Genome-wide Identification and Expression Analysis of the YTH Domain-containing RNA-binding Protein Family in *Citrus Sinensis*

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ABSTRACT. In eukaryotic systems, messenger RNA regulations, including splicing, 3'-end formation, editing, localization, and translation, are achieved by different RNA-binding proteins and noncoding RNAs. The YTH domain is a newly identified RNA-binding domain that was identified by comparing its sequence with that of splicing factor YT521-B. Previous study showed that the YTH gene plays an important role in plant resistance to abiotic and biotic stress. In this study, 211 YTH genes were identified in 26 species that represent four major plant lineages. Phylogenetic analysis revealed that these genes could be divided into eight subgroups. All of the YTH genes contain a YT521 domain and have different structures. Ten YTH genes were identified in navel orange (*Citrus sinensis*). The expression profiles of these CitYTH genes were analyzed in different tissues and at different fruit developmental stages, and CitYTH genes displayed distinct expression patterns under heat, cold, salt, and drought stress. Furthermore, expression of the CitYTH genes in response to exogenous hormones was measured. Nuclear localization was also confirmed for five of the proteins encoded by these genes after transient expression in *Nicotiana benthamiana* cells. This study provides valuable information on the role of CitYTHs in the signaling pathways involved in environmental stress responses in *Citrus*.

Pre-messenger RNA (mRNA), synthesized from a DNA template in the cell nucleus by transcription, is modified by several processes to become a mature functional mRNA, which can be subsequently translated (Herzel and Neugebauer, 2015). In eukaryotic systems, pre-mRNA processing, including splicing (Braunschweig et al., 2013), 3'-end formation, editing, and polyadenylation (Shi and Manley, 2015), is achieved via different RNA-binding proteins (RBPs) and noncoding RNAs (such as microRNAs) in the nucleus and cytoplasm. RBPs existed in association with RNA to form ribonucleoprotein complexes, and are crucial players in all aspects of mRNA regulation by binding RNA with different RNA-sequence specificities and affinities (Calabretta and Richard, 2015; Glisovic et al., 2009). RBPs are widely distributed among species. More than 200 putative RBP genes have been identified in *Arabidopsis thaliana*, and ≈250 have been identified in rice (*Oryza sativa*) (Cook et al., 2010; Lorkovic, 2009). Predicted structure analyses have revealed that RBPs contain several common motifs, including RNA recognition motifs (RRMs), double-stranded RNA-binding domains (Tam et al., 2010), heterogeneous nuclear ribonucleoprotein K homology domains (Lorkovic and Barta, 2002), Pumilio/FBF domains (Cheong and Hall, 2006; Wang et al., 2013), Piwi Argonaute and Zwillie domains (Song et al., 2003), DEAD/DEAH boxed (Owtrim, 2006), ZnF domains (mainly the CCCH type) (Kim et al., 2007;

Qu et al., 2014), and auxiliary domains (Alba and Pagès, 1998; Ambrosone et al., 2012).

The regulatory mechanisms of RBPs, which are structurally and functionally important for various cellular processes, involve brief interactions or stable binding of target RNAs (Sharan et al., 2017). The human HuR protein is a well-studied RBP. Two RRM within the HuR N-terminal region mediate the recognition of adenosine-uridine (AU)-rich elements located primarily within the 3'-untranslated region (UTR) of target mRNA transcripts. Another RRM in the C-terminus region of HuR has affinity for polyadenosine [poly(A)] RNA and is thought to bind to the poly(A) tail of target mRNAs. This binding could increase the stability of the HuR-bound target mRNA, and further increase the steady-state protein level (Wigington et al., 2014). In addition to their role in normal cellular functions, RBPs are emerging as a class of proteins involved in a wide range of posttranscriptional regulatory events that are important in providing plants with the ability to respond rapidly to changes in environmental conditions (Marondedze et al., 2016).

The YTH (YT521 homologous) domain, which is composed of 100 to 150 amino acids, is a newly identified RNA-binding domain that was identified by comparing its sequence with that of splicing factor YT521-B (Stoilov et al., 2002). Homology searches have shown that the YTH domain is typical for eukaryotes and is particularly abundant in plants (Stoilov et al., 2002; Zhang et al., 2010). The predicted secondary structure of the YTH domain consists of four α -helices and six β -strands, and, via its conserved amino acid sequences (Trp380, Phe412, and Gly414), shows the ability to bind with single-stranded mRNA (Zhang et al., 2010). The first gene discovered to carry this domain was YT521, which was cloned from the astrocytes of rats (*Rattus norvegicus*), the expression of this

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gene was upregulated in reoxygenated astrocytes (Hartmann et al., 1999). Human YTH domain family proteins include five members, YTHDF1-3 and YTHDC1-2. YTHDF2 promotes mRNA degradation by binding with m6A-modified mRNA and transporting it to processing bodies (P-bodies, cytoplasmic decay sites) in the cell cytoplasm. However, the regulatory function of YTHDF1 binding is opposite that of YTHDF2. YTHDF1 enhances the translation of its targets by interacting with initiation factors (eIF3) and facilitating ribosome loading. A third member of the YTH family, YTHDF3 promotes protein synthesis synergistically with YTHDF1 and affects methylated mRNA decay via YTHDF2, suggesting that these three YTHDF proteins may act in an integrated and cooperative manner to impact fundamental biological processes related to m6A RNA methylation (Shi et al., 2017).

Proteins with a YTH domain have been reported to play roles in multiple aspects of plant life, such as developmental progress, and responses to abiotic and biotic stress. However, direct evidence is limited. Wang et al. (2017a) reported that overexpressing *MhYTP1* or *MhYTP2* in *Malus hupehensis* led to sensitivity to heat stress, high salinity, and *Diplocarpon mali* infection, but increased resistance to water logging, chilling stress, drought stress, and nutrition deficiency conditions. However, no biological functions of YTH domain-containing proteins have been reported in other plant species. Many plant genome projects have recently been completed, making it possible to identify YTH domain-containing proteins in different species. Recently, most studies have focused on the identification and characterization of YTH domain-containing protein family in plants. Several YTH genes have been identified in *A. thaliana*, rice, apple (*Malus domestica*), green algae (*Chlamydomonas reinhardtii*), moss (*Physcomitrella patens*), selaginella (*Selaginella moellendorffii*), norway spruce (*Picea abies*), grape (*Vitis vinifera*), tomato (*Solanum lycopersicum*), and cucumber (*Cucumis sativus*) (Li et al., 2014; Wang et al., 2014; Zhou et al., 2018); however, few reports of the YTH gene family in *Citrus* exist. In the present study, we identified and cloned 10 YTH genes in *C. sinensis* and analyzed their expression patterns in response to the most common abiotic stresses, including salt, drought, heat, and cold stresses, as well as hormone treatments, such as abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA). We also characterized the functions of these YTH genes in fruit development via transcriptome analyses. The results of this study will be helpful for future investigations aimed at the functional characterization of these YTH genes and their utilization for genetic improvement.

Materials and Methods

PLANT MATERIALS. *Citrus sinensis* (L.) Osbeck cv. Gannan Zao was used for expression assays. ‘Gannan Zao’ navel orange seedlings were grafted and grown in plastic pots that contained a composted soil mixture (1 sand:1 soil:1 plant ash) under fluorescent light ($200 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) with a 14/10 h light/dark photoperiod at 22 to 24 °C. For tissue or organ-specific expression of *C. sinensis* YTH genes (CitYTHs), the mature leaves, stems that had spring shoots (≈ 2 months after grafting to trifoliate orange plant), flowers at full bloom and whole fruits at 140, 160, and 200 d after flowering were collected from 4-year-old ‘Gannan Zao’ trees grown in a culture chamber. Lateral roots of seedlings were collected from

trifoliate orange plant. Tissue materials were frozen in liquid nitrogen and stored at -80°C until use.

