Characterization and analysis of some chilling-response WRKY transcription factors in tomato

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Abstract

WRKY transcription factors play various important roles in biotic and abiotic stress. In present study, a total of 81 WRKYs in tomato (*Solanum lycopersicum*) was identified and their gene structure, phylogeny and sub-location were analyzed. Here, we further analyzed their expression and potential roles under chilling stress. Nevertheless, the predicted chloroplast-located WRKYs are failed to be detected in the chloroplast. Then, 27 *SlWRKYs* with high chilling-induced mRNA levels (more than 3 fold to the control) are selected from these *WRKYs*. Promoter analysis showed that some cold stress-related cis-acting elements (CBFs binding site) existed in many promoter regions of these chilling response *WRKYs* (*WRKY2*, *WRKY50*, *WRKY59* etc.), implying that these WRKY transcription factors may participate in *CBFs* mediated pathway under chilling stress. The interaction proteins of WRKYs are essential to affect the DNA binding and transcription regulatory activities of WRKYs, thus controlling its downstream genes expression. Therefore, we predicted and analyzed the protein-protein interactions of those chilling related WRKY transcription factors and then speculated the complex regulatory and functional network of WRKY transcription factors under chilling stress. A better understanding of SlWRKYs would be helpful for providing a theoretical basis for further illustrating the regulatory mechanism of SlWRKYs under chilling stress.

Background

Chilling stress is a major environmental factor that limits the agricultural productivity and geographical distribution of many plant species (Sanghera et al., 2011). Tomato (*Solanum lycopersicum*), as a typical warm-season vegetable crops, is very sensitive to chilling stress (0–12 °C) which is common during their growing season. The chilling stress will rigidify the cell membrane, destabilize protein complexes, and impair photosynthesis of tomato (Martin et al., 1981; Ruelland et al., 2009). Under chilling stress, the complex mechanisms have been evolved to improve plant chilling tolerance including multiple signal transduction, transcription factor regulation and protein-protein interactions (Hannah *et al*., 2015; Chinnusamy et al., 2007; Shi et al., 2018).

As one of the largest transcriptional factors (TFs) families in plants, WRKY TFs with a conserved WRKYGQK motif and a novel zinc-finger-like motif contain more than 70 memebers in *Arabidopsis thaliana* and *S. lycopersicum* (Wu et al., 2005; Madhunita *et al*., 2014; Chen et al., 2015). Based on its number of WRKY domain and structure of zinc-finger motifs, WRKY proteins are initially classified into three groups (Group I-III) (Eulgem et al., 2000). Following analyses have shown that Group II WRKY proteins are further divided into five subgroups (IIa, IIb, IIc, IId, and IIe) based on the primary amino acid sequence (Rushton et al., 2010; Madhunita *et al*., 2014). The green algae *Chlamydomonas reinhardtii*, non-photosynthetic slime mold *Dictyostelium discoideum*, and unicellular protest *Giardi alamblia* all contain a single Group I *WRKY* gene, suggesting that Group I WRKY proteins with two WRKY domains are the ancestors to the other groups of WRKY proteins and the WRKYs origin is before the emergence of photosynthetic eukaryotes (Zhang and Wang, 2005). In *Arabidopsis*, AtWRKY40, a Group IIa member, can be recruited from nucleus to cytosol to interact with a chloroplast envelope and the cytosolic C-terminus

spanned magnesium-protoporphyrin IX chelatase H subunit (ABAR), thus playing a negative role in response to ABA signaling (Shang et al., 2010). This suggests that WRKY family may evolve to be involved in maintaining the connection between the chloroplast and nucleus during the long evolutionary process. However, the roles of WRKYs in chloroplasts are unclear.

WRKY transcription factors function as important components in the regulation of transcriptional reprogramming during plant stress responses (Madhunita *et al*., 2014). Extensive studies show that many WRKYs are responsive to pathogens or pathogen elicitors. Studies using overexpression lines or mutants of *WRKYs* have shown that WRKYs can positively or negatively regulate the expressions of hormone-related or pathogen defence genes (Dong et al., 2003; Lai et al., 2008, 2011). In addition, WRKYs are involved not only in biotic stress responses but also in abiotic stress responses and adaptations. In *Arabidopsis*, AtWRKY25, AtWRKY26, and AtWRKY33 enhance the tolerance to heat stress through regulating the expression of heat-induced genes, such as *AtHSPs* (Li et al., 2011). Overexpression of *GsWRKY20* reduces the stomatal density and water loss efficiency, thus improving plant drought tolerance (Luo et al., 2013). While GhWRKY68 reduces the resistance to salt and drought stress (Jia et al., 2015).

Transcription factors are significant for cold signaling and tolerance by modulating the expression of related functional genes (Jiang *et al*., 2020). WRKY family members are also essential to function in cold response. CsWRKY46 of cucumber, OsWRKY71 of rice, and VbWRKY32 of *Verbena bonariensis* all play roles in improving the cold tolerance (Kim et al., 2016; Ying *et al*., 2016; Wang et al., 2020). Besides, in mature pollen of *Arabidopsis*, AtWRKY34 negatively regulated its cold sensitivity through regulating the expression of *CBFs* (C-repeat binding factors) (Zou et al., 2010). However, the specific molecular mechanism of how *WRKYs* respond to cold signals and regulate the expression of downstream genes is still very limited.

