Cloning and characterization of WRKY gene homologs in Chieh-qua (*Benincasa hispida* Cogn. var. Chieh-qua How) and their expression in response to fusaric acid treatment

Yizhou Mao^{1,2,3} · Biao Jiang^{1,2} · Qingwu Peng^{1,2} · Wenrui Liu^{1,2} · Yue Lin^{1,2} · Dasen Xie^{1,2} · Xiaoming He^{1,2} · Shaoshan Li³

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Abstract The WRKY transcription factors play an important role in plant resistance for biotic and abiotic stresses. In the present study, we cloned 10 WRKY gene homologs (*CqWRKY*) in Chieh-qua (*Benincasa hispida* Cogn. var. *Chieh-qua*) using the rapid-amplification of cDNA ends (RACE) or homology-based cloning methods. We characterized the structure of these *CqWRKY* genes. Phylogenetic analysis of these sequences with cucumber homologs suggested possible structural conservation of these genes among cucurbit crops. We examined the expression levels of these genes in response to fusaric acid (FA) treatment between resistant and susceptible Chieh-qua lines with quantitative real-time PCR. All genes could be upregulated upon FA treatment, but four *CqWRKY* genes exhibited differential expression between resistant and susceptible lines before and after FA application. *CqWRKY31* seemed to be a positive regulator while *CqWRKY1*, *CqWRKY23* and *CqWRKY53* were negative

regulators of fusaric resistance. This is the first report of characterization of WRKY family genes in Chieh-qua. The results may also be useful in breeding Chieh-qua for Fusarium wilt resistance.

Keywords Chieh-qua · *Benincasa hispida* · WRKY transcription factor · Fusarium resistance · Gene expression

Introduction

Chieh-qua, *Benincasa hispida* Cogn. var. *Chieh-qua* How., is an important vegetable crop in the Cucurbitaceae family, which is widely cultivated all over South China and Southeast Asian countries (He et al. 2007). It is a subspecies of the wax gourd [*Benincasa hispida* (Thunb.) Cogn.]. Chieh-qua has a vigorous annual vine and often grown with a trellis support (Cantwell et al. 1996). Its immature fruit is consumed that can be baked, fried, boiled or pickled. Fusarium wilt (FW herein after), caused by *Fusarium oxysporum* Sch., is one of the most serious diseases in Chieh-qua production (He et al. 2007). Fusaric acid (5-butylpyridine-2-carboxylic acid, FA) is the major toxin which is produced by *F. oxysporum* pathogen. Several studies have shown that FA plays a critical role in boosting FW symptom development in plants (Löffler and Mouris 1992; Liu et al. 2011). After *F. oxysporum* infection, FA was present in the whole banana plant and there was a positive correlation between its concentration and the incidence rate of disease symptoms (Dong et al. 2012). Similar correlation was also found in Chieh-qua, and FA treatment can be used as an effective method in screening of FW resistance (He et al. 2009). Breeding of resistant varieties is an effective and environmental friendly approach to solve the problem of Fusarium wilt in Chieh-

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Yizhou Mao and Biao Jiang contributed equally to this work.

✉ Xiaoming He
xiaominghe626@163.com

✉ Shaoshan Li
lishsh@scnu.edu.cn

- ¹ Vegetable Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
- ² Guangdong Key Lab for New Technology Research of Vegetables, Guangzhou 510640, China
- ³ Key Laboratory of Ecology and Environmental Science in Guangdong Higher Education, School of Life Science, South China Normal University, Guangzhou 510631, China

qua whereas identifying resistance-related genes presents an important significance in breeding of resistant variety.