IDENTIFICATION OF YTH PROTEINS IN *C. SINENSIS*. BLASTP searches were performed against the National Center for Biotechnology Information (NCBI, Bethesda, MD) database and Phytozome databases (Goodstein et al., 2011) using *A. thaliana* and rice YTH proteins as queries. The protein, predicted complementary DNA (cDNA), and genomic sequences for the putative YTH genes were downloaded. Each of the YTH candidate genes was then queried against The *Arabidopsis* Information Resource (Lamesch et al., 2012) using the BLASTP program, and those associated with the greatest *E* value with at least one known *A. thaliana* YTH gene were considered members of the *C. sinensis* YTH family. The Compute pI/Mw (isoelectric point/molecular weight) tool of ExPASy (Gasteiger et al., 2005) was used to calculate the Mw and pI of the deduced *C. sinensis* YTH proteins. The queries for the conserved domains were performed using the NCBI Conserved Domain Database [NCBI-CDD (Marchler-Bauer et al., 2014)], and the gene structures were analyzed with the online Gene Structure Display Server [GSDS (Li et al., 2014)].

MULTIPLE SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSIS. Protein sequences of other plants, including *A. thaliana*, rice, tomato, and rapeseed were obtained from the NCBI database, the *Vitis vinifera* Genome Database [VvGDB (Duvick et al., 2008)] and the Phytozome database. Multiple sequence alignments of the full-length YTH proteins from representative plant species were performed using the ClustalX program (Larkin et al., 2007), and were viewed by GeneDoc (Nicholas et al. 1997). A phylogenetic tree was constructed using MEGA 6.0 software via the neighbor-joining (NJ) method with 1000 bootstrap replicates (Tamura et al., 2013).

ABIOTIC STRESS AND HORMONE TREATMENTS. After they were grafted, three pots of 3-week-old navel orange seedlings (three biological replicates) with a consistent growth status for each group were chosen and treated with abiotic stresses and hormone treatments. With respect to drought and salinity stress, the seedlings were transferred from hydroponic cultivation to a layered filter for fast dehydration or maintained on liquid media supplemented with 200 mM NaCl for salinity stress. With respect to cold and heat treatments, culture tubes containing navel orange seedlings were placed in incubators that were maintained at 4 ± 1 or $42 \pm 1^\circ\text{C}$, respectively. The navel orange seedlings maintained at $28 \pm 1^\circ\text{C}$ were used as negative controls. With respect to hormone treatments, 3-week-old navel orange seedlings with expanded leaves were sprayed with 100 mM SA (Sigma-Aldrich, Poole, UK), 100 μM methyl jasmonate [MeJA (Sigma-Aldrich)], or 100 μM ABA (Sigma-Aldrich) in solutions containing 0.1% ethanol and 0.02% polyoxyethylene sorbitol anhydride monolaurate (Tween-20; Sigma-Aldrich) and then maintained under normal growth conditions. Plants sprayed with solutions that contained only 0.1% ethanol and 0.02% Tween-20 were used as negative controls. All leaf samples harvested at the indicated times were stored at -80°C until future use.

RNA EXTRACTION AND GENE EXPRESSION ANALYSES. Total RNA was isolated from plants using TRIZOL reagent (Invitrogen, Shanghai, China) and subsequently reverse transcribed into cDNA using Superscript III RT (Invitrogen) in accordance with the manufacturer’s protocols. Fluorescence quantitative polymerase chain reaction (qPCR) was used to

investigate the expression patterns of CitYTHs. The qPCR was conducted in a total volume of 25 μ L, which consisted of 1-mg aliquots of cDNA, 12.5 μ L of SYBR Green Master Mix Reagent (TaKaRa, Kyoto, Japan), and specific primers (3 pmol). qPCR was performed on a 96-well plate qPCR system (CFX96; Bio-Rad, Hercules, CA) in accordance with the following profile: 5 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 30 s at 57 °C, and 1 min at 72 °C. Quantification analysis was carried out via CFX Manager Software (Bio-Rad, Hercules, CA). The qPCR primers used are listed in Supplemental Table 1, and *C. sinensis* *CitActin* (accession no. XM_006464503) was used as an internal control in conjunction with primers *CitActin-F* and *CitActin-R* (Lugassi et al., 2015). Each experiment was performed in at least three technical replications and three biological replicates were run per sample (using three independent sets of RNA).

SUBCELLULAR LOCALIZATION. The coding sequences of the CitYTH genes were amplified by PCR with specific primers (Supplemental Table 1). The PCR products were then digested with *Xba*I and *Sam*I restriction enzymes and subsequently inserted into the appropriate sites in pFGC-Egfp vectors to obtain pFGC-Egfp:CitYTH constructs. The sequence-verified constructs and pFGC-Egfp vectors were transformed into *Agrobacterium tumefaciens* GV3101. Four-week-old *N. benthamiana* plants were inoculated with the transformed *A. tumefaciens* cultures and then grown at 25 °C for 36 h. Fluorescence signals were excited at 488 nm and detected using a 500- to 530-nm emission filter, both of which were performed with a confocal laser scanning microscope (LSM 510 Meta confocal microscope; Zeiss, Oberkochen, Germany).

RNA SEQUENCING. Transcriptome sequencing (RNA-Seq) analysis was used to study the expression of CitYTH genes during different fruit development stages. Transcriptome sequencing and assembly were performed by Meiji Biological Medicine Technology Co., Ltd. (Shanghai, China). A heatmap for the expression of CitYTH genes was generated in R using the heatmap 2 function from the gplots CRAN library (Warnes et al., 2013).

STATISTICAL ANALYSIS. SAS version 9.4 (SAS Institute, Cary, NC) was used for the statistical analyses. The experiments were repeated three times, and three replicates were included per experiment. The data from three independent experiments were analyzed via Dunnett's *t* test, taking $P < 0.05$ as a significant difference.

Results

IDENTIFICATION OF YTH DOMAIN-CONTAINING FAMILY MEMBERS IN 26 PLANT SPECIES. Based on domain composition analyses of sequences retrieved from BLAST searches using *A. thaliana* YTH domain-containing proteins as queries, 211 YTH sequences were identified among 26 plant species, including six Chlorophyta, one moss, one Lycopodiatae, four monocot, and 15 eudicot species (Fig. 1). Detailed information on these YTH family genes is provided in Supplemental Table 2. With the exception of that of the reported YTH gene, the identified sequences were named in accordance with their identity with AtYTH1. The evolutionary history of the plants suggested that the first plant YTH gene occurred in Chlorophyta, but only half of the Chlorophyta species contained the YTH gene. The presence of YTH domain-containing proteins varied in all other tested plant species (Fig. 1), suggesting that the YHT

domain-containing family is universally present in multicellular land plants but not in unicellular eukaryotes. In addition, the size of the YHT family differed among the moss, lycophyte, and flowering higher plant species. The lycophyte *S. moellendorffii* and the moss *P. patens* have only two and four YHT genes, respectively, whereas excluding grand eucalyptus (*Eucalyptus grandis*), potato (*Solanum tuberosum*), and cucumber, flowering higher plant species generally carry 8 to 20 YTHs. Many plants were found to have more than 10 YTH genes (Fig. 1, Supplemental Table 2).

PHYLOGENETIC ANALYSES OF YTH GENES. To investigate the phylogenetic relationships among YTH proteins, a phylogenetic tree of YTH proteins from 26 plant species was constructed based on the alignment of full-length proteins by the NJ method. The phylogenetic analyses revealed a clear clustering of the 211 YTH family members into three groups, I, II, and III; group I was further divided into two subgroups, whereas both group II and III were separated into three subgroups (Fig. 2). With respect to the eudicot plants, all YTH proteins were distributed in group Ia, IIc, IIIa, IIb, and IIc, except for *E. grandis*, *S. tuberosum*, and *C. sativus*. Similarly, apart from those of maize (*Zea mays*), the YTH proteins of grass species were clustered in group Ia, IIb, IIc, IIIa, IIb, and IIc. *Z. mays* has the second largest YTH family, and its members distributed in almost all subgroups excluding group Ia. Two species, *S. moellendorffii* and *P. patens*, have fewer YTH members, all of which are distributed in group Ia and IIb; these members share high identity among their protein sequences. Among six select Chlorophyta species, only three were found to contain YTH proteins (one YTH each), and they were all clustered in the group Ib. Interestingly, one ZmYTH (*ZmYTH18*) was also congregated in this group.