Tomato is one of the most sensitive crops to chilling stress. Although the function of many individual WRKY genes have been analyzed in tomato (Table 1, most of them function in responses to biotic stress), the chilling responsive pathway mediated by WRKYs remain unclear. Here, we identified 27 chilling-response *WRKY* genes by qRT-PCR and RNA-seq assay. Then, the prediction analysis of their functional interaction networks, phosphorylation sites and cis-acting element suggested their potential roles under chilling stress.

Table 1
The function analysis of some individual WRKY genes in tomato.

Gene	Locus	Function	Refs
SlWRKY78	Solyc12g014610	Plant immunity	Cui et al., 2019
SlWRKY2	Solyc01g079260	Plant growth and development	Singh et al., 2020
SlWRKY15	Solyc02g093050	Plant immunity, response to drought and salt stress	Gao et al., 2019
SlWRKY14	Solyc02g088340	Plant immunity	Chinnapandi et al., 2019
SlWRKY58	Solyc07g066220	Plant immunity	Hong et al., 2018
SlWRKY63	Solyc08g067360	Plant growth and development	Chinnapandi et al., 2017
SpWRKY14	Solyc02g088340	Plant immunity	Cui et al., 2017
SlWRKY9	Solyc02g032950	Fruit ripening	Wang et al., 2017
SlWRKY54	Solyc07g051840		
SlWRKY5	Solyc01g095100		
SlWRKY74	Solyc10g011910		
SlWRKY49	Solyc06g066370		
SlWRKY68	Solyc09g014990		
SlWRKY60	Solyc08g008280		
SlWRKY66	Solyc08g082110		
SlWRKY69	Solyc09g015770	Response to salt stress	Hichri et al., 2017
SlWRKY78	Solyc12g014610	Plant immunity	Li et al., 2015
SlWRKY49	Solyc06g066370	Plant immunity	Liu et al., 2014
SlWRKY57	Solyc07go65260	Response to salt and drought stress	Li et al., 2012
SlWRKY21	Solyc03g095770	Plant immunity	Atamian et al., 2012
SlWRKY72	SGNU150340 SGNU151380	Plant immunity	Bhattarai et al., 2010
SlWRKY68	Solyc09g014990	Plant immunity	Hofmann et al., 2008
SlWRKY49	Solyc06g066370	Plant immunity, response to heat stress	Zhou et al., 2015
SlWRKY68	Solyc09g014990		

Materials And Methods

Data collection of SlWRKYs

The DNA, protein and promoter sequences of 81 *SlWRKYs* were obtained from PlantTFDB (http://planttfdb.cbi.pku.edu.cn/) and Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html), respectively.

Gene structure analysis, phylogenetic tree and motif composition of SlWRKYs

The gene structures of *SlWRKYs* were determined by comparing their genomic sequences with predicted coding sequences using the Gene Structure Display Server (http://gsds.cbi.pku.edu.cn/). The conserved motifs analysis of SlWRKY proteins were done by MEME (http://meme-suite.org/tools/meme). In addition, the phylogenetic tree of SlWRKYs was constructed using MEGA7 (Kumar *et al*., 2016).

The sub-location, post-translational modification and protein interaction analysis of SlWRKYs

The sub-locations of all SlWRKYs were predicted by LOCALIZER (http://localizer.csiro.au/) and TargetP 1.1 Server (http://www.cbs.dtu.dk/services/TargetP-1.1/index.php). To confirm the subcellular localization of SlWRKY14, SlWRKY33, SlWRKY49, SlWRKY68, SlWRKY78, the full-length sequence of them without the termination codon were subcloned into a 35S-driven pPZP211-green fluorescent protein (GFP) vector using *BamH*I and *Sal*I, *Kpn*I and *Sal*I, *BamH*I and *Sal*I, *Kpn*I and *Sal*I, *Kpn*I and *Sal*I, respectively. The constructed SlWRKYs-GFP vector were then transformed into Agrobacterium *GV3101* cells by the freeze-thaw method. The tobacco transient expression method and the observation of fluorescence were carried out following the methods described by Zhuang *et al*. (2020). The post-translational modification of 27 chilling response SlWRKY proteins was conducted by P3DB (http://www.p3db.org/). The construction of 27 chilling response SlWRKYs interactions network was based on STRING (https://string-db.org/cgi/network.pl).