The WRKY protein family is named after the characteristic amino acid sequence WRKYGQK (Rushton et al. 2010). According to the quantity of WRKY domains and the type of zinc-finger motifs in the sequence, WRKY proteins can be classified into three major groups and five subgroups (Eulgem et al. 2000). Through combining its conserved WRKY domain to the cognate *cis*-acting elements, W box, WRKY protein performs its functions specifically (Eulgem et al. 2000). W box contains an invariable TGAC bases core and whichever base replaced will decline the DNA-binding activities (Dong et al. 2003). It is well known that WRKY transcription factors act on a variety of plant biochemical reactions with a rapid and instant feature, such as growth and senescence, metabolism, biotic and abiotic stresses (Zhang et al. 2008; Miao et al. 2004; Suttipanta et al. 2011; Wang et al. 2013; Li et al. 2013). In *Arabidopsis thaliana*, for instance, *WRKY53* is implicated in leaf senescence process (Miao et al. 2004), while *WRKY25*, *WRKY26* and *WRKY33* are coordinately in response to heat stress (Li et al. 2011). Sun and Yu (2015) found that *AtWRKY53* controls stomata open and close by regulating H_2O_2 contents and starch degradation.

There are many studies on the resistant functions of WRKY transcription factors in response to pathogen infection. For example, Lai et al. (2008) found that the *Arabidopsis AtWRKY3* and *AtWRKY4* played a positive role in resistance to necrotrophic pathogens, and *AtWRKY4* played a negative role in resistance to biotrophic pathogens. In rice, *OsWRKY71* overexpression plant exhibited enhanced resistance to *Xanthomonas oryzae* pv. *oryzae* (Xoo), a virulent bacterial pathogen (Liu et al. 2007). The *Arabidopsis* transgenic mutant overexpressing *CIWRKY70* (cloned from *Citrullus lanatus*) showed a significantly decreased impaired area upon *Botrytis cinerea* or *Erwinia carotovora* (Cho et al. 2012).

In the present study, we cloned 10 Chieh-qua WRKY sequences using RACE (rapid-amplification of cDNA ends) and homology-based cloning. We characterized these *CqWRKY* sequences and investigated their expression in response to FA treatment in FW resistant and susceptible Chieh-qua lines. Four *CqWRKY* genes were found to be potentially associated with FW resistance.

Materials and methods

Materials

The Fusarium-resistant A39FA and susceptible H5 Chieh-qua (*Benincasa hispida* Cogn. var. Chieh-qua How) inbred lines used in the research were provided by the

Vegetable Research Institute, Guangdong Academy of Agricultural Sciences, China. Chieh-qua seedlings were growing in 1:1 mixture of sterile soil and vermiculite at 25 ± 2 °C and 60% relative humidity under a 16-h light/8-h dark cycle (Xie et al. 2006). Twenty-day seedlings were used for fusaric acid (FA) treatment. The fusaric acid was purchased from Tokyo Chemical Industry, Japan.

Cloning of full-length cDNA of CqWRKY gene homologs

Total RNA was isolated from A39FA leaves with the TransZol™ Plant reagent (TRANSGEN, China) following the manufacturer's instructions. The first cDNA strand was synthesized from 1 µg total RNA using PrimeScript™ RT kit followed with treatment of gDNA Eraser (TaKaRa, Japan). Primer pairs (Table 1) used for amplification of Chieh-qua WRKY gene homologs (*CqWRKY*) were designed with Oligo v7.37 based on sequences of the wax gourd transcriptome developed in our lab (Jiang et al. 2013). Homolog-based cloning of the *CqWRKY* genes followed Dubé et al. (2008).

Bioinformatics analysis of CqWRKY sequences

The ORF (open reading frame) sequences of these cloned *CqWRKY* genes were searched with the ORF Finder (<https://www.ncbi.nlm.nih.gov/orffinder/>). *Arabidopsis* WRKY sequences were downloaded from TAIR (<http://www.arabidopsis.org/>). Cucumber WRKY sequences were obtained from cucumber genome database (<http://www.cucumber.genomics.org.cn/>). Multiple sequence alignment of cucumber and Chieh-qua WRKY sequences was performed with ClustalW v2.1 (Larkin et al. 2007) and GeneDoc (Nicholas et al. 1995). A phylogenetic tree was constructed with MEGA5.0 software using the Neighbor-Joining method with 1000 bootstrap replicates (Tamura et al. 2011).