GENE STRUCTURE ANALYSIS OF THE YTH-CONTAINING FAMILY MEMBERS. To determine the possible gene structure relationships among YTH gene orthologs and paralogs, the exon/intron structures of individual YTH genes identified from 26 plant species were analyzed using GSDS software. The number of introns in these genes varied widely from 2 to 13 (Fig. 3). Two groups (Ib and IIb) with relatively few YTH gene members also contained few introns. Group Ib members had 3 or 4 introns, and group IIb members had 5 or 6 introns. Group Ia YTH genes contained introns whose numbers ranged from 4 (*AtYTH1*) to 9 (*PpYTH4*), although most had 6 (53%) or 7 (21%), and group IIc YTH genes had 6 (5 genes) to 10 (*VvYTH9*) introns, although most had 8 (28%). Group IIIa YTH genes had 4 (*CitYTH5*) to 9 (*VvYTH4* and *PpYTH4*) introns, while most had 8 (57%) or 6 (32%), and group IIb YTH genes had 2 (*PvYTH2*) to 13 (*PpYTH2*) introns, although most had 6 (28%). The structure of the YTH genes in group IIc distinctly differed from that in the other groups, with more introns at different phases and with different lengths. The number of introns in group IIc ranged from 2 (*ZmYTH6*) to 9 (*VvYTH8*); most members had 7 (55%). Moreover, the intron phases within the YTH gene families varied greatly among the different groups. For instance, most group Ia YTH genes had the unique phase pattern 2-2-0-0-0-0 (45%) for 6-intron members and 1-0-0-2-0-0-2 (18%) for 7-intron members, whereas the pattern 1-0-0-0-0-0-0 for 6-intron members and 1-0-1-0-0-0 (48%) for 7-intron members were observed in group IIIa and group IIc genes, respectively. Notably, the phase patterns of different introns within the same group were very similar. For instance, the phase patterns for 7-intron, 8-intron, and 9-intron members were 1-0-1-0-0-0-0, 1-0-1-0-0-0-0-1 and

Fig. 1. Species of plant investigated in this study. Left column: phylogenetic tree of plant species generated according to the classification of plants in the Phytozome database (Goodstein et al., 2011). Right column: YTH domain-containing proteins identified. A total of 211 YTH sequences were identified among 26 plant species.

1-0-1-0-1-0-0-0-1 in group IIIa, respectively, whereas the patterns were 1-0-0-0-0-1 and 2-1-0-0-0-1 for 5-intron members and 6-intron members in group Ib, respectively.

All of the identified YTH proteins contain a YT521-B-like domain (pfam04146) (Fig. 3). In addition, ZmYTH16 possesses a prephenate dehydratase domain (PDT PF00800) (Fig. 3). Prephenate dehydratase plays function in amino acid transport and metabolism (Pascual et al., 2016). Moreover, a UBA/TS-N domain (PF00627) was found in PpYTH1 and PpYTH2 protein (Fig. 4). Ubiquitin-mediated proteolysis is involved in the regulated turnover of proteins required for controlling cell

cycle progression (Liu et al., 2006). These results suggested that the YHT proteins may have other functions in addition to binding with RNA.

TISSUE-SPECIFIC EXPRESSION PATTERNS OF CitYTH GENES. To determine the possible physiological roles of CitYTH genes (listed in Supplemental Table 2) in growth and development, we analyzed their tissue-specific expression patterns in five different tissues (roots, stems, leaves, flowers, and fruits) by qPCR. The results revealed different expression patterns of the CitYTH genes in the five tissues. As shown in Fig. 4, 10 CitYTH genes were constitutively expressed in all tested tissues but exhibited different expression patterns. Both *CitYHT1* and *CitYHT10* were expressed specifically in the leaves, but in the roots, stems, flowers, and fruits, their expression levels were very low. On the other hand, *CitYTH5* and *CitYTH9* were highly expressed in the stems, leaves, flowers, and fruits, but their expression levels in the roots were low. *CitYHT3* and *CitYHT6* were highly expressed in the stems and leaves, but their expression levels in the roots, flowers, and fruits were low. In addition, the expression of *CitYHT4* was relatively high in the leaves, flowers, and fruits but low in the roots and stems. In general, with the exception of those of *CitYHT10*, the expression levels of the CitYTH genes were lowest in the roots, whereas the levels of all 10 CitYTH genes, 5 CitYTH genes (*CitYTH2*, 3, 4, 5, 9), and 3 CitYTH genes (*CitYTH2*, 5, 9) were relatively high in the leaves, flowers, and fruits, respectively.

EXPRESSION OF CitYTH GENES IN FRUITS AT DIFFERENT DEVELOPMENTAL STAGES. To study the potential role of CitYTH genes

in fruit development, we evaluated their expression patterns during three different developmental stages in two different cultivars of navel orange. The time to maturity of ‘Gannan Zao’ is shorter than that of ‘Newhall’ navel orange. The results showed that the expression in two different parts of the fruits (peel and pulp) exhibited different patterns (Fig. 5). In both the ‘Newhall’ and ‘Gannan Zao’, *CitYTH3*, 4 and 6 were relatively highly expressed in peels, but their expression levels were low in the pulp during all three developmental stages. On the other hand, the expression levels of *CitYTH8* and *CitYTH9* were relatively high in the navel orange peels but low in the pulp

Fig. 3. Phylogenetic relationships, conserved motif compositions, numbers of introns, and gene structure of YTH domain-containing proteins. Left column: phylogenetic tree of YTH domain-containing proteins. Middle left column: exon/intron gene structure (filled box = exon, solid line = intron). The intron phases are indicated as the numbers 0, 1, and 2. Excluding *DsYTH1*, *PvYTH11*, *SIYTH7*, *SIYTH8*, *SIYTH9*, *VvYTH2*, *VvYTH7*, *VvYTH9*, and *ZmYTH19*, whose long introns are denoted by a “/”, the exons and introns are drawn to scale. Middle right column: numbers of introns. Right column: conserved domains within the YTH domain-containing proteins. The domains and motifs are drawn to scale.

major environmental stresses that affect plant growth and development and ultimately crop yields (Shahbaz and Ashraf, 2013; Zhu, 2016). To investigate the potential involvement of CitYTH genes in abiotic stress responses, the expression

patterns of CitYTHs in navel orange in response to salt, drought, cold, and heat stresses were analyzed.

We first analyzed the expression of the CitYTH genes in the leaves of navel orange seedlings after they were treated with

Fig. 3. (Continued)

salt (NaCl) and drought. In our study, 10 *CitYTH* genes exhibited different expression patterns under salt (NaCl) and drought stress (Fig. 6). Compared with those in the control plants, the expression levels of *CitYTH2*, *CitYTH3*, *CitYTH5*, *CitYTH7*, and *CitYTH8* were dramatically up-regulated by different amounts and peaked at 24 to 48 h after

the drought treatment. The expression of *CitYTH9* peaked at 24 h after drought stress treatment but then decreased afterward. In addition, the expression of *CitYTH1* and *CitYTH4* was strongly repressed in response to drought stress, especially that of *CitYTH1*, whose expression decreased by ≈ 10 -fold at 48 h after treatment. In the salt

Fig. 4. Expression analysis of *Citrus sinensis* YTH domain-containing protein genes in the different tissues of 'Gannan Zao' navel orange plants (R = roots, S = stems, L = leaves, F = flowers, FR = mature fruit pulp, FP = mature fruit peels). Root, stem, and leaf samples were collected from spring shoots ($\approx$ 2 months after grafting). Flowers at full bloom and whole fruits at 140 d after full bloom were collected from adult navel orange trees grown in a culture chamber. The transcript levels of CitYTH genes were detected using the real-time polymerase chain reaction. The data presented are mean $\pm$ SD of three independent experiments. The x- and y-axes represent the different tissues of navel orange and relative expression (fold change relative to actin transcript values), respectively.

treatment assay, only increased expression of *CitYTH4* was observed. In contrast, the expression of *CitYTH2* was downregulated during the 24- to 48-h test period. Moreover, the expression of *CitYTH6* and *CitYTH8* increased at 24 h but then decreased at 48 h after treatment. The expression of *CitYTH5* increased but then decreased at 24 and 48 h after treatment, respectively. The expression of the other five CitYTH genes (*CitYTH1*, 3, 7, 9) exhibited no detectable changes within 48 h period.

