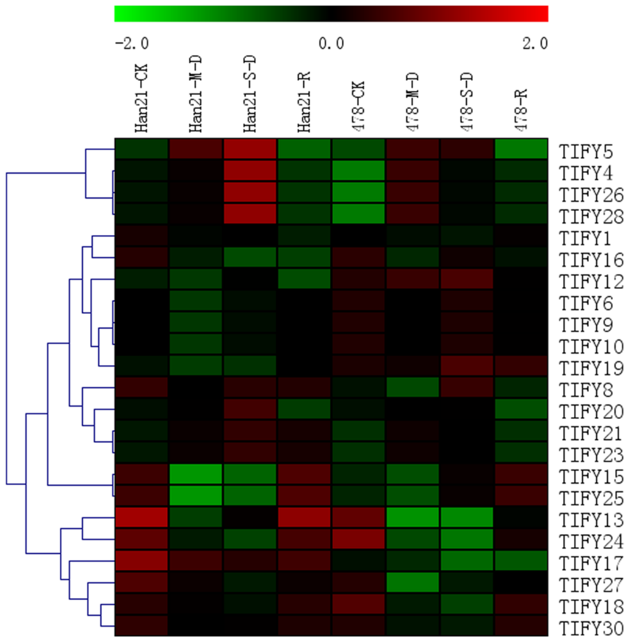

Fig. 6 Expression levels of *ZmTIFY* genes under drought stress determined using microarray data. Log2 ratios of expression were used to produce this heat map. *Red* indicates higher expression, while *green* signifies lower expression (color figure online)

2004). *AtTIFY4a* (*PPD1*) and *AtTIFY4b* (*PPD2*) play a role in the coordination of leaf growth (White 2006). Regarding rice, the transcript levels of the 10 *ZmTIFY* genes differed

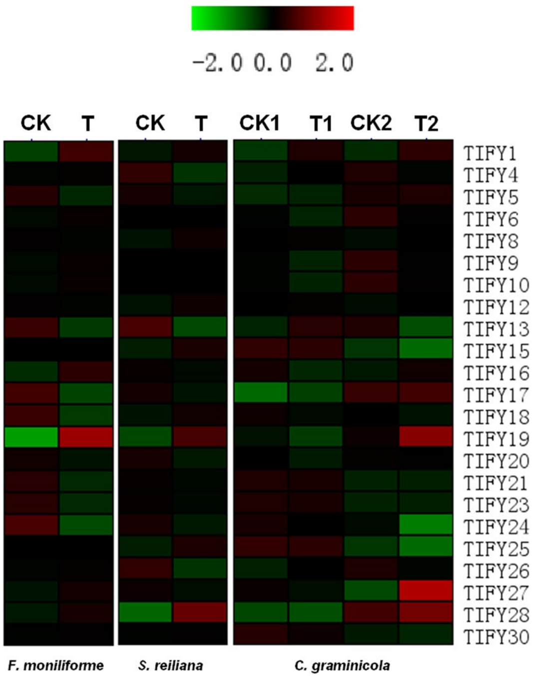

Fig. 7 Expression levels of *ZmTIFY* genes under abiotic stresses. Log2 ratios of expression were used to produce this heat map. *Red* indicates higher expression, while *green* signifies lower expression. *CK1*: Samples from uninfected control plants were taken at the same time points of *T1*. *T1*: Samples from infected leaves were taken at 36 h post-infection. *CK2*: Samples from uninfected control plants were taken at the same time points of *T2*. *T2*: Samples from infected leaves were taken at 96 h post-infection (color figure online)

in reproductive and vegetative organs (Fig. 5). We noted that *ZmTIFY8* and *ZmTIFY19*, which were closely grouped in the phylogenetic tree, had similar expression patterns and were expressed at higher levels in reproductive organs. An additional two genes, *ZmTIFY15* and *ZmTIFY25*, were grouped together in the phylogenetic tree and showed similar expression levels in vegetative organs.

Recent discoveries have shown that the TIFY family may play important roles in the response to abiotic stresses. *Arabidopsis* ZIM (At3g17860) was induced after 6 h of NaCl treatment (Jiang and Deyholos 2006). Most *OsTIFY* genes were responsive to at least one abiotic stress, such as drought, salinity, or low temperature. Overexpression of *OsTIFY11a* significantly increased the tolerance of rice transgenic lines to salt and dehydration stresses (Ye et al. 2009). *GsTIFY10* was induced by salinity, bicarbonate stress, and the plant hormone JA. Overexpression of *GsTIFY10* in *Arabidopsis* enhanced plant tolerance to bicarbonate stress during most developmental stages (Zhu et al. 2011). Some grape TIFY genes (*VvTIFY*) were regulated by osmotic, cold, drought, salinity, JA, and ABA treatments. Only a single *VvTIFY* gene responded to heat stress and no *VvTIFY* gene responded to biotic stress (Zhang et al. 2012). *PvTIFY* genes might play a vital role in bean adaptation to P-starvation mediated by JA signaling (Aparicio et al. 2013). Our findings indicated that *ZmTIFY*4, *5, 8, 26,* and *28* were induced, while *ZmTIFY16, 13, 24, 27, 18,* and *30* were suppressed, by drought stress in the maize inbred lines Han21 and Ye478 (Fig. 6). *ZmTIFY1*, *ZmTIFY19,* and *ZmTIFY28* were upregulated after infection by three pathogens (*F. moniliforme*, *S. reiliana* and *C. graminicola*), while *ZmTIF*Y4, 13, 21, 23, 24, and *26* were suppressed (Fig. 7).

These studies in plants indicated that TIFY family genes might be vital not only for plant development but also for the response and adaptation to stresses. In this study, we have systematically investigated the putative TIFY gene family and revealed that the maize genome contained 30 gene members encoding TIFY transcription factors. The detailed information on the genomic structures, chromosomal locations, protein architecture, promoter component, and phylogenetic analysis among TIFY genes in maize, *Arabidopsis,* and rice were presented. In addition, the expression profiles of the genes were measured in various maize tissues and seedling leaves under various abiotic and biotic stresses. The results presented herein provide vital clues for further investigating the functions of the genes in the *ZmTIFY* family.

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