The motifs presented in 10 *CqWRKY* proteins were identified with MEME v4.11.2 (<http://meme-suite.org/tools/meme/>) with the following parameters: number of repetitions: any; maximum number of motifs: 50; optimum width of each motif: 6–300 residues (Huang et al. 2012).

Fusaric acid treatments

Twenty-day-old resistant (A39FA) and susceptible (H5) Chieh-qua seedlings with two fully expanded leaves were treated with FA solution (200 mg/L) using the root-soaking method with sterile water as the blank control (Zhang et al. 2014). Briefly, the roots of seedlings were cut into ~5 cm length and submerged in the FA solution. The treated plants were maintained in the culturing room at 25 ± 2 °C.

Table 1 Primers used in polymerase chain reactions (PCR) in this study

Primers	Sequence (5′–3′)	Length (bp)
CqWRKY-1 F	ATGGCCTCCTCTTCCGGG	18
CqWRKY-1 R	CTAACATAGGAGAGATTGGATGAACA	26
CqWRKY-21 F	TAAATCCCTTCTGGGTCGTCCTC	23
CqWRKY-21 R	TATCTTCCACCTCCTAAAATCCTTC	25
CqWRKY-23 F	AGCCTTCATTCTGTAAGTCTTTC	24
CqWRKY-23 R	CTATTCTATTCTACTCATCATCCCC	25
CqWRKY-31 F	CCTACTTCCATGTTTGTGTCTC	23
CqWRKY-31 R	GTTGCTTAGTCTAATGTTGATGC	23
CqWRKY-34 F	TATTTTCATTTACATCCAAACTCC	24
CqWRKY-34 R	TATGTCCCAGTGGAACAAATTT	25
CqWRKY-37 F	CCAGAAACGCCTGTTGATTAC	21
CqWRKY-37 R	ATGGCTTTTGAAGTGGGAATT	21
CqWRKY-45 F	AGAGTGGGCAGTGGTTGTATT	21
CqWRKY-45 R	CATTAGGGAGATTAAGATGAAGG	23
CqWRKY-50 F	ATTGAAAACCTTGAATGACC	21
CqWRKY-50 R	CTTCTCAAAGTTATCTTACATGCG	25
CqWRKY-53 F	AAAGGCCATTTCCATAGCCAACC	23
CqWRKY-53 R	CCAAAGTCATTACAAGAAAGTTACC	25
CqWRKY-54 F	CTTGTTTTACCCCAAGTCCAT	22
CqWRKY-54 R	TAAAAAGTCAATCCAAATCGAGTGT	25

Leaf samples were collected from each seedling at 0, 6, 12 and 24 h after FA treatment. There were three replications per treatment. All samples were flash frozen in liquid nitrogen and stored at -70°C until total RNA extraction.

CqWRKY gene expression analysis

Quantitative real-time PCR (qRT-PCR) was performed for selected *CqWRKY* genes in both resistant and susceptible Chieh-qua lines before and after FA treatments. The qRT-PCR primers are provided in Table 2, which were designed with Oligo v7.37. The qRT-PCR reactions were performed on the CFX96TM Real-Time PCR Detection System (Bio-Rad, Germany). Each 10 μL mixture contained 5 μL of SYBR[®] Premix Ex TaqTM II (Tli RNaseH Plus) (TaKaRa, Japan), 0.25 μL each of forward and reverse primer (10 μM), 4 μL of cDNA template (50 ng/ μL) and 0.5 μL of sterile water. The PCR program included an initial denaturation (95 $^{\circ}\text{C}$ /30 s), then followed by 40 cycles of 95 $^{\circ}\text{C}$ /5 s, 60 $^{\circ}\text{C}$ /20 s, 72 $^{\circ}\text{C}$ /30 s. A melting curve was obtained from 65 to 95 $^{\circ}\text{C}$, increment of 0.5 $^{\circ}\text{C}$ for 5 s. There were three biological and technical replicates for each sample. The *actin* gene was used as the internal standard to normalize each sample (Zhang et al. 2014). Relative gene expression was calculated with the $2^{-\Delta\text{Ct}}$ method (Livak and Schmittgen 2001).