As shown in Fig. 7, the expression of four CitYTH (*CitYTH2*, 4, 5, 9) genes was significantly induced and peaked at 24 h after heat treatment; compared with that in the control plants, the expression in the treated plants increased 2.3- to 8.0-fold. However, the expression of *CitYTH3*, *CitYTH6*, *CitYTH7*, and *CitYTH10* was downregulated at 24 or 48 h after heat treatment. Among the expression of these genes, the expression of *CitYTH6* was repressed gradually and reached a minimum of a decrease of 10-fold at 48 h after heat treatment. The expression of the other two CitYTH genes (*CitYTH1* and *CitYTH8*) was not markedly affected under heat stress conditions. In the cold stress treatment group, the expression levels of all 10 CitYTH genes were higher (to varying degrees) than those in the control plant group under low temperature (4 °C) conditions. However, the patterns among them were not the same. Compared with those in the control plants, the expression levels of seven CitYTH (*CitYTH1*, 3, 5, 7, 8, 9, 10) genes in the treated plants increased 1.8- to 3.3-fold and peaked at 24 h after the cold treatment. Furthermore, the expression levels of the other three CitYTH genes increased 3.5- to 7.2-fold and peaked at 24 h after cold treatment.

EXPRESSION OF CITYTH GENES IN RESPONSE TO HORMONE TREATMENTS. To examine the possible effects of SA, JA, and ABA on CitYTH transcript levels, we analyzed the expression of these genes in response to spraying with JA, SA, and ABA. As shown in Fig. 8, the expression patterns of

Fig. 5. Heatmap representation of *Citrus sinensis* YTH domain-containing protein genes in fruit peels and pulp during fruit development. Whole fruits at 140, 160, and 200 d after full bloom (DAFB) were collected from 4-year-old 'Gannan Zao' navel orange trees. Transcriptome sequencing (RNA-Seq) analysis was used to analyze the expression of CitYTH genes, and each experiment contains three biological replications. The heatmap was generated in R using the heatmap 2 function from the gplots CRAN library (Warnes et al., 2013). The bar on the right of heatmap represents the relative expression value. 1P_WT, 2P_WT, and 3P_WT = peels of 'Newhall' fruit at 160, 180, and 200 DAFB, respectively; 1P_MT, 2P_MT, and 3P_MT = peels of 'Gannan Zao' fruit at 160, 180, and 200 DAFB, respectively; 1R_WT, 2R_WT, and 3R_WT = pulp of 'Newhall' fruit at 160, 180, and 200 DAFB, respectively; 1R_MT, 2R_MT, and 3R_MT = pulp of 'Gannan Zao' fruit at 160, 180, and 200 DAFB, respectively.

CitYTH3, *CitYTH5*, *CitYTH6*, *CitYTH7*, *CitYTH8*, and *CitYTH9* were similar from 24 to 48 h and displayed no detectable changes after applications of SA, JA, and ABA, compared with those in the control plants. Increased expression of *CitYTH2* and *CitYTH10* was observed after applications of JA and SA, whereas the expression of *CitYTH1* was downregulated. In response to ABA, the expression levels of both *CitYTH2* and *CitYTH4* were downregulated after 24 and 48 h.

C. SINENSIS YTH DOMAIN-CONTAINING PROTEINS TARGET TO NUCLEUS AND CYTOPLASM. To investigate the subcellular localization of CitYTH proteins, the coding sequences of the CitYTH genes were amplified and fused to the N-terminus of green fluorescent protein (GFP) under the control of the CaMV 35S promoter, after which the constructs were transiently expressed in *N. benthamiana* leaves via agroinfiltration. The subcellular localization of GFP in *N. benthamiana* leaves was subsequently examined by confocal microscopy. The results showed that both the CitYTH:GFP and GFP alone were localized to in the nucleus and the cytoplasm (Fig. 9). These results demonstrate that CitYTH proteins do not target specific compartments and are likely to distribute ubiquitously in cells.

Discussion

YT521-B is present only in vertebrate genomes (Zhang et al., 2010). However, genome-wide identification of gene family members containing YT521-B-like domain has been reported in several plant species (Li et al., 2014; Wang et al.,

Fig. 6. Expression analysis of *Citrus sinensis* YTH domain-containing protein genes in 'Gannan Zao' navel orange leaves treated with drought or salt stress. Drought stress was applied to 3-week-old navel orange seedlings after grafting by transferred to three layers of filter papers for fast dehydration. Salt stress was applied to 3-week-old seedlings by drenching the plants with a 200-mM NaCl solution. Navel orange leaves were collected for quantitative polymerase chain reaction analysis at 0, 24, and 48 h after treatment. Relative expression folds were calculated after normalization with *CitActin* transcript values. The x- and y-axes represent the treatment time (units = hours) and relative expression, respectively. The data presented are mean $\pm$ SD of three independent experiments run in triplicate. The * above the columns indicates significant differences at the $P < 0.05$ level.

Fig. 7. Expression analysis of *Citrus sinensis* YTH domain-containing protein genes in 'Gannan Zao' navel orange leaves treated with cold or heat stress. Cold and heat stress was applied by placing 3-week-old navel orange seedlings after grafting in growth chambers whose temperatures were set at 4 and 42 °C, respectively; navel orange trees grown at 28 °C were used as controls. Leaf samples for quantitative polymerase chain reaction analysis were collected at 0, 24, and 48 h after treatment. The x- and y-axes represent the treat time (units = hours) and relative expression (fold change relative to actin transcript values), respectively. The data presented are mean $\pm$ SD of three independent experiments run in triplicate. The * above the columns indicates significant differences at the $P < 0.05$ level.

2014; Zhang et al., 2010). Our sequence search in which *A. thaliana* and *O. sativa* YTH proteins were used as queries for YTH domain-containing genes from unicellular green algae, mosses, lycophytes, monocots, and eudicots suggested that YTH genes existed not only in land plants, but also in an aquatic plant (Fig. 1). Among the plant species we studied, the most ancestral to present a YTH domain-containing gene was a green alga. However, not all green algae contained the YTH gene; three green alga species (*Coccomyxa subellipsoidea*, *Micromonas pusilla*, *Ostreococcus lucimarinus*) lacked the YTH gene, and

another three green alga species had only one YTH gene, respectively. In contrast, all other higher plant possesses YTH genes.

A combined NJ tree was also constructed to investigate the phylogenetic relationships of YTH genes in plants and their evolutionary relationships. The YTH members were divided into three groups, and group a, b, and c contained two, three, and three subgroups, respectively. The phylogenetic tree suggested that a close relationship exists among YTH genes in plant species (Fig. 2). All of the YTHs in green algae were clustered in subgroup Ib. It appeared that subgroup Ib members (apart from *ZmYTH*) were present in the lower plant species. The lycophyte *S. moellendorffii* and moss *P. patens* had only two and four YTH genes, respectively, and those genes were clustered into group Ia and group IIIb, whereas flowering higher plant species generally harbored 8 to 20 YTH genes. Interestingly, the proportion of total YTH genes between *S. moellendorffii* and *P. patens* was 1:2, which is consistent with the proportion of the YTH genes in the corresponding subgroups. Excluding four species (*S. tuberosum*, *E. grandis*, *C. sativus*, and *Z. mays*), most angiosperm plants had group Ia, IIc, IIIa, IIIb, and IIIc YTH genes. We found 10 YTH genes in *C. sinensis*, which is a smaller number than that in *A. thaliana* (13 members), *O. sativa* (12 members), *Z. mays* (19 members), and cabbage (*Brassica rapa*) (20 members), suggesting that the number of YTH genes is not proportional to genome size.

Gene fusion is a well-known process in molecular evolution (Enright and Ouzounis, 2001). We analyzed the gene structure of YTH.

The YTH genes that clustered in the same group had similar motif distributions and exon/intron organizations (Fig. 3), implying that the YTH gene family in different species may evolve independently. For example, the phase patterns of 7-intron members, 8-intron members, and 9-intron members are 1-0-1-0-0-0-0, 1-0-1-0-0-0-0-1, and 1-0-1-0-1-0-0-0-1 in group IIIa, respectively, whereas the patterns for 5-intron members and 6-intron members are 1-0-0-0-1 and 2-1-0-0-0-1 in group Ib, respectively. Similar to all of the YTH genes characterized in various species, all eight family members contain conserved YT521-B-like domains (Fig. 3). However, other motifs were also found within the YTH

Fig. 8. Expression analysis of *Citrus sinensis* YTH domain-containing protein genes in 'Gannan Zao' navel orange leaves treated with salicylic acid (SA), methyl jasmonate (JA), and abscisic acid (ABA). Three-week-old navel orange seedlings after grafting were treated by foliar sprays of 1 mM SA, 100 μ M JA, 100 μ M ABA, or an equal volume of solvent [as a control (ck)]. The expression of CitYTH genes was analyzed by quantitative polymerase chain reaction after the seedlings were treated for 0, 24, and 48 h. The x- and y-axes represent the treat time (units = hours) and relative expression (fold change relative to actin transcript values), respectively. The data presented are mean $\pm$ SD of three independent experiments run in triplicate. The * above the columns indicates a significant difference at the $P < 0.05$ level.