Plant growth conditions, chilling treatment and qRT-PCR analysis

The tomato plants (*Solanum lycopersicum*, Micro-TOM) were used in this work. Sterilized and incubated method of seeds and the culture conditions of young seedlings were described as Zhuang et al., (2019). Seedlings with five fully developed leaves were used for the chilling treatment (4 °C) in the illuminated incubation chamber (E-41L2, Percival) with a PFD of approximately 400 μmol m^{-2} s^{-1} and relative humidity of 60–70% for 12 h. The functional leaves of the treated plants were harvested and frozen in liquid nitrogen. The methods of total RNA extraction, reverse transcription, and qRT-PCR were all performed as described by Zhuang et al., (2019). This assay was performed with three biological replicates. All primers used in this study are listed in Supporting Information Table S3.

Results

The phylogenetic analysis, protein and gene structure analysis of WRKY family members of tomato

The DNA and protein sequences of 81 SlWRKYs were obtained from PlantTFDB (http://planttfdb.cbi.pku.edu.cn/). The 81 transcripts were named from *SlWRKY1* to *SlWRKY81* based on their order on the tomato chromosomes. Based on these, phylogenetic tree, conserved protein domains and gene structure analysis were shown in Fig. 1. This analysis showed that SlWRKYs with two WRKY domains (motif1 and motif5) were clustered together except WRKY32 and WRKY8 whose motif5 is not very conservative (Fig. 1). WRKY proteins with two WRKY domains are the ancestors to the WRKY proteins with one WRKY domain (Eulgem et al., 2000; Zhang and Wang, 2005). SlWRKY32 and SlWRKY8 may have specific roles in terms of evolution. In addtion, according to gene structure analysis of the *SlWRKYs*, the SlWRKY members with motif 4 and motif 10 have at least two introns besides *SlWRKY46* and *SlWRKY10*, but most of the other members have only two introns (Fig. 1).

The subcellular localization analysis of SlWRKYs

The subcellular localization of each SlWRKY was predicted by LOCALIZER and TargetP 1.1 Server. Previous studies showed that most WRKY family members were targeted to nucleus and cytoplasm (Ruelland et al., 2009; Bakshi *et al.*, 2014). Chloroplast is one of the most sensitive sites to chilling stress in plant cells. Here, we want to identify whether there is some chloroplast-located SlWRKYs. Five SlWRKYs (SlWRKY14, SlWRKY33, SlWRKY49, SlWRKY68, SlWRKY78) were predicted to target in the chloroplast (Fig. 2Table). However, the transient expression assay in *Nicotiana benthamiana* confirmed that SlWRKY14, SlWRKY33, SlWRKY68, SlWRKY78 were all located to the nucleus (Fig. 2A) and we failed to detect the subcellular localization of SlWRKY49.

The up-regulation SlWRKYs **under chilling treatment**

In tomato, SlWRKYs were found to play roles in heat, drought, salt and biotic stress (Table 1). While the specific functions of them under chilling stress were unclear. 27 up-regulation *SlWRKYs* (*SlWRKY1*, *SlWRKY2*, *SlWRKY6*, *SlWRKY8*, *SlWRKY11*, *SlWRKY15*, *SlWRKY21*, *SlWRKY23*, *SlWRKY24*, *SlWRKY27*, *SlWRKY29*, *SlWRKY35*, *SlWRKY43*, *SlWRKY45*, *SlWRKY49*, *SlWRKY50*, *SlWRKY56*, *SlWRKY59*, *SlWRKY60*, *SlWRKY61*, *SlWRKY62*, *SlWRKY63*, *SlWRKY64*, *SlWRKY69*, *SlWRKY74*, *SlWRKY79*, *SlWRKY80*) were selected by RNA-seq analysis (Zhuang et al., 2019) and qRT-PCR under 0 h, 1.5 h, 3 h, 6 h, 9 h and 12 h chilling treatment. All these genes were up-regulated over 3-fold at least in one time site under chilling stress. And the greatest expression increase (nearly 186.54 fold compared with the expression of 0 h) was found at 6 h of *SlWRKY43* under chilling treatment (Fig. 2). Moreover, the expression of most of the chilling response *SlWRKY* genes were peaked at 9 h under chilling stress except *SlWRKY59* and *SlWRKY60* at 3 h, *SlWRKY43* at 6 h and *SlWRKY49* at 12 h (Fig. 2). The results suggest that these chilling response *SlWRKY* genes may participate in different pathway under chilling stress.

Analysis of putative relationship between these chilling response SlWRKYs and CBFs

In *Arabidopsis*, C-repeat/DREB binding factors (CBFs) as key transcription factors that function in cold stress (Shi et al., 2018). CBF proteins recognize the CRT/DRE cis-acting element (for example, CCGAC). In addition, WRKY gene have CRT/DRE cis-acting element. Thus, we speculate WRKY gene function as cold response by CBF combining with CRT/DRE cis-acting element of WRKY gene. Most of the promoters of 27 *SlWRKYs* contain these CBFs binding elements, while *SlWRKY6*, *SlWRKY11*, *SlWRKY15*, *SlWRKY27*, *SlWRKY29*, *SlWRKY56*, *SlWRKY60*, *SlWRKY63*, *SlWRKY74* do not contain those elements (Table. 2). By contrast, we further analyzed whether the promoter regions of *SlCBFs* contained WRKY bind element (TTGACC/T, Ciolkowski *et al*., 2008). The analysis showed that all the three *SlCBFs* promoter regions process the WRKY binding region suggesting that some SlWRKYs, especially these WRKYs without the CBFs binding elements as described above, were the pontensial upstream regulator of *SlCBFs* (data was not shown).