Data analysis

All statistical analyses were performed in R (v3.2.0). The standard deviation of fold changes of expression levels was calculated from three replicates; significance tests were performed with the Tukey's multiple-range test ($P < 0.05$). Origin Pro 8 (v8.0724) was used for visualization of relative transcription levels of *CqWRKY* genes.

Results

The WRKY genes of Chieh-qua

In total, we identified 10 *CqWRKY* sequences through rapid-amplification of cDNA ends (RACE) (*CqWRKY1*) or homology-based cloning method (9). Based on sequence homology with the cucumber WRKY genes, the 9 *CqWRKY* genes were designated as *CqWRKY21*, *CqWRKY23*, *CqWRKY31*, *CqWRKY34*, *CqWRKY37*, *CqWRKY45*, *CqWRKY50*, *CqWRKY53* and *CqWRKY54*, respectively. The full-length cDNA sequences varied from 612 to 1746 bp and the predicted proteins ranged from 204 to 582 amino acid residues. The domain features of these WRKY genes are provided in Table 3.

Table 2 Oligonucleotide sequences of primers used in qRT-PCR assays

Gene	Sequence (5'–3')	Length (bp)	Amplified fragment (bp)
CqWRKY-1 F qRT	ACTACGGCAATAATTACCCTC	21	197
CqWRKY-1 R qRT	AACAATCTCCGTGATCTGTCC	21	
CqWRKY-21 F qRT	GCTGCCTTTTCCTCTTGGTTG	21	186
CqWRKY-21 R qRT	CTTGTTCTCTGCACTTACCCTC	22	
CqWRKY-23 F qRT	TTCAGTCCTCTTCTTCCAA	19	179
CqWRKY-23 R qRT	CCAGTTATATCCATCGTTCTC	21	
CqWRKY-31 F qRT	TAGCCCTACCATGTCATTACAC	22	158
CqWRKY-31 R qRT	AGATGCCTTGTTTTTCGAGCTT	21	
CqWRKY-34 F qRT	CCTGCTTCTCCGATTGTCTCC	21	102
CqWRKY-34 R qRT	AACCTCGGTCAGTTTATCGTT	21	
CqWRKY-37 F qRT	ACGCCAAGAGACAGAATACTGAG	23	120
CqWRKY-37 R qRT	AGCCATCATCCAATAGATCGAC	22	
CqWRKY-45 F qRT	CCTCGCCTTTTCTAATTACC	20	105
CqWRKY-45 R qRT	ATCCCAAGTTATCGGAGCTAC	21	
CqWRKY-50 F qRT	GCCCTCTAAATGATGGTTATAG	22	183
CqWRKY-50 R qRT	TGTGTCTTCTCTGTATGTT	20	
CqWRKY-53 F qRT	ACATCCACTCCAAATCTATGGC	22	179
CqWRKY-53 R qRT	ACATCAACATCGTCATGGTC	21	
CqWRKY-54 F qRT	GACAGTGGTGGTAGTTCT	18	168
CqWRKY-54 R qRT	AGCCATCATCCATAATCTCT	20	
Actin F qRT	ACTCAACCCAAAGGCTAACAGA	22	155
Actin R qRT	GACTCACACCATCACCAGAATC	22	