Fig. 9. Subcellular location of *Citrus sinensis* YTH domain-containing proteins. *Nicotiana benthamiana* leaves were transiently transformed with constructs harboring pFGC-Egfp: CitYTHs and pFGC-Egfp via agro-infiltration. Fluorescence of the target proteins was observed with confocal microscopy 36 h post infection.

protein domain, suggesting the YT521-B-like domains is a necessary and not just sufficient condition for judging YTH proteins.

developmental stages (such as germinating seeds, seedlings, and rosettes) (Li et al., 2014). In apple, expression of *MhYTP1* and *MhYTP2* is induced during natural leaf senescence;

A comprehensive gene expression analysis of YTH gene family members revealed that the YTH genes exhibited distinct expression patterns in various tissues of several plant species (Li et al., 2014). Some members of the YTH family exhibit organ-specific expression patterns or are not expressed in special tissues (e.g., *CsYTH1* is not expressed in any tissues in cucumber) (Zhou et al., 2018). Furthermore, neither *MdYTP9* nor *MdYTP12* was detected in any of those tissues (Wang et al., 2014). In contrast, some YTH members, such as *AtYTH* genes, are highly expressed; however, there are exceptions; for example, the potential for *AtYTH06* expression is relatively high in both the seeds, including in the embryo, endosperm, and testa and in the shoots (Li et al., 2014). The expression of *MdYTH2* was highest in unfertilized ovaries and lowest in tendril bases. In addition, *MdYTP1* transcripts were higher in the shoot apex and flowers than in other tissues (Wang et al., 2014). In the present study, 10 CitYTH genes were constitutively expressed in all tested tissues. For example, with the exception of those of *CitYTH9*, the expression levels of most of the CitYTH genes were lowest in the roots, whereas the expression levels of all 10 CitYTH genes were relatively high in the leaves; five CitYTH genes (*CitYTH2*, 5, 7, 8, 9) were expressed in the flowers, and three CitYTH genes (*CitYTH2*, 5, 9) were expressed in the fruits (Fig. 4). YTH genes were expressed in all tested tissues and exhibited different expression patterns, suggesting that they may play different potential roles in different tissues.

The gene expression profiles at the different developmental stages or in response to different stresses may indicate gene function in relation to developmental and stress responses (Li et al., 2014). Previous studies have shown that the expression of YTH genes is highly developmentally regulated in plants. OsYTHs and AtYTHs exhibit high and low expression potential at the flowering stage and during different

compared with wild-type plants, the leaves of overexpression plants showed earlier yellowing and significantly lower chlorophyll concentrations in *A. thaliana* and apple. *MhYTP2* can interact with acireductone dioxygenase 4 [an ethylene (ET) biosynthesis-related protein] to promote the progress of maturity (Wang et al., 2017a). The fruits of both *MhYTP1* and *MhYTP2* transgenic tomato plants turned yellow earlier than did those of wild-type plants, indicating that these two YTH domain-containing genes can promote fruit ripening in tomato (Wang et al., 2017c). In two different cultivars, the expression of *CitYTH5* and *CitYTH10* was higher in peels of early cultivars, whereas the expression of *CitYTH1*, 7, 8, and 9 was lower (Fig. 5). Because the maturity period of the ‘Gannan Zao’ is shorter than that of the ‘Newhall’ by ≈ 1 month, the results suggested that these affected genes may have a potential role played in peel and pulp development. However, the function of YTH genes in navel orange development and fruit ripening requires validation by further study.

In many species, YHT-containing genes can transcriptionally respond to abiotic stress, including high salinity, drought, heat, cold, and polyethylene glycol stress (Wang et al., 2014; Zhou et al., 2018). However, there is limited direct evidence showing that YTH genes play a role in different stress responses in plants. Overexpression of *MhYTP1* or *MhYTP2* makes plants more sensitive to NaCl, but more resistant to nutrient deficits (Wang et al., 2017b). In addition, Wang et al. (2017c) reported that, compared with wild-type plants, plants overexpressing *MhYTP1* or *MhYTP2* were more sensitive to heat stress and high salinity but were more resistant to waterlogging, chilling, drought, and nutrient deficiency conditions. Moreover, both the *MhYTP1* and *MhYTP2* genes in those overexpression plants could be induced by various treatments (e.g., water logging, water deficit, and high salinity). In contrast, the abiotic stress also can affect the expression of YTHs. Several stress-related *cis*-elements, such as heat stress-responsive elements, drought-responsive elements, defense- and stress-responsive elements (TC-rich repeats), anaerobic induction elements, and low temperature-responsive elements, all of which respond to external environmental stresses, have been found in the upstream regions of the promoters of YTH members (Li et al., 2014; Wang et al., 2017a), and the *MhYTP* family was sensitive to chilling and H_2O_2 conditions and responsive to heat, water deficit, and salt treatment (Wang et al., 2014). Several *AtYTH* and *OsYTH* genes presented altered expression in response to abiotic stress. The expression profile of *AtYTH07* and *AtYTH10* in the seedlings and flowers was dramatically induced at early stages during heat stress. In addition, increased expression levels of *AtYTH05* under cold stress, of *AtYTH02* under hypoxic stress, and of *AtYTH01* and *AtYTH02* under submergence stress also have been reported (Li et al., 2014). ABA is involved in the response to drought, salt, low temperature, and heat stress by inducing a series of signaling cascades. Studies suggest that osmotic stress imposed by high-salt or drought stress is transmitted through at least two pathways: ABA-dependent and ABA-independent pathways (Xiong and Zhu, 2002; Yamaguchi-Shinozaki and Shinozaki, 2006). ABA can also induce gene expression associated with stress responses (Busk and Pages, 1998). YTH members encompass a classic ABA-responsive element (ABRE, ACGTGT) found in the promoter region (Zhou et al., 2018). Thus, ABA could bind to the ABRE promoter to regulate gene expression. Several lines of evidence support the involvement of ABA in

the YTH family in response to abiotic stresses. Expression of both *MhYTP1* and *MhYTP2* can be induced when ABA treatment is applied. Compared with wild-type plants, *MhYTP1*, 2 overexpression in plants was less sensitive to ABA treatment and has greater fresh weight and more roots. The expression patterns of *AtYTH06* in *A. thaliana* were also altered by treatment with cold, drought, heat, and osmotic stresses and by treatment with ABA (Li et al., 2014). In the present study, *CitYTH2/4* was induced/suppressed by salt, drought, cold, and heat stresses; the expression of these genes was also induced by ABA, suggesting that *CitYTH2/4* has various functions in response to multiple abiotic stresses via ABA signaling. However, several *CitYTH* genes were affected by stress and not by ABA. This finding may suggest that *CitYTH* is also involved in ABA-independent pathways. Furthermore, we observed the induction or suppression of *CitYTH* family gene expression in response to the tested stress treatments, suggesting that crosstalk occurs between different stress signaling pathways.

SA and JA involved in defense against different biotic stressors have been well established (Atkinson and Urwin, 2012; Glazebrook, 2005; Laluk et al., 2012). Li et al. (2014) reported that treatment with ACC (a precursor of ET), ET, MeJA, or SA did not affect the expression patterns of all *AtYTH* genes in *A. thaliana* plants. However, in overexpressing plants *MhYTH1* and *MhYTH2*, the expression of both of these genes was induced by treatment with SA and MeJA. Furthermore, transgenic plants showed more sensitivity to *Diplocarpon mali* infection, and expression of the JA signaling-related genes *PLD* and *COI1*, the SA signaling-related genes *PR1* and *PR5*, and the ET-responsive gene *ERF3* in transgenic plants was altered, suggesting that *MhYTH1* and *MhYTH2* are involved in SA and MeJA/ET signaling (Wang et al., 2017a). The present results also showed that the expression of three *CitYTH* genes (*CitYTH1*, *CitYTH2*, and *CitYTH10*) was significantly altered in response to JA and SA. These findings were in accordance with that of *MhYTH1/2*, suggesting that these genes may be involved in JA and SA signaling. However, further studies are required to reveal the molecular mechanisms of *CitYTHs* in response to biotic stress by biochemical and genetic approaches.