Interaction network of these chilling response SlWRKY proteins

Exploring the interaction proteins of chilling response SlWRKYs is important to understand their regulatory function mechanism under chilling stress. Therefore, we constructed an SlWRKY proteins interaction network based on the data of *Arabidopsis* homologous proteins using STRING 11.0 to systematically analyze the interaction proteins of these chilling response SlWRKYs (Fig. 4). Chi *et al*., (2013) summarized that WRKY-WRKY, WRKY-VQ and WRKY-MAPK interactions were the most common interaction relationship of WRKY. Their corresponding genes in *Arabidopsis* were shown in Table. S1. As shown in Fig. 4, SlWRKY21 seems a central factor to interact with a large number of other chilling response WRKYs including SlWRKY6, SlWRKY24, SlWRKY43, SlWRKY49, SlWRKY60 and SlWRKY61, which may play the most important roles under chilling stress. Besides, MPK3, MPK2 and MPKA1 may function as a key complex to interact with a large number of chilling response SlWRKYs directly (SlWRKY21, SlWRKY24, SlWRKY49, SlWRKY50, SlWRKY64, SlWRKY74 and SlWRKY79) and then phosphorylated them to affect their transcription regulatory activities (Fig. 4). The post-translational modification analysis showed that all chilling response WRKYs had phosphorylation sites (Table. S3). As expected, these WRKYs, which interact with MPKs as description above, process at least 8 phosphorylation sites, suggesting their interaction relationship (Table. S3). VQ proteins were found to specifically interact with the WRKY domain of WRKY proteins (Cheng et al., 2012). In this analysis, VQ proteins are also predicted to interact with chilling response SlWRKYs (SlWRKY11, SlWRKY24, SlWRKY49, SlWRKY56, SlWRKY68 and SlWRKY74) (Fig. 4). In addition, many other chilling related transcription factors (like ICE1, ERF, AP2, JAZ1 etc.) were found to interact with chilling response SlWRKYs, which was independent with the interaction as described above (Fig. 4). The interaction network of these chilling response SlWRKYs provides new research ideas for exploring the new chilling related mechanism of tomato in the future.

Conclusion

Almost all studied WRKY proteins can bind to the core W-box promoter elements of their downstream genes in the nucleus to participate in plant growth, development, and responses to biotic and abiotic stress, respectively (Bakshi *et al.*, 2014). As a nucleus and cytosol dual-located WRKY member, AtWRKY40 could be from nucleus to cytosol to interact with a chloroplast envelope located cytosolic C-terminus spanned magnesium-protoporphyrin IX chelatase H subunit (ABAR) (Shang et al., 2010). This suggests that WRKY family may be involved in maintaining the connection between the chloroplast, cytosol and nucleus. However, the affection of WRKYs to the chloroplast is not directly. In tomato, SlWRKYs were predicted to target to the nucleus, cytosol, chloroplasts and mitochondria (Chen et al., 2015). Our result also showed five candidate chloroplast-located SlWRKYs (Fig. 2). Nevertheless, four of them were confirmed to target to the nucleus (Fig. 2). Only the sub-location of SlWRKY49 remains unclear.

CBF, also known as dehydration responsive element (DRE) binding factor (DREB) proteins, are key factors in plant cold response (Liu et al., 1998; Shi et al., 2018). In rice, *WRKY* genes expressed highly under cold stress and may be involved in ICE-CBF-COR pathway (Guo et al., 2019). In this study, we mainly identified 27 chilling response *SlWRKY* genes based on RNA-seq and qRT-PCR analysis (Fig. 3). Promoter analysis of *SlWRKYs* and *SlCBFs* showed most *SlWRKYs* may have close regulatory relationship with *SlCBFs* mediated chilling response pathway. The promoter regions of 18 chilling response *SlWRKYs* (*SlWRKY1*, *SlWRKY2*, *SlWRKY8*, *SlWRKY21*, *SlWRKY23*, *SlWRKY24*, *SlWRKY35*, *SlWRKY43*, *SlWRKY45*, *SlWRKY49*, *SlWRKY50*, *SlWRKY59*, *SlWRKY61*, *SlWRKY62*, *SlWRKY64*, *SlWRKY69*, *SlWRKY79* and *SlWRKY80*) all contained the CBFs binding element, suggesting that they may be the directly downstream signaling factors under chilling stress (Table 2). The SlWRKY6, SlWRKY11, SlWRKY15, SlWRKY27, SlWRKY29, SlWRKY56, SlWRKY60, SlWRKY63, SlWRKY74, which were left behind, may act as upstream regulatory factors of *SlCBFs* due to the W-box elements in *SlCBFs* promoters. Meanwhile, we can not fully rule out that these chilling response SlWRKYs function in a CBFs independent pathway under chilling stress.