Table 3 Structural features of cloned *CqWRKY* gene sequences

Gene	# of WRKY domains	Zinc-finger motifs	WRKY group	cDNA Length (bp)	Amino acid length (aa)
CqWRKY1	2	C ₂ H ₂	Group 1	1746	582
CqWRKY21	1	C ₂ H ₂	Group 2a	942	314
CqWRKY23	2	C ₂ H ₂	Group 1	1431	477
CqWRKY31	1	C ₂ HC	Group 3	1086	362
CqWRKY34	1	C ₂ HC	Group 3	876	292
CqWRKY37	2	C ₂ H ₂	Group 1	1440	480
CqWRKY45	1	C ₂ H ₂	Group 2d	882	294
CqWRKY50	1	C ₂ HC	Group 3	1005	335
CqWRKY53	1	C ₂ H ₂	Group 2c	729	243
CqWRKY54	1	C ₂ H ₂	Group 2c	612	204

C₂H₂ C–X_{4–5}–C–X_{22–23}–H–X₁–H, C₂HC C–X₇–C–X₂₃–H–X₁–C

Phylogenetic relationships of Chieh-qua and cucumber WRKY sequences

We examined the phylogenetic relationship of 10 *CqWRKY* and 57 cucumber *CsWRKY* genes, and the unrooted phylogenetic tree is presented in Fig. 1. All branching nodes were supported with high bootstrap values. From Fig. 1, it can be seen that all 10 *CqWRKYs* belonged to Groups I, IIa, IIc, IId and III, each of which contained 3, 1, 2, 1, 3 genes,

respectively. Group I members were closer to those in Group II, especially Group IIc, and Group IId seemed more distant to other groups and subgroups except for Group IIe.

CqWRKY motif structure analysis

WRKY proteins are characterized by the conserved polypeptide WRKYGQK (Eulgem et al. 2000), and variations of this heptapeptide have been rarely demonstrated

Fig. 1 An unrooted phylogenetic tree of the WRKY peptide sequences of Chieh-qua and cucumber. The *CqWRKY* and *CsWRKY* protein sequences were aligned with Clustal W and the neighbor-join tree was constructed with MEGA5. Blue diamonds WRKY proteins of Chieh-qua

among large number of WRKY proteins (Zhang and Wang 2005; Xie et al. 2005). We compared the WRKY domains in 10 *CqWRKY* proteins with those from Arabidopsis (*AtWRKY*) and cucumber (*CsWRKY*). The representative alignment is presented in Fig. 2. As shown in Fig. 2, sequences in these WRKY domains of different species were highly conserved. Most heptapeptide sequences in the *CqWRKY* genes contained were WRKYGQK, and only *CqWRKY54* had WRKYGKK. Compared with other groups and subgroups, the WRKY domains in Group III were less conserved.

We compared the amino acid motifs presented in *CqWRKY* and *AtWRKY* proteins, which is illustrated in Fig. 3. The number of location of motifs among these proteins was largely consistent. Except for *CqWRKY34*, motif 2 presented in the remaining 9 *CqWRKY* proteins. The *CqWRKYs* in Groups II d and III shared common conserved motifs with motifs 5 and 7 located downstream of the WRKY domain and motif 2 upstream of it. However, in Group 2a, motif 7 was located in before the WRKY domain. Motif 3 only appeared in Group 2a.

Expression of *CqWRKY* genes in response to FA treatment

We examined expression of *CqWRKY* genes in A39FA (FW resistant) and H5 (FW susceptible) before and after FA treatment with qPCR, and results are presented in Fig. 4. The expression of all 10 *CqWRKY* genes was strongly induced by FA application. The expression of *CqWRKY1*, *CqWRKY23*, *CqWRKY31* and *CqWRKY53* did not show significant differences between the resistant and susceptible lines before treatment ($P > 0.05$); all of them showed significant increase in expression after FA treatment. Among them, *CqWRKY31* had a higher expression in A39FA than H5, and the transcription level reached to the peak at 12 h after treatment. For *CqWRKY1*, *CqWRKY23* and *CqWRKY53*, the expression level was higher in H5 than in A39FA. Both *CqWRKY1* and *CqWRKY53* reached their peak expression at 12 h after treatment whereas that of *CqWRKY23* peaked at 6 h after FA application. Although *CqWRKY34* and *CqWRKY45* responded positively to FA stress, their expressions did not show