RBP coordinate the life of mRNAs, from birth in the nucleus to death in the cytoplasm, and play important roles in many regulatory aspects of cellular RNA metabolism (Wurth and Gebauer, 2014; Zhang et al., 2010), including RNA synthesis, folding/unfolding, modification, processing, and degradation. Thus, RBPs may function in both the nucleus and cytoplasm. *MhYTP1* and *MhYTP2* occur throughout the entire cell rather than within only specific compartments (Wang et al., 2017c). Otherwise, *AtYTH5* and *AtYTH7* in *A. thaliana* have also been shown to localize throughout the leaf cells of *N. benthamiana* (Li et al., 2014). Similarly, our subcellular results showed that the *CitYTH* proteins we selected were located in the nucleus and cytoplasm, which is consistent with their functional location.

In conclusion, 211 YTH genes were identified in 26 species that represent four major plant lineages in this study. Phylogenetic analysis revealed that these genes could be divided into eight subgroups. All of the YTH genes contain a YT521 domain and have different structures. Ten YTH genes were identified in *C. sinensis*. The expression profiles of these *CitYTH* genes were analyzed in different tissues and at different fruit developmental stages, and *CitYTH* genes

displayed distinct expression patterns under heat, cold, salt, and drought stress. Furthermore, expression of the CitYTH genes in response to exogenous hormones was measured. Nuclear localization was also confirmed for five of these proteins encoded by these genes after transient expression in *N. benthamiana* cells.

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Supplemental Table 1. Primers used for quantitative polymerase chain reaction (qPCR) analysis of *Citrus sinensis* YTH genes expression and subcellular localization. For qPCR, *CitActin* was used as reference gene (Lugassi et al., 2015).

Primer name	Primer 5'–3'	Size (base pairs)
qPCR		
CitYTH1-1F	CTCGGCATTTTCTAACCAAA	130
CitYTH1-1R	TCCAAACCTCCAGCTCTAC	
CitYTH2-1F	TGCGATCCATCATTTATGGTAG	118
CitYTH2-1R	ATTGAGATTGAGGGAACTTTT	
CitYTH3-1F	TGCTCCAGATTTACTTGCCA	196
CitYTH3-1R	CGACTCGACAGCTTCAGAGT	
CitYTH4-1F	TCTAATGGTAATGGCTTTTCTGAC	148
CitYTH4-1R	CCCGATTCTGTTCACTAAACATAC	
CitYTH5-1F	CGAAGCAACACCAGTTACAGAG	182
CitYTH5-1R	ATTCTCCTCCAACCTTCTCACA	
CitYTH6-1F	GGGTAGTGGTTCTTTATGTGGTTG	142
CitYTH6-1R	AGACTTGCTTTCTTTGTTTGGTC	
CitYTH7-1F	TGGTCGTGGATGGTTGTCTG	136
CitYTH7-1R	GTTTTTGTCTCCCTTGGCTC	
CitYTH8-1F	GGATCATTTTTTGGGTTTGG	164
CitYTH8-1R	CATCTTCCTCCTTGCTGGC	
CitYTH9-1F	GATGTGGTGGTGATGCTGAA	164
CitYTH9-1R	GCTGGCAAGAGGACTGTCTG	
CitYTH10-1F	GGTAGAGGGAGGGGCAGAG	189
CitYTH10-1R	TGGAGCAAAAGGACGAGGA	
CitActin-F	CATCCCTCAGCACCTTCC	195
CitActin-R	CCAACCTTAGCACTTCTCC	
Subcellular localization		
CitYTH1-2F	GAT GGATCC ATCGAT ATTCTCATTACATGGGTTTG	1719
CitYTH1-2R	GAT GGATCC GACATCATCCTCACTGTACG	
CitYTH2-2F	GAT GGATCC ATGTATGTCATGCCAGCATTTTC	2429
CitYTH2-2R	TCC CCCGGG TTATTCCTTGCAGCCCTCAACA	
CitYTH3-2F	GAT GGATCC CTTCCAGTTTCACCATCTGAGA	1329
CitYTH3-2R	TCC CCCGGG TTAATTCTGATTCTCACTACCTGT	
CitYTH4-2F	GAT GGATCC ATGGAAATGTATAATGCGTCTG	2091
CitYTH4-2R	TCC CCCGGG TTAATATTGATTTTGGGATGAGAT	
CitYTH5-2F	GAT GGATCC ATGGCAGACAAAGTGTCTTTGT	1554
CitYTH5-2R	TCC CCCGGG TTAGCATGCGCTTCCAACCC	
CitYTH6-2F	GAT GGATCC ATGGCGGCAACCCAATCACA	2010
CitYTH6-2R	TCC CCCGGG CTAAGTGGACTGAGCAGAAG	
CitYTH7-2F	GAT GGATCC ATGGCTACTACTGTTGCTCC	2136
CitYTH7-2R	TCC CCCGGG CTAACAACCATTTGCAACCCCA	
CitYTH8-2F	GAT GGATCC ATGGCACAGCCAACCTCCGA	1893
CitYTH8-2R	TCC CCCGGG TTAATCGCCGTGAGCCACAG	
CitYTH9-2F	GAT GGATCC ATGTCGTCTGATACTGCTAAAGAA	1206
CitYTH9-2R	TCC CCCGGG TCATTTTGGGACGACTGATGT	
CitYTH10-2F	GAT GGATCC ATGGAGGACTCGGAAGGAGG	2106
CitYTH10-2R	TCC CCCGGG CTAGTTATCAGAACTGGCAGCA	

Supplemental Table 2. Information of YTH domain-containing proteins identified in this study. Protein sequences of YTH domain-containing proteins were obtained from the Phytozome database (Goodstein et al., 2011).