Table 2
The chilling related cis-acting element prediction in the promoter region of the 27 *SlWRKYs*

Chilling related cis-acting element		**Gene names of *WRKYs***
Core sequence	**Cis-acting element**	
RCCGAC	**DRECRTCOREAT**	**WRKY2, WRKY21, WRKY24, WRKY35, WRKY43, WRKY50, WRKY59, WRKY69**
GTCGAC	**CRTDREHVCBF2**	**WRKY2, WRKY23, WRKY43, WRKY50, WRKY59, WRKY62**
CCGAC	**LTRECOREATCOR15**	**WRKY1, WRKY2, WRKY8, WRKY21, WRKY23, WRKY24, WRKY35, WRKY43, WRKY49, WRKY50, WRKY59, WRKY61, WRKY69, WRKY79**
CCGAAA	**LTRE-1**	**WRKY1, WRKY2, WRKY8, WRKY21, WRKY23, WRKY24, WRKY35, WRKY43, WRKY49, WRKY50, WRKY59, WRKY61, WRKY69, WRKY79**

The interaction proteins of WRKYs are essential to affect the DNA binding and transcription regulatory activities of WRKYs, thus controlling its downstream genes expression. WRKY-WRKY, WRKY-VQ and WRKY-MAPK interactions are the most common interaction relationship of WRKY (Chi *et al*., 2013). In *Arabidopsis*, MPK3, MPK4, and MPK6 could be activated by biotic and abiotic stresses and their functional analyses indicate their critical roles in plant disease resistance and stress tolerance (Pitzschke et al., 2009). AtWRKY33 acts as a downstream component of MPK3/MPK6 cascade in regulation of the pathogen-induced defense responses (Mao et al., 2011). In our analysis, these chilling response SlWRKYs were also predicted to interact with some kinase (MPK3, MPK2 and MPKA1), which may function as a key complex in whole SlWRKYs interaction network under chilling stress in tomato (Fig. 4). In addition, SlWRKY21 seems a central factor due to its interaction with the most chilling response WRKYs. As its homologous gene in *Arabidopsis*, AtWRKY70 can play a role in plant immunity and response to salicylic acid (SA), reactive oxygen species (ROS) and jasmonic acid (JA) which are also induced by chilling stress (Chen et al., 2017; Lortzing et al., 2018; Zhou et al., 2018; Chae et al., 2020). These suggest that SlWRKY21 may play an essential and complex role in chilling response pathway. Otherwise, SlWRKY23 was predicted to interact with a critical cold-responce factor ICE1 (Induced of CBF expression1, Lee *et al*., 2005) which showed its potential function under chilling stress.

Although chilling response WRKYs were studied in several species, the mechanism of how WRKYs respond to chilling stress and the specific regulatory mechanism to their downstream genes are remain poorly understood. In this study, we identified 27 obvious chilling-induced *SlWRKYs* and analyzed their potential roles and interaction network under chilling stress, which provides an important reference for future studies on their biological functions under chilling stress in tomato.

Abbreviations

WRKYs, WRKY transcription factors; TFs, transcriptional factors; CBFs, C-repeat binding factors; qRT-PCR, quantitative real-time polymerase chain reaction; SA, salicylic acid; ROS, reactive oxygen species; JA, jasmonic acid; ICE1, induced of CBF expression1

Declarations

Availability of data and material:

The data that support the findings of this study are available from the corresponding author upon request.

Competing interests:

There is no competing interests about this research and all authors agree to publish.

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

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Author contributions:

W. M., Y. X. W., C. M. and K. Y. Z. conceived and designed this experiment. K. Y. Z. and C. M. carried out the experiments and analyzed the data. Y. X. W. helped to analyze the data and make the figures. K. Y. Z. and C. M. wrote the manuscript. Q. W. M. and Y. X. W. helped to revise the manuscript. All authors read and approved the manuscript.

