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ORIGINAL ARTICLE

Genome-wide Analysis of WRKY Transcription Factor Family in Melon (*Cucumis Melo* L.) and Their Response to Powdery Mildew

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Abstract

The WRKY family is a large group of transcription factors found in higher plants; it plays an important role in many aspects of biological processes. However, there is very little information about this family in melon (*Cucumis melo* L.). In our research, 57 candidate WRKY genes in the melon genome were identified. According to their structural and phylogenetic features, the 57 *CmWRKY* genes were classified into three groups, I, II, and III, and the group II was further divided into five subgroups. Group I included 11 members that all have two conservative WRKY domains and a C_2H_2-type zinc finger motif; Group II contains 41 WRKY gene family members, that all have a WRKY domain and a C_2H_2-type zinc finger motif. Five members that all have a conservative WRKY domain and a C_2HC-type zinc finger motif are classified as Group III. The expression of 16 selected melon *WRKY* genes was detected by quantitative real-time PCR after sprayed with salicylic acid (SA) or powdery mildew infection. qRT-PCR analysis showed that 16 *CmWRKY* genes exhibited distinct expression patterns upon powdery mildew infection, and the expression levels of nine genes were inhibited, and seven genes were induced. After being sprayed with SA, the expression levels of 11 genes were inhibited, and five genes were induced. The data here provide an important basis for further functional studies of the *WRKY* gene in melon resistance.

Keywords Bioinformatics analysis · Melon · Powdery mildew · WRKY transcription factor

Introduction

As an important cash crop of the Cucurbitaceae family, melon (*Cucumis melo* L.) is widely planted in China and worldwide. It is very popular due to its sweet taste and nutritional value (Wang et al. 2018). Since the reform and opening-up of China, the country has made great progress in the melon industry and become the world's largest melon producer and consumer (Lu and Hong 2014). Powdery mildew is a common disease of Cucurbitaceae vegetables worldwide, and its incidence can reach 90% in spring and autumn, and the yield can be reduced by 40% when the disease is serious, especially in melon and cucumber (Casulli et al. 2008). For melon, powdery mildew caused by the plant pathogen *Podosphaera xanthii* and a lesser degree of *Golovinomyces cichoracearum* limit the yield (Fukino et al. 2008). The WRKY transcription factor (TF) is a new type of transcription regulatory factor that has been found in plants in recent years. Studies have shown that the WRKY protein plays a key role in plant responses to pathogens (Song et al. 2014). In 1994, the first WRKY transcription factors, i.e., SPF1, were

Key Message
• The 57 *CmWRKY* genes were classified into three groups, I, II, and III, and the group II was further divided into five subgroups. qRT-PCR analysis showed that 16 *CmWRKY* genes exhibited distinct expression patterns in powdery mildew infection

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obtained from sweet potato (Ishiguro and Nakamura 1994). Since then, *WRKY* genes have been identified in various plant species and have been shown to be involved in many biological processes, including biotic and abiotic stress responses and plant pathogen interactions (Cheng et al. 2016). So far, the *WRKY* gene has been isolated from many plants. For example, the *Arabidopsis thaliana* WRKY family has 71 genes (Song and Gao 2014). A total of 171 *WRKY* genes were identified from wheat (Ning et al. 2017) and 54 *WRKY* genes were identified from pineapple (Xie et al. 2018). One hundred sixty-four putative WRKY proteins were identified in the tobacco genome (Xiang et al. 2016), whereas 82 genes were found in the potato WRKY family (Liu et al. 2017). In addition, over 70 WRKY proteins were identified in the peanut wild ancestor diploid genome (Song et al. 2016) and 85 *WRKY* genes were found in *Salix suchowensis* (Bi et al. 2016). Sixty-six *WRKY* genes were responsive to salt stress in soybean root (Yu et al. 2016). The number of *WRKY* genes that were detected in tomatoes (Huang et al. 2012), cotton (Ding et al. 2015), castor oil plants (Li et al. 2012) were 81, 109, and 47, respectively. *WRKY* genes were also detected in rice and lotus roots (Ramamoorthy et al. 2008; Song et al. 2014).