Group 1-N
 AtWRKY33: -----DGYNWRKYGQKQVKGSENPSSYYKCTFPNCPTKKKVERSLGQITEIVYKGSNNHPKQOSTRRSSSSSST
 CsWRKY2 : VNRRSDDGYNWRKYGQKQVKGSENPSSYYKCTFPNCPTKKKVERSLGQITEIVYKGSNNHPKQOSTRRSSLSAG
 CqWRKY1 : LNRRSDDGYNWRKYGQKQVKGSENPSSYYKCTFPNCPTKKKVERSLGQITEIVYKGSNNHPKQOSTRRSSLSAG
 CqWRKY23: -----DGYNWRKYGQKQVKGSENPSSYYKCTFPNCPTKKKVERSLGQITEIVYKGSNNHPKQOSTRRSG-----
 CqWRKY37: -----DGYNWRKYGQKQVKGSENPSSYYKCTFPNCPTKKKVERSLGQITEIVYKGSNNHPKQOSTRRSG-----

Group 1-C
 AtWRKY33: RIVVQTTSDIDILDDGYRWRKYGQKQVKGSENPSSYYKCTFPNCPTKKKVERSLGQITEIVYKGSNNHPKQOSTRRSSLSAG
 CsWRKY2 : RIVVQTTSDIDILDDGYRWRKYGQKQVKGSENPSSYYKCTFPNCPTKKKVERSLGQITEIVYKGSNNHPKQOSTRRSSLSAG
 CqWRKY1 : RIVVQTTSDIDILDDGYRWRKYGQKQVKGSENPSSYYKCTFPNCPTKKKVERSLGQITEIVYKGSNNHPKQOSTRRSSLSAG
 CqWRKY23: RIVVQTTSEIDILDDGYRWRKYGQKQVKGSENPSSYYKCTFPNCPTKKKVERSLGQITEIVYKGSNNHPKQOSTRRSSLSAG
 CqWRKY37: RIVVQTTSEIDILDDGYRWRKYGQKQVKGSENPSSYYKCTFPNCPTKKKVERSLGQITEIVYKGSNNHPKQOSTRRSSLSAG

Group 2a
 AtWRKY40: ASDTILVVKDGYQWRKYGQKQVTRDNPSPRAYFKCACAPSCSVKKKVQSVSDQSVLVATYEGEHNHMPSPQIDSN
 CsWRKY21: ASDSNLVVKDGYQWRKYGQKQVTRDNPSPRAYFKCACAPSCSVKKKVQSVSDQSVLVATYEGEHNHMPSPQIDSN
 CqWRKY21: ASDSNLVVKDGYQWRKYGQKQVTRDNPSPRAYFKCACAPSCSVKKKVQSVSDQSVLVATYEGEHNHMPSPQIDSN

Group 2c
 AtWRKY13: SEVDVLDDGYRWRKYGQKQVKNTOHPSYYRCTQDRCRVKKRVERLAEDPRMVITTYEGHHSNHLDDDSLST
 CsWRKY53: SEVDVLDDGYRWRKYGQKQVKNTOHPSYYRCTQDRCRVKKRVERLAEDPRMVITTYEGHHSNHLDDDSLST
 CqWRKY53: SEVDVLDDGYRWRKYGQKQVKNTOHPSYYRCTQDRCRVKKRVERLAEDPRMVITTYEGHHSNHLDDDSLST
 CqWRKY54: SEVDVLDDGYRWRKYGQKQVKNTOHPSYYRCTQDRCRVKKRVERLAEDPRMVITTYEGHHSNHLDDDSLST