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Figures

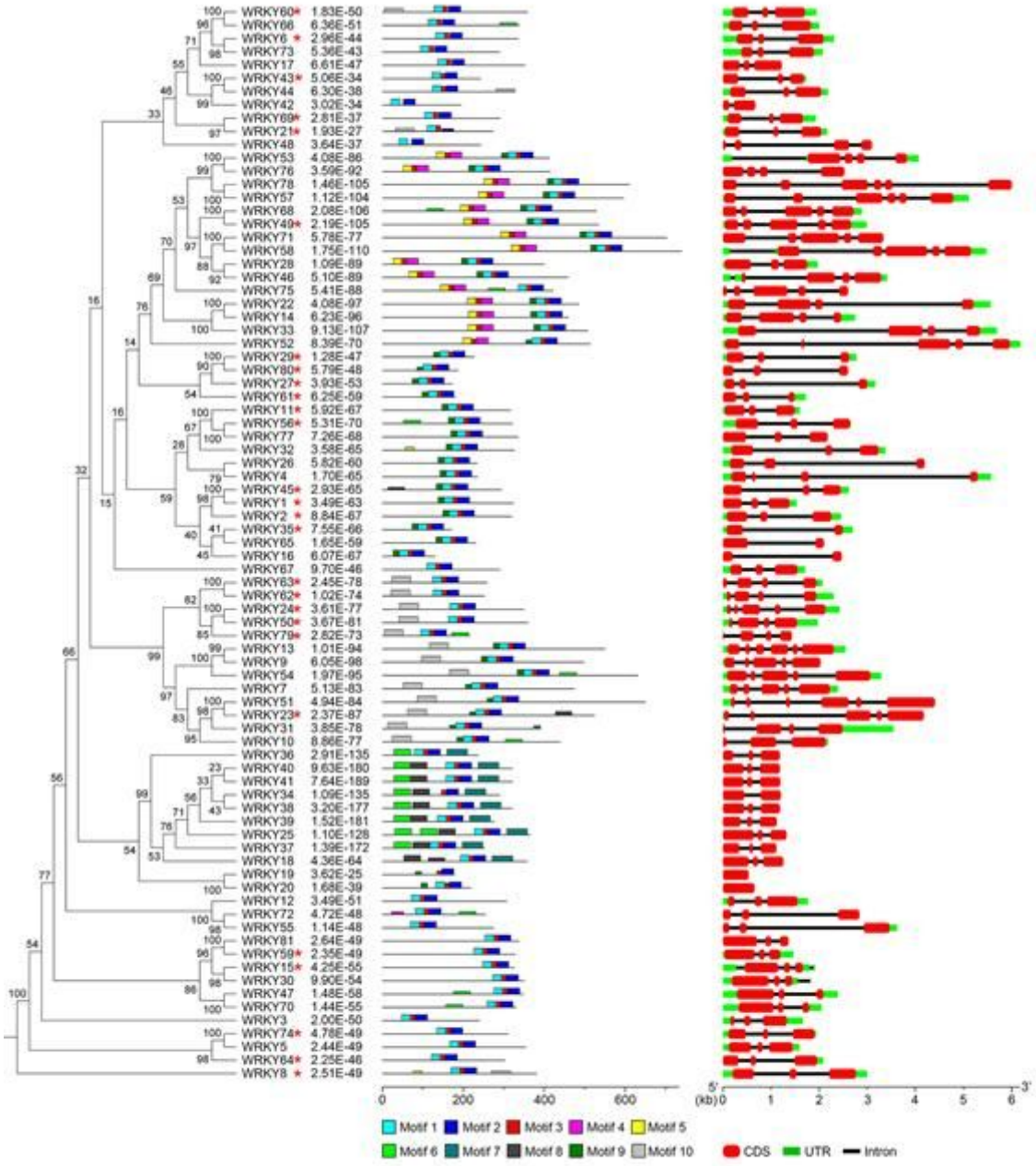

Figure 1

The phylogenetic analysis, protein and gene structure analysis of SlWRKY family members The phylogenetic analysis shown on the left and the red * indicated the 27 up-regulation SlWRKYs under chilling treatment. The motif composition analysis shown on the middle. WRKY domain was in the motif1 and motif5. Gene structure of SlWRKYs was shown on the right part.

Table. The prediction and analysis of WRKY subcellular localization.

Gene ID	Gene name	Subcellular location	
		LOCALIZER	TargetP 1.1 Server
Solyc01g079360	WRKY3	nucleus	mitochondrion
Solyc02g088340	WRKY14	nucleus	chloroplast
Solyc05g012770	WRKY33	nucleus	chloroplast
Solyc06g066370	WRKY49	chloroplast and nucleus	-（Any other location）
Solyc09g014990	WRKY68	nucleus	chloroplast
Solyc12g014610	WRKY78	nucleus	chloroplast