Family and species	Gene symbol	Protein no.	Gene location	Protein length (amino acids)
Funariaceae				
<i>Physcomitrella patens</i>	PpYTH1	Pp3c10_17810V3.1	Chr10:12137972..12139094	878
	PpYTH2	Pp3c3_33560V3.1	Chr03:22578711..22580073	982
	PpYTH3	Pp3c20_9290V3.1	Chr20:5579213..5585252	774
	PpYTH4	Pp3c8_11880V3.1	Chr08:7612847..7613382	562
Selaginellaceae				
<i>Selaginella moellendorffii</i>	SmYTH1	6427	Scaffold_1:5762194..5762817	168
	SmYTH2	77664	scaffold_1:2289264..2289783	152
Grass				
<i>Brachypodium distachyon</i>	BdYTH1	Bradi3g06027.2	Bd3:4323560..4326704	586
	BdYTH2	Bradi5g01317.2	Bd5:1241164..1244329	707
	BdYTH3	Bradi2g46590.1	Bd2:46788296..46789682	646
	BdYTH4	Bradi1g05970.1	Bd1:4027107..4028272	609
	BdYTH5	Bradi3g18190.1	Bd3:16645681..16648259	590
	BdYTH6	Bradi3g42640.2	Bd3:43888082..43888644	666
	BdYTH7	Bradi4g02150.1	Bd4:1409062..1409632	656
	BdYTH8	Bradi1g64100.2	Bd1:63508088..63514486	740
	BdYTH9	Bradi1g74560.2	Bd1:71995191..71997470	751
	BdYTH10	Bradi2g12380.1	Bd2:10732820..10737832	694
	BdYTH11	Bradi2g34785	Bd2:34612920..34617148	732
	BdYTH12	Bradi1g32560.1	Bd1:28033974..28037763	653
<i>Oryza sativa</i>	OsYTH1	LOC_Os01g22630.1	Chr1:12725141..12731526	708
	OsYTH2	LOC_Os01g48790.1	Chr1:27983688..27990383	609
	OsYTH3	LOC_Os03g06240.1	Chr3:3125933..3130483	708
	OsYTH4	LOC_Os03g20180.2	Chr3:11402649..11408106	709
	OsYTH5	LOC_Os03g53670.2	Chr3:30777720..30781832	662
	OsYTH6	LOC_Os04g04000.1	Chr4:1843006..1848258	675
	OsYTH7	LOC_Os04g51940.1	Chr4:30821134..30825520	574
	OsYTH8	LOC_Os05g01520.1	Chr5:304131..311132	638
	OsYTH9	LOC_Os06g46400.1	Chr6:28151360..28156784	665
	OsYTH10	LOC_Os07g07490.1	Chr7:3726574..3731170	602
	OsYTH11	LOC_Os08g12760.1	Chr8:7559098..7563188	577
	OsYTH12	LOC_Os08g44200.1	Chr8:27825032..27830316	624
Panicoideae				
<i>Zea mays</i>	ZmYTH1	GRMZM2G314176_T01	2:4328051..4328948	119
	ZmYTH2	GRMZM2G303312_T01	9:154512218..154519531	666
	ZmYTH3	GRMZM2G025488_T02	1:205729284..205737582	516
	ZmYTH4	GRMZM2G340130_T01	2:234409795..234414268	635
	ZmYTH5	GRMZM2G442195_T01	10:66329570..66333487	496
	ZmYTH6	GRMZM2G144726_T01	7:8310841..8315240	637
	ZmYTH7	GRMZM2G126338_T02	1:276138867..276143356	660
	ZmYTH8	GRMZM2G076062_T02	1:12162892..12167581	691
	ZmYTH9	GRMZM2G330019_T01	9:153128002..153132868	702
	ZmYTH10	GRMZM2G004997_T02	4:26320085..26324458	594
	ZmYTH11	GRMZM2G102069_T01	2:11208368..11212888	552
	ZmYTH12	GRMZM2G056573_T01	4:40005803..40012977	720
	ZmYTH13	GRMZM2G169116_T01	8:150552217..150559397	609
	ZmYTH14	GRMZM2G127780_T02	3:208862305..208867531	686
	ZmYTH15	GRMZM2G098174_T01	6:1281903..1288156	688
	ZmYTH16	GRMZM2G363789_T01	9:113957381..113961138	562
	ZmYTH18	GRMZM2G145770_T01	4:166518141..166521453	230
	ZmYTH17	GRMZM2G433616_T01	8:72326272..72328873	492
	ZmYTH19	GRMZM2G020704_T02	2:130572564..130589249	727
<i>Sorghum bicolor</i>	SbYTH1	Sobic.001G060500.1.p	Chr01:4523110..4527828	572
	SbYTH2	Sobic.007G172700.1.p	Chr07:60761969..60767071	639
	SbYTH3	Sobic.002G047000.2.p	Chr02:4379219..4383210	646
	SbYTH4	Sobic.007G078600.1.p	Chr07:9201830..9206035	594

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Supplemental Table 2. Continued.

Family and species	Gene symbol	Protein no.	Gene location	Protein length (amino acids)
	SbYTH5	Sobic.001G090500.1.p	Chr01:7004122..7008251	659
	SbYTH6	Sobic.001G498200.2.p	Chr01:76810098..76814761	711
	SbYTH7	Sobic.009G004300.1.p	Chr09:377484..383567	653
	SbYTH8	Sobic.003G257100.1.p	Chr03:59529078..59534732	671
	SbYTH9	Sobic.006G208800.1.p	Chr06:55865933..55869663	552
	SbYTH10	Sobic.007G005500.1.p	Chr07:475260..480608	733
	SbYTH11	Sobic.009G054400.2.p	Chr09:5514380..5520238	708
	SbYTH12	Sobic.010G228700.1.p	Chr10:57165982..57172412	645
Ranunculaceae				
<i>Aquilegia coerulea</i>	AcYTH1	Aqcoe6G010000.1	Chr_06:599442..605166	541
	AcYTH2	Aqcoe5G227200.1	Chr_05:14733862..14736836	388
	AcYTH3	Aqcoe1G157200.1	Chr_01:8896859..8902124	587
	AcYTH4	Aqcoe5G460600.1	Chr_05:43534455..43538843	700
	AcYTH5	Aqcoe1G060600.1	Chr_01:3035623..3040007	700
	AcYTH6	Aqcoe7G241300.1	Chr_07:22181287..22188010	647
	AcYTH7	Aqcoe2G257600.1	Chr_02:31570646..31576886	717
	AcYTH8	Aqcoe1G183700.1	Chr_01:10710466..10718706	668
Pentapetalae				
<i>Mimulus guttatus</i>	MgYTH1	Migut.N00839.1	scaffold_14:5225248..5227492	461
	MgYTH2	Migut.B00769.1	scaffold_2:4519952..4524021	539
	MgYTH3	Migut.D02575.1	scaffold_4:21078853..21083207	665
	MgYTH4	Migut.N00538.1	scaffold_14:2665296..2669474	698
	MgYTH5	Migut.J00855.1	scaffold_10:5147050..5153048	610
	MgYTH6	Migut.O00389.1	scaffold_94:1245..5691	714
	MgYTH7	Migut.G00015.1	scaffold_7:94508..98689	714
	MgYTH8	Migut.A00893.1	scaffold_1:5460439..5468103	391
	MgYTH9	Migut.M01886.1	scaffold_13:20623743..20629715	662
	MgYTH10	Migut.H01337.1	scaffold_8:15527249..15532258	681
<i>Solanum lycopersicum</i>	SIYTH1	Solyc01g103540.2	SL2.40ch01:83887917..83893923	555
	SIYTH2	Solyc01g028860.2	SL2.40ch01:33625778..33629920	706
	SIYTH3	Solyc05g032850.2	SL2.40ch05:45405216..45415463	604
	SIYTH4	Solyc12g099090.1	SL2.40ch12:64706410..64710192	690
	SIYTH5	Solyc08g007760.2	SL2.40ch08:2275280..2278977	299
	SIYTH6	Solyc08g007730.2	SL2.50ch08:2230870..2237873	395
	SIYTH7	Solyc08g007750.2	SL2.40ch08:2251358..2271383	369
	SIYTH8	Solyc02g070240.2	SL2.40ch02:34567760..34582177	671
	SIYTH9	Solyc02g021760.2	SL2.40ch02:14319129..14335346	689
<i>Solanum tuberosum</i>	StYTH1	PGSC0003DMG400024891	chr01:86293895..86301458	570
	StYTH2	PGSC0003DMG400024008	chr01:30337389..30341163	736
	StYTH3	PGSC0003DMG400004607	chr12:66496287..66504929	699
	StYTH4	PGSC0003DMG400012521	chr08:687962..693195	232
Rosid				
<i>Vitis vinifera</i>	VvYTH1	GSVIVT01010494001	chr1:21046883..21052994	572
	VvYTH2	GSVIVT01019895001	chr2:4307378..4323174	306
	VvYTH3	GSVIVT01036696001	chr19:23780412..23784906	591
	VvYTH4	GSVIVT01025494001	chr6:326465..331217	675
	VvYTH5	GSVIVT01033395001	chr8:20978678..20984054	694
	VvYTH6	GSVIVT01029460001	chr9:22603668..22613667	666
	VvYTH7	GSVIVT01021617001	chr10:8166088..8208339	485
	VvYTH8	GSVIVT01032236001	chr11:13546524..13554835	615
	VvYTH9	GSVIVT01023248001	chr12:20595791..20611182	708
	VvYTH10	GSVIVT01017649001	chr5:2377860..2383123	570
<i>Eucalyptus grandis</i>	EgYTH1	Eucgr.B03591.1	Chr02:55683004..55687386	764
	EgYTH2	Eucgr.B03590.1	Chr02:55676022..55680499	690
	EgYTH3	Eucgr.J03195.1	Chr10:37634110..37640689	652
	EgYTH4	Eucgr.H04096.1	Chr08:55550252..55555746	701
	EgYTH5	Eucgr.D01949.1	Chr04:33050717..33056935	397

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Supplemental Table 2. Continued.