Group 2d
 AtWRKY15: KMSDVPDDYSWRKYGQKQVKNTOHPSYYRCTQDRCRVKKRVERLAEDPRMVITTYEGHHSNHLDDDSLST
 CsWRKY45: KMSDVPDDYSWRKYGQKQVKNTOHPSYYRCTQDRCRVKKRVERLAEDPRMVITTYEGHHSNHLDDDSLST
 CqWRKY45: KMSDVPDDYSWRKYGQKQVKNTOHPSYYRCTQDRCRVKKRVERLAEDPRMVITTYEGHHSNHLDDDSLST

Group 3
 AtWRKY41: RGLGEPHDDIFSWRKYGQKQVKNTOHPSYYRCTQDRCRVKKRVERLAEDPRMVITTYEGHHSNHLDDDSLST
 CsWRKY31: RGLGEPHDDIFSWRKYGQKQVKNTOHPSYYRCTQDRCRVKKRVERLAEDPRMVITTYEGHHSNHLDDDSLST
 CqWRKY31: RGLGEPHDDIFSWRKYGQKQVKNTOHPSYYRCTQDRCRVKKRVERLAEDPRMVITTYEGHHSNHLDDDSLST
 CqWRKY34: VITRATAEDYAWRKYGQKQVKNTOHPSYYRCTQDRCRVKKRVERLAEDPRMVITTYEGHHSNHLDDDSLST
 CqWRKY50: VITRATAEDYAWRKYGQKQVKNTOHPSYYRCTQDRCRVKKRVERLAEDPRMVITTYEGHHSNHLDDDSLST

Fig. 2 Alignment of WRKY domain amino acid sequences among CqWRKY, selected AtWRKY and CsWRKY proteins. Alignment was performed with Clustal W and GeneDoc. The suffix 'N' and 'C' of Group 1 indicates the N-terminal and C-terminal WRKY domain,

respectively. The conserved residues are highlighted in black and gray boxes. The conserved WRKY amino acid signatures are underlined in red and the zinc-finger motifs are underlined in blue

Fig. 3 The amino acid motifs presented in CqWRKY and AtWRKY proteins characterized with the MEME 4112 Program. The grey solid line represents the corresponding WRKY protein and the red boxes

represent WRKY motifs. The various colored boxes represent different motifs and their location in each WRKY sequence

significant differences between these two cultivars. Besides, the expression levels of *CqWRKY21*, *CqWRKY37* and *CqWRKY54* did not show consistent change across

different time points between A39FA and H5. Lastly, *CqWRKY50* had significant differential expression between the two lines even before FA treatment.

Fig. 4 Differential expression patterns of *CqWRKY* genes in leaves under FA treatment. Y-axis is the relative gene expression level. The Chieh-qua *Actin* gene was used as an internal control for data

Discussion

A number of studies have shown that WRKY transcription factors participate in defense responses against various abiotic and biotic stresses (Eulgem et al. 2000; Dong et al. 2003; Xie et al. 2005; Wu et al. 2005; Eulgem and Somssich 2007; Yang et al. 2009; Rushton et al. 2010; Dang et al. 2013, 2014; Liu et al. 2014). For example, in *Arabidopsis*, *AtWRKY28* and *AtWRKY75* overexpression mutants exhibited increased resistance to oxalic acid and *Sclerotinia sclerotiorum* stresses (Chen et al. 2013). Kim et al. (2016) found that the expression of two cold-responsive genes, *OsTGFR* and *WSI76*, were elevated in rice *OsWRKY71*-overexpressing lines under cold stress suggesting *OsWRKY71* as a positive regulator in cold tolerance. In potato, *StWRKY1*-overexpressing lines showed an enhanced resistance to *Phytophthora infestans* and an

normalized. X-axis is the hours after FA treatment (0, 6, 12 and 24 h). F FA treatment, H H₂O control. Error bars are calculated according to three replicates

elevated tolerance to dehydration (Shahzad et al. 2016). In addition, broccoli *BoWRKY6*-overexpressing mutant exhibited a highly resistance to downy mildew (*Hyaloperonospora parasitica*) (Jiang et al. 2016).