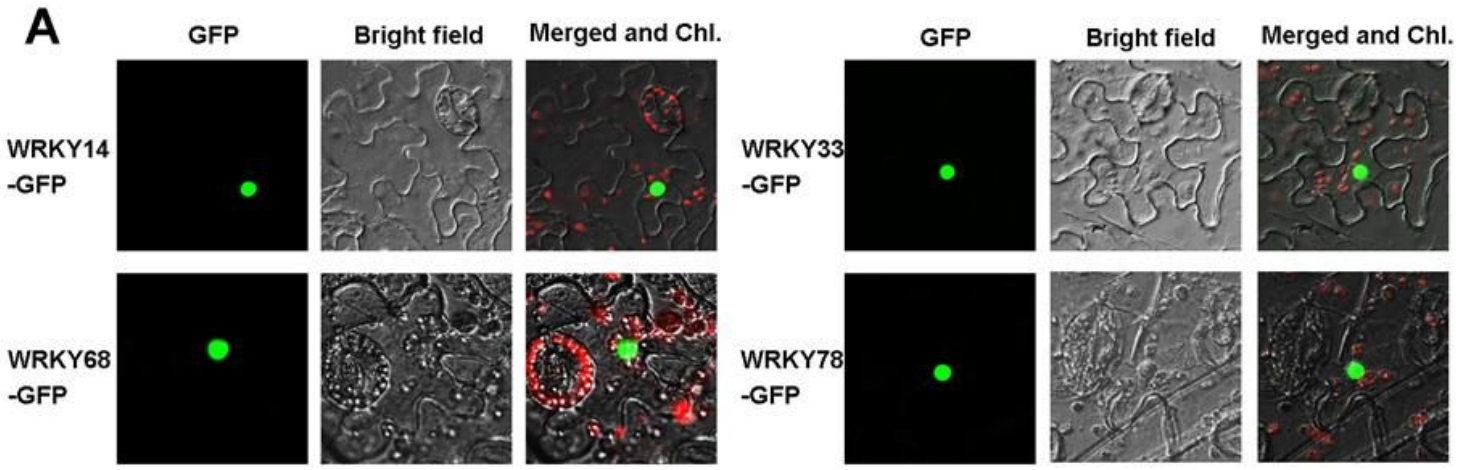

Figure 2

The sub-localization prediction and analysis of SlWRKYs The upper part showed the prediction results of some chloroplast-located SlWRKYs. The bottom showed the nuclear location of SlWRKY14, SlWRKY33, SlWRKY68, SlWRKY78 in Nicotiana benthamiana. Green fluorescence was produced by 35S drived SlWRKY14-GFP, SlWRKY33-GFP, SlWRKY68-GFP, SlWRKY78-GFP.

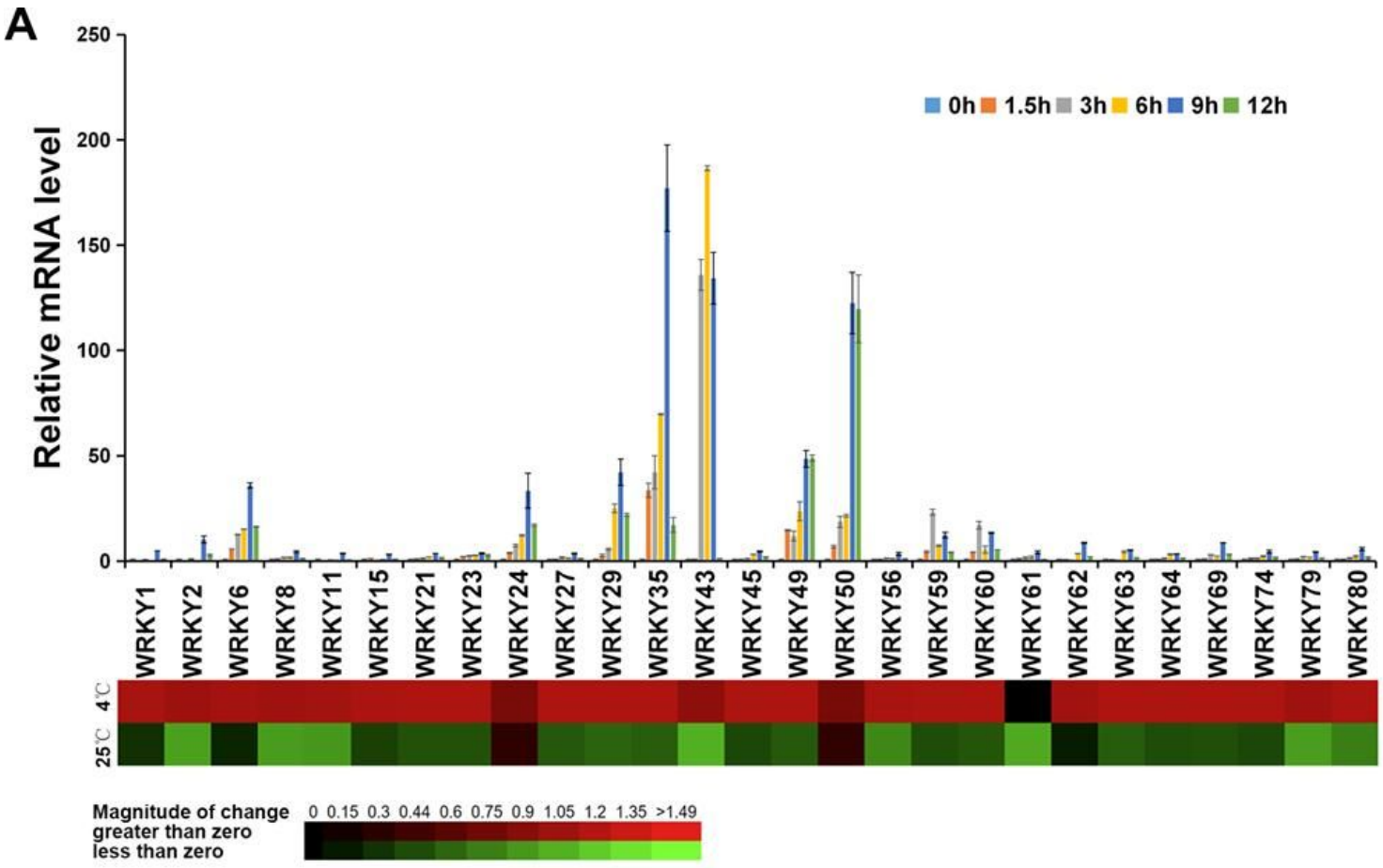

Figure 3

The expression of some SlWRKYs under chilling treatment in tomato The upper part showed the detection of qRT-PCR. All these expression was normalized to ACTIN. Error bars represent the SD of three independent replicates. The bottom part showed the corresponding RNA-seq data in Zhuang et al., 2019.