Family and species	Gene symbol	Protein no.	Gene location	Protein length (amino acids)
Rutaceae	EgYTH6	Eucgr.E02496.1	Chr05:32701248..32710600	723
<i>Citrus sinensis</i>	CitYTH1	orange1.lg008255m	7,160,400..7,170,600	701
	CitYTH2	orange1.lg048663m	6,196,000..6,210,300	572
	CitYTH3	orange1.lg038459m	2,895,200..2,908,800	442
	CitYTH4	orange1.lg005441m	1,636,500..1,647,700	711
	CitYTH5	orange1.lg010141m	29,401,300..29,414,400	517
	CitYTH6	orange1.lg005934m	22,614,100..22,629,900	669
	CitYTH7	orange1.lg005158m	352,700..364,000	696
	CitYTH8	orange1.lg006798m	7,023,100..7,040,400	630
	CitYTH9	orange1.lg015749m	6,833,100..6,847,700	401
	CitYTH10	orange1.lg005338m	106,600..118,100	472
<i>Citrus clementina</i>	CcYTH1	Ciclev10000713m	scaffold_5:5309895..5315706	572
	CcYTH2	Ciclev10004597m	scaffold_9:31193246..31199360	594
	CcYTH3	Ciclev10010381m	scaffold_1:26181273..26186058	604
	CcYTH4	Ciclev10014439m	scaffold_2:188504..193028	711
	CcYTH5	Ciclev10004458m	scaffold_9:378361..382628	696
	CcYTH6	Ciclev10027973m	scaffold_8:24954823..24961235	658
	CcYTH7	Ciclev10019348m	scaffold_3:41345629..41350936	611
	CcYTH8	Ciclev10030951m	scaffold_4:21192983..21199939	630
	CcYTH9	Ciclev10020457m	scaffold_3:6546647..6552193	401
	CcYTH10	Ciclev10014454m	scaffold_2:31489811..31497594	701
Brassicales-Malvales				
<i>Theobroma cacao</i>	TcYTH1	Thecc1EG006167t1	scaffold_2:1059280..1066411	573
	TcYTH2	Thecc1EG039865t1	scaffold_9:24125408..24130041	742
	TcYTH3	Thecc1EG026461t1	scaffold_5:38374403..38378754	622
	TcYTH4	Thecc1EG027659t6	scaffold_6:5912196..5918919	652
	TcYTH5	Thecc1EG021457t1	scaffold_4:33165146..33169610	659
	TcYTH6	Thecc1EG008783t1	scaffold_2:16988579..16996466	634
	TcYTH7	Thecc1EG039931t1	scaffold_9:24941549..24946182	352
	TcYTH8	Thecc1EG016131t1	scaffold_3:31434495..31439651	373
	TcYTH9	Thecc1EG006095t1	scaffold_2:768314..774162	698
<i>Gossypium raimondii</i>	GrYTH1	Gorai.005G265300.1	Chr05:63865504..63871029	572
	GrYTH2	Gorai.004G020600.1	Chr04:1530886..1534693	670
	GrYTH3	Gorai.007G288900.1	Chr07:49501978..49505697	605
	GrYTH4	Gorai.004G244600.1	Chr04:58179613..58182566	599
	GrYTH5	Gorai.009G120700.1	Chr09:8935247..8942392	654
	GrYTH6	Gorai.011G001700.1	Chr11:161861..168102	648
	GrYTH7	Gorai.007G289200.1	Chr07:49545172..49551745	622
	GrYTH8	Gorai.013G123200.1	Chr13:31722245..31726598	606
	GrYTH9	Gorai.005G070500.1	Chr05:7641980..7646032	654
	GrYTH10	Gorai.002G031800.1	Chr02:2370851..2374924	654
	GrYTH11	Gorai.002G182900.1	Chr02:48153196..48160671	625
	GrYTH12	Gorai.004G205800.1	Chr04:53583839..53588598	386
	GrYTH13	Gorai.008G046800.1	Chr08:6332699..6338419	701
Brassicaceae				
<i>Brassica rapa</i>	BrYTH1	Brara.F00638.1	A06:3655226..3658829	502
	BrYTH2	Brara.G02587.1	A07:21382343..21384514	421
	BrYTH3	Brara.A03796.1	A01:30159742..30162154	406
	BrYTH4	Brara.G03650.1	A07:27015669..27018099	443
	BrYTH5	Brara.E03479.1	A05:27549589..27551472	397
	BrYTH6	Brara.B02362.1	A02:15455762..15458405	448
	BrYTH7	Brara.B02570.1	A02:18182697..18184972	447
	BrYTH8	Brara.G02088.1	A07:18521873..18524730	494
	BrYTH9	Brara.E02771.1	A05:23983684..23987129	644
	BrYTH10	Brara.C03469.1	A03:17568871..17572245	639
	BrYTH11	Brara.A03258.1	A01:27170859..27174022	655

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Supplemental Table 2. Continued.

Family and species	Gene symbol	Protein no.	Gene location	Protein length (amino acids)
<i>Arabidopsis thaliana</i>	BrYTH12	Brara.B01161.1	A02:5813195..5816193	529
	BrYTH13	Brara.E02805.1	A05:24181561..24185011	618
	BrYTH14	Brara.A03292.1	A01:27341438..27344946	614
	BrYTH15	Brara.C01152.1	A03:5352371..5355084	510
	BrYTH16	Brara.F00450.1	A06:2692275..2695502	610
	BrYTH17	Brara.H02025.1	A08:18408896..18412209	556
	BrYTH18	Brara.B02704.1	A02:19829691..19832449	355
	BrYTH19	Brara.H01885.1	A08:17669528..17674194	630
	BrYTH20	Brara.G00734.1	A07:9342196..9346526	547
	AtYTH1	AT1G09810	Chr1:3180936..3184224	470
	AtYTH2	AT1G27960	Chr1:9742188..9745787	539
	AtYTH3	AT3G13460	Chr1:10770813..10775402	631
	AtYTH4	AT1G48110	Chr1:17769938..17773493	639
	AtYTH5	AT1G55500	Chr1:20719548..20722739	599
	AtYTH6	AT1G79270	Chr1:29815506..29819104	528
	AtYTH7	AT3G03950	Chr3:1021260..1024009	428
	AtYTH8	AT3G13060	Chr3:4180573..4183975	634
	AtYTH9	AT1G30460	Chr3:4384714..4388525	667
	AtYTH10	AT3G17330	Chr3:5916940..5920188	595
	AtYTH11	AT4G11970	Chr4:7180437..7183677	444
Fabidae	AtYTH12	AT5G58190	Chr5:23546373..23549549	528
	AtYTH13	AT5G61020	Chr5:24557201..24559878	459
<i>Phaseolus vulgaris</i>	PvYTH1	Phvul.006G218800.1	Chr06:31791191..31796424	557
	PvYTH2	Phvul.004G132700.1	Chr04:40999492..41000430	231
	PvYTH3	Phvul.001G110200.1	Chr01:29981937..29986345	552
	PvYTH4	Phvul.002G247000.1	Chr02:41345444..41349656	677
	PvYTH5	Phvul.004G080300.1	Chr04:13760325..13764853	638
	PvYTH6	Phvul.010G165400.1	Chr10:43198372..43205017	634
	PvYTH7	Phvul.006G121600.1	Chr06:23734449..23739716	649
	PvYTH8	Phvul.003G119300.1	Chr03:29746822..29751712	698
	PvYTH9	Phvul.005G045600.1	Chr05:4866485..4871433	575
	PvYTH10	Phvul.002G152600.1	Chr02:29349475..29353426	379
	PvYTH11	Phvul.006G130200.1	Chr06:24478265..24492524	697
<i>Cucumis sativus</i>	CsYTH1	CuCita.135220.1	scaffold01044:81083..85863	675
	CsYTH2	CuCita.142200.1	scaffold01079:493556..498316	704
	CsYTH3	CuCita.356240.1	scaffold03577:957570..966762	547
	CsYTH4	CuCita.237700.1	scaffold02023:475332..481160	707
	CsYTH5	CuCita.167130.1	scaffold01154:506377..512678	650
<i>Ricinus communis</i>	RcYTH1	29765.m000746	29765:265502..269351	582
	RcYTH2	29955.m001158	29955:221274..224628	575
	RcYTH3	29890.m000285	29890:12818..19075	559
	RcYTH4	30169.m006347	30169:699445..703160	636
	RcYTH5	29889.m003364	29889:682318..685943	706
	RcYTH6	29929.m004643	29929:865918..870999	595
	RcYTH7	29950.m001140	29950:129336..133634	667
	RcYTH8	30190.m011088	30190:1744873..1748176	358
	RcYTH9	30075.m001190	30075:495510..505766	702