The *WRKY* gene is named for its encoded transcription regulator WRKY protein which contains at least a highly conserved domain of about 60 amino acids. At the N-terminal of the structure, almost all members have seven peptides, i.e., WRKYGQK, so it is abbreviated as WRKY (Liu et al. 2017; Robatzek and Somssich 2001). The WRKY protein contains one or two WRKY domains and zinc finger motifs at the C-terminal (Rushton et al. 2010). According to the classification standard of *Arabidopsis thaliana*, that is, the number of WRKY domains and the pattern of zinc finger motifs, members of the WRKY family can be divided into three groups. Group I contains two highly conserved "WRKYGQK" sequences and a C_2H_2-type zinc finger structure ($C–X_{4-5}–C_{22-23}–H–X–H$); Group II contains a highly conserved "WRKYGQK" sequence and a C_2H_2-type zinc finger structure ($C–X_{4-5}–C–X_{22-23}–H–X–H$); Group III contains a highly conserved "WRKYGQK" sequence and a C_2HC-type zinc finger structure ($C–X_7–C–X_{23}–H–X–C$) (Eulgem et al. 2000). WRKY proteins control the transcription of target genes by binding to the promoter region, which contains a DNA element called the W-box, the core sequence of which is TTGACY (Y is C or T) (Yamasaki et al. 2012; Xu et al. 2017).

While many reports have been published on the WRKY family, relatively few have investigated melon. The results of melon genome sequencing and gene annotation were completed and published in June 2012 (Garcia-Mas et al. 2012); however, the WRKY gene family is not well studied in melons. In this study, 57 melon WRKY gene family members were identified, and their chromosome location, conserved WRKY domain, gene structure, and expression response to powdery mildew infection and salicylic acid (SA) treatment were analyzed. The result provides a basis for future research on the biological function of the melon *WRKY* gene family.

Materials and Methods

Identification of the WRKY Proteins in Melon

The WRKY TF family sequences were downloaded from the Melon Genomics website (http://cucurbitgenomics.org/); 72 Arabidopsis WRKY TF family sequences were searched from the NCBI. The downloaded melon and Arabidopsis WRKY amino acid sequences were compared through the software BioEdit7.2. Then, the sequence of the WRKY TF family was selected after the alignment, and the repeat sequence and the portion without the WRKY domain of "WRKYGQK" were deleted. Finally, 57 WRKY sequences were obtained. The relevant information was located in the Plant Transcription Factor Database, such as the isoelectric point, amino acid length, CDS length, and molecular weight of the 57 genes.

Chromosome Location and Naming

The location of 57 genes in the chromosome were found on the melon genome website (http://cucurbitgenomics.org/). The chromosomes were located and mapped by using the software MapInspect. The 57 genes were distributed on 13 chromosomes separately and named according to their positions.

Conservative Motif Analysis of the Melon WRKY TF Family

According to Eulgem et al. (2000), 57 genes were identified and divided into 3 groups. The amino acid sequences of 57 genes were compared by MEGA6.0 software (Zhang et al. 2017). The conservative motif of the melon WRKY family protein was analyzed and identified by the online tool WebLogo (http://weblogo.berkeley.edu/logo.cgi).

Construction of the Phylogenetic Tree

The phylogenetic tree was constructed using the software MEGA6.0, and Neighbor-Joining (NJ) was used as the construction method (Yu et al. 2016). The phylogenetic tree contains 57 melon genes and 72 Arabidopsis genes.

Gene Structural Analysis of Melon WRKY Genes

The coding sequences and their corresponding genome sequences were compared through an online GSDS2.0 program to achieve the purpose of predicting the structure of the *CmWRKY* gene (http://gsds.cbi.pku.edu.cn) (Xiang et al. 2016).

RNA Extraction and Gene Expression Analysis

The experiment was carried out in the artificial climate chamber of Shandong Agricultural University. The melon variety used in this experiment is "Yangjiaomi," which is a common commercial species that is sensitive to powdery mildew. After the melon seeds were soaked and germinated by the conventional method, they were sown in a 50-hole seedling tray containing the seedling substrate (charcoal: vermiculite: perlite = 2:1:1, V:V). When the seedlings grew to three true leaves, the seedlings were sprayed with a spore suspension concentration of 10^6 spores/mL powdery mildew, a concentration of 75 μmol/L SA. Seedling was sprayed with sterile water, which served as the control. Each treatment was repeated three times with 50 melons per repetition. Samples were taken at 0, 3, 12, and 24 h after treatment. The samples were frozen immediately in liquid nitrogen and stored at − 80 °C for further analyses.

Total RNA was extracted from leaves using the TRIzol (Vazyme Biotech, Nanjing, China) method according to the manufacturer's instructions. Total RNA was reverse transcribed to cDNA using *EasyScript*® One-Step gDNA Removal and cDNA Synthesis SuperMIX (TransGen Biotech, Beijing, China). The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed on a LightCycler® 480 detection system (Roche, Indianapolis, IN, USA) using *TransStart*® Tip Green qPCR SuperMix (TransGen Biotech, Beijing, China) and primers. The total volume used for the reaction was 20 μL, of which the cDNA was 200 ng, both the forward primer and the reverse primer were 0.4 μL, and the 2× TransStart® Tip Green qPCR SuperMix was 10 μL; less than 20 μL part was replenished with double distilled water. The PCR cycle conditions were set as follows: pre-denaturation was carried out for 30 s at 94 °C, followed by 40 cycles of 94 °C for 5 s and 60 °C for 30 s, and finally, the dissociation stage was used to detect the fluorescence signal to verify whether the target product was single. The primers used in the qRT-PCR analyses are shown in Table 1. The housekeeping gene ACTIN was used as the internal control. Difference analysis was performed using SPSS20.0 software with three replicates. Values presented are the means ± SE of three replicates. Differences between treatments were determined by the least significant difference with $P < 0.05$.

Table 1 Primer sequences

Annotation ID	Primer sequences
CmWRKY1	F: TGGAGGAAGAGAAGAAGGAA R: GGAATAATCGCATCGGAG
CmWRKY11	F: CTTCTCCCAACTCTAAACAACG R: CTCATCATCCTCTTCCTTCC
CmWRKY15	F: ACCCAAGGTTATGAGAGTCC R: GACCGTATTTCCTCCATTG
CmWRKY20	F: GCACAACAACTACGAGCGT R: GAAAGAGGATAGGATTCTGGTG
CmWRKY21	F: GCACACACTTAGCCTACAAGC R: AAGCACCAACACCAATCC
CmWRKY25	F: CAGGGTGTGGTGTGAAGAAG R: GCATTATCGGACTTTGGTGAGT
CmWRKY26	F: CCTTTATGCTACGACCGTGT R: AACCCTTCACTCAACCCA
CmWRKY35	F: GCTCTCCATACCCAAGAAGC R: TCGGCTGTGTAGGTTACGA
CmWRKY40	F: AGGGTCTATGTCAAGTGCG R: TGGGTTTGGACTGTGAGTG
CmWRKY41	F: AAGAGAGTAGAAAGGTTAGCCG R: AGGTGGGAAGAGGGTAAGTG
CmWRKY42	F: GAGAGAGGAAATGGGTGTAATC R: TGGATGAGGGCTGTTCTT
CmWRKY49	F: CTCCAACGATTGTAACGCT R: CTGTCTCTCCCACCTTGATT
CmWRKY51	F: GTGAAGAAGAAGGTGGAAAGAG R: GGGTAGAGGTTGTAAAGGATGG
CmWRKY52	F: CTTACTCCGCTGCTCCTACT R: AGGCTTAGAGACAACAGACGA
CmWRKY54	F: TTCCTCGTAATCACACCACA R: CTGTTTCTCATCTCCCATTCTC
CmWRKY56	F: CAACTCCACCTTCAGATTCA R: TCTACTTGCTTCCTCGCTG
ACTIN	F: GGCAGTGGTGGTGAACATG R: TTCTGGTGATGGTGTGAGTC

Results

Genome-wide Exploration and Phylogenetic Analysis of the Melon WRKY Gene

After the search for WRKY by using a local search in the Cucurbitaceae website GuGenDB, 62 non-redundant protein sequences containing typical WRKY domains were obtained. It was found that five sequences have undergone variable shear under the analysis. For example, *CmWRKY19* is divided into three different protein sequences after variable shear; one sequence has only one WRKY domain, and the other two has two WRKY domains, which should belong to different groups, as shown in Table 2. However, variable shear belongs to the same gene, so only 57 genes were analyzed in the following analysis. Fifty-seven *WRKY* genes were found to be located on

Table 2 The WRKY transcription factor family in melon

Groups	Name	Annotation ID	Chromosome location	CDS length (bp)	Protein length (aa)	MW (Da)	PI
I	MELO3C012130	CmWRKY48	chr10	1491	497	54,354	8.5294
I	MELO3C014066	CmWRKY30	chr6	2307	769	83,454	6.6683
I	MELO3C025686	CmWRKY54	chr11	984	328	36,306	9.8613
I	MELO3C022014	CmWRKY45	chr9	1521	507	55,151	8.1559
I	MELO3C024209P1	CmWRKY3	chr1	1335	445	48,885	6.5673
I	MELO3C024209P2	CmWRKY3	chr1	1326	442	48,468	6.3517
I	MELO3C009302	CmWRKY18	chr4	1413	471	51,725	8.8616
I	MELO3C009127P1	CmWRKY19	chr4	1431	477	52,034	6.6706
I	MELO3C009127P2	CmWRKY19	chr4	1119	373	41,413	5.5716
I	MELO3C009127P3	CmWRKY19	chr4	858	286	31,315	4.8181
I	MELO3C020489	CmWRKY55	chr12	1734	578	63,423	8.1373
I	MELO3C010057	CmWRKY8	chr2	1482	534	58,729	6.094
I	MELO3C014134	CmWRKY29	chr6	1602	534	58,534	6.4629
I	MELO3C010223	CmWRKY9	chr2	1539	513	55,810	6.458
II a	MELO3C011295	CmWRKY15	chr3	957	319	35,054	9.1944
II a	MELO3C016966	CmWRKY31	chr7	978	326	36,475	6.7876
II a	MELO3C026740	CmWRKY17	chr4	942	314	34,437	9.062
II b	MELO3C007409	CmWRKY40	chr8	1869	623	67,214	7.2188
II b	MELO3C002202	CmWRKY57	chr12	1848	616	66,692	7.0361
II b	MELO3C019494	CmWRKY27	chr6	777	259	27,851	9.279
II b	MELO3C011296	CmWRKY14	chr3	291	97	10,777	9.2112
II b	MELO3C017157	CmWRKY11	chr2	1758	586	63,693	6.8196
II c	MELO3C007157	CmWRKY39	chr8	789	263	28,753	6.3681
II c	MELO3C014896	CmWRKY28	chr6	513	171	19,206	9.2883
II c	MELO3C006078	CmWRKY23	chr6	540	180	20,963	9.8163
II c	MELO3C006725	CmWRKY25	chr6	525	175	19,334	8.2472
II c	MELO3C007458	CmWRKY41	chr8	459	153	17,878	10.267
II c	MELO3C000030	CmWRKY1	chr0	978	326	36,739	8.8106
II c	MELO3C012635	CmWRKY5	chr1	894	298	33,511	8.263
II c	MELO3C016337	CmWRKY34	chr7	441	147	16,667	10.28
II c	MELO3C017743	CmWRKY36	chr7	336	112	13,159	10.281
II c	MELO3C019679	CmWRKY53	chr11	918	306	34,378	6.1848
II c	MELO3C005937	CmWRKY22	chr6	663	221	25,355	8.1638
II c	MELO3C008175	CmWRKY12	chr3	444	148	17,227	9.6685
II c	MELO3C015910	CmWRKY7	chr1	723	241	27,432	6.7141
II c	MELO3C003140	CmWRKY44	chr8	843	281	31,184	6.6241
II c	MELO3C020963	CmWRKY51	chr11	639	213	23,524	6.2708
II c	MELO3C020967	CmWRKY50	chr11	747	249	28,584	9.8975
II c	MELO3C011432	CmWRKY13	chr3	930	310	33,974	8.0546
II c	MELO3C007470	CmWRKY42	chr8	606	202	22,589	7.2168
II d	MELO3C017415	CmWRKY10	chr2	852	284	31,058	10.185
II d	MELO3C014305	CmWRKY21	chr5	1032	344	38,641	10.196
II d	MELO3C015776	CmWRKY6	chr1	732	244	27,098	9.7414
II d	MELO3C003622	CmWRKY16	chr4	1053	351	37,711	10.136
II d	MELO3C017912	CmWRKY37	chr7	867	289	32,681	4.3797
II d	MELO3C007576	CmWRKY43	chr8	888	296	31,995	10.192
II d	MELO3C024787P1	CmWRKY52	chr11	885	295	31,762	10.097
II d	MELO3C024787P2	CmWRKY52	chr11	882	294	31,675	10.097
II d	MELO3C016774P1	CmWRKY32	chr7	1173	391	42,334	10.098

Table 2 (continued)

Groups	Name	Annotation ID	Chromosome location	CDS length (bp)	Protein length (aa)	MW (Da)	PI
II d	MELO3C016774P2	CmWRKY32	chr7	1170	390	42,247	10.098
II e	MELO3C016338	CmWRKY35	chr7	816	272	30,447	6.942
II e	MELO3C005843	CmWRKY47	chr9	1044	348	38,018	5.7668
II e	MELO3C007048	CmWRKY38	chr8	1560	520	57,871	6.6502
II e	MELO3C006081	CmWRKY24	chr6	996	332	36,686	6.3471
II e	MELO3C002675	CmWRKY56	chr12	852	284	30,999	5.4004
II e	MELO3C002875	CmWRKY46	chr9	1221	407	44,010	5.5356
II e	MELO3C018717	CmWRKY2	chr1	894	298	31,758	5.2261
III	MELO3C026932	CmWRKY26	chr6	1140	380	41,843	4.7591
III	MELO3C024135	CmWRKY4	chr1	891	297	32,823	6.0309
III	MELO3C020166	CmWRKY49	chr10	879	293	32,408	5.4024
III	MELO3C009097	CmWRKY20	chr4	888	296	33,240	5.9916
III	MELO3C016281	CmWRKY33	chr7	984	328	36,572	4.834

chromosome 0–12 and were renamed from *CmWRKY1* to *CmWRKY57* according to their order on the chromosome (Fig. 1). There is only one variant (WRKYGKK) including a small amount of amino acid differences in these 57 sequences. The amino acid length of these 57 WRKY TFs ranges from 97 to 769, which means that they are structurally different and functionally diversified. Then, the 57 WRKY TFs were further analyzed. The name of the 57 WRKY TFs was searched on the Plant Transcription Factor Database website, and the MW of the 57 WRKY TFs ranged from 10,777 to 83,454 Da, and the PI ranged from 4.38 to 10.28 (Table 2).

The *WRKY* gene family has 72 members in *A. thaliana* (*AtWRKY*) (Song and Gao 2014), 100 members in *Oryza sativa* (*OsWRKY*) (Song and Gao 2014), and 61 members in *Cucumis sativus* (*CsWRKY*) (Chen et al. 2020). In this study, we identified 57 members in *Cucumis melo* L. (*CmWRKY*). Compared with Arabidopsis and rice, the melon WRKY family has a smaller scale which is equivalent to the cucumber WRKY family. We compared the number of *WRKY* genes in different groups of Arabidopsis, rice, cucumber, and melon (Table 3). As shown in Table 3, the main difference is that the number of *CmWRKY* genes (5) in Group III is much fewer than that of Arabidopsis (14) and rice (36). However, compared with cucumbers, the number of members in the three groups is basically the same, which may be the characteristic of Cucurbitaceae crops.

As shown in Figs. 1 and 2, the 57 amino acid sequences of all identified melon WRKY proteins were compared by using MEGA 6.0 software. A total of 57 WRKY proteins were found to have highly conserved sequences of WRKYGQK, one of which was mutated, i.e., WRKYGKK. A phylogenetic tree based on conservative WRKY domain was successfully established by using MEGA 6.0 software. To obtain a better classification within the different clades, the representative WRKY domains from *A. thaliana*, *Cucumis sativus* L., and *Cucumis melo* L. were included in our analysis (Fig. 3). According to the A. thaliana classification criteria, the 57 WRKY genes were divided into three groups: I, II, and III. As shown in Fig. 2, eleven members including two conservative WRKY domains and a C_2H_2-type zinc finger motif were classified as the first group. The third group includes five members with a conservative WRKY domain and a C_2HC-type zinc finger motif; The second group contains 41 WRKY gene family members with a WRKY domain and a C_2H_2-type zinc finger motif. According to the changes of conservative structural motifs, the members of the second group can be divided into five subgroups: II a, II b, II c, II d, and II e (Song and Gao 2014; Xie et al. 2018). Among them Groups II a, II b, II c, II d, and II e include 3, 5, 18, 8, and 7 WRKY family members, respectively.

Gene Structure of the Melon WRKY Gene Family

Zou et al. (2016) found that the transcription sequence is longer than the gene sequence and introduced the concept of exon-intron. The exon-intron of 57 *WRKY* genes was analyzed to further understand the evolution of the WRKY family in melon. As shown in Fig. 4, almost all *WRKY* genes have more than one intron (except *CmWRKY32*, no intron). Most genes usually have 1–5 introns. Among them, half of the genes contain 2 introns, 10 genes contain 1 intron, 6 genes contain 3 introns, 8 genes contain 4 introns, and 4 genes contain 