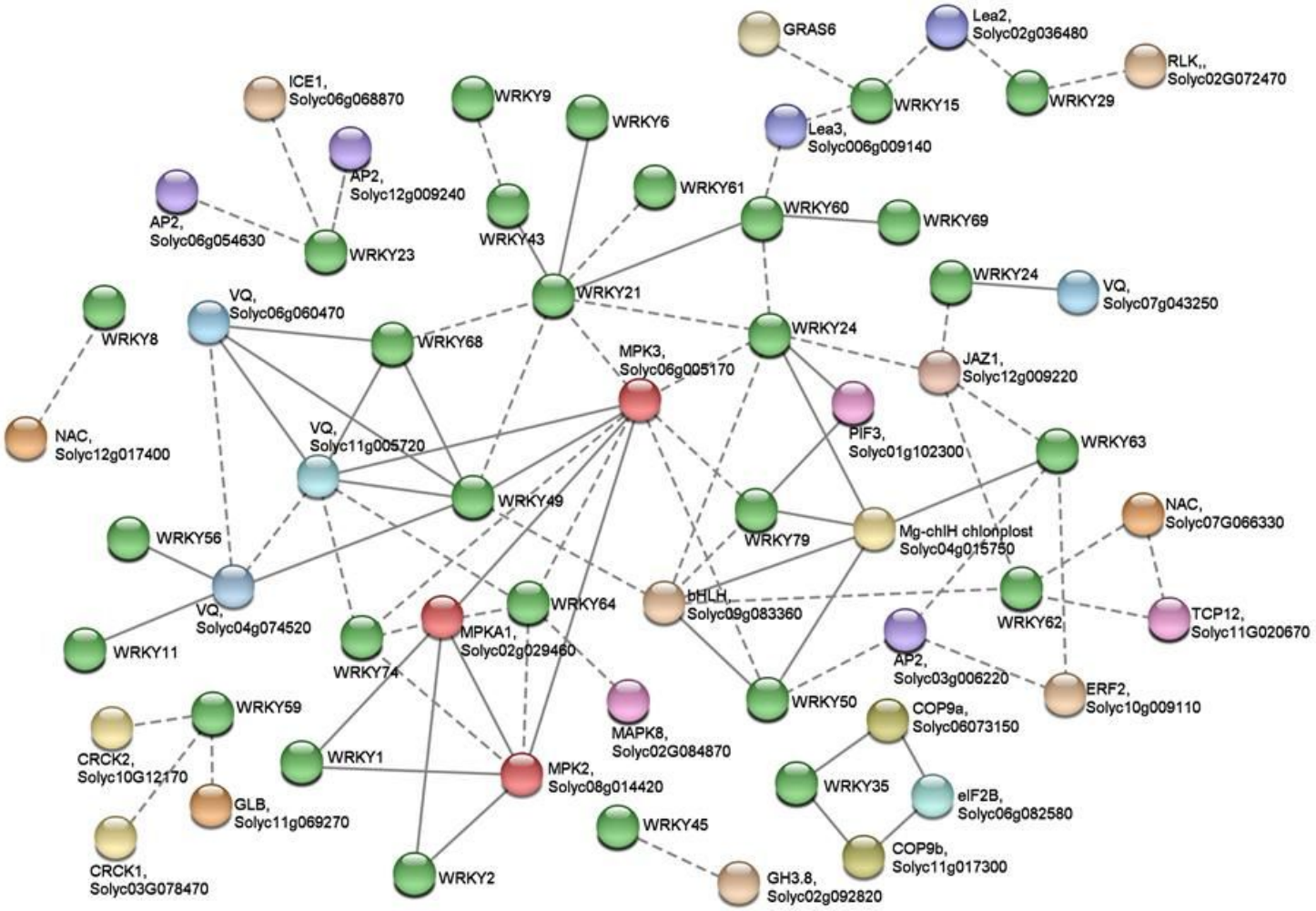

Figure 4

The interaction network of 27 chilling response SlWRKY proteins The interaction networks of SlWRKY proteins in tomato. Solid line represent confirmed interaction of their corresponding homology proteins in Arabidopsis, dashed line represent unproven protein-protein associations. The green ball indicated SlWRKYs.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table.S3.doc
- Table.S2.docx
- Table.S1.doc