There are also several studies about WRKY transcription factors in the Cucurbitaceae family. For example, in cucumber, Ling et al. (2011) identified 55 WRKY sequences in the cucumber genome and found that 23 of them displayed differential expression under at least one abiotic stress (drought, cold or salinity). Xu et al. (2015) investigated expression of these cucumber WRKY genes in response to inoculation of *Phytophthora melonis* and found that only six of them showed significant differential expression. Besides, the expression levels of *CsWRKY30* and *CsWRKY6* were elevated after infection with the powdery mildew pathogen (Alfandi et al. 2012). In watermelon, Yang et al. (2014) found that the expression of

CIWRKY1 was elevated after infection with *Fusarium oxysporum* f. sp. *niveum*.

In the present study, for the first time, we cloned 10 *CqWRKY* genes, and found that they shared high-degree homology with the cucumber homologs (Fig. 1). We show that some of these *CqWRKY* genes exhibited differential expression in FW-resistant and -susceptible lines before and after FA treatment (Fig. 4). A large number of WRKY genes have been found to be involved in plant resistances against pathogen infection (Zheng et al. 2006; Zhang et al. 2012). For example, transgenic *AtWRKY18 Arabidopsis* showed an outstanding resistance to the bacterial pathogen *Pseudomonas syringae* (Chen and Chen, 2002) while overexpressing *BnWRKY33* transgenic *Brassica napus* showed remarkable enhanced resistance to *Sclerotinia sclerotiorum* (Wang et al., 2014). In cotton, Guo et al. (2011) found that the transcription level of *GhWRKY3* dramatically increased when inoculated with *Fusarium oxysporum* f. sp. *vasinfectum*. Also in cotton, Yu et al. (2012) detected the accumulation of the *GhWRKY15* transcripts when treated with *Fusarium oxysporum* f. sp. *vasinfectum*. In this study, we found four *CqWRKY* genes with response to FA treatment and their expression was significantly different between resistant and susceptible lines (Fig. 4). *CqWRKY31* showed a higher level of expression in A39FA than H5, which may suggest that this gene might be a positive regulator of fusaric resistance. On the contrary, *CqWRKY1*, 23 and 53 were strongly induced by FA in the susceptible H5, implying that they might play a negative role for regulating resistant to fusaric wilt. In other studies, the expression patterns of WRKY family genes presented different trends under the same treatment. Ling et al. (2011) found that *CsWRKY2* was upregulated in response to cold and salt, while *CsWRKY28* was down-regulated in cold and *CsWRKY23* was down-regulated in salt in cucumber. Similarly, Yang et al. (2009) found that *BnWRKY20* and 32 were suppressed by *S. sclerotiorum* infection while other several *BnWRKYs* were highly induced after the inoculation in canola. These results showed that WRKY family genes responded to biotic and abiotic stresses in both positive and negative way. In summary, our results indicated that *CqWRKY31* was positively related, while *CqWRKY1*, 23 and 53 were negatively correlated to fusaric resistance. These results may have potential application in breeding of resistance to Fusarium wilt in Chieh-qua.

Conclusions

For the first time, we cloned 10 *CqWRKY* family genes in Chieh-qua including *CqWRKY1*, 21, 23, 31, 34, 37, 45, 50, 53 and 54. Four of them exhibited differential expression in

resistant and susceptible Chieh-qua varieties in response to FA treatment. *CqWRKY31* may be a positive regulator whereas *CqWRKY1*, *CqWRKY23* and *CqWRKY53* may be negative regulators of fusaric resistance. Results from this study may be useful in breeding Chieh-qua for Fusarium wilt resistance.

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflicts of interest to this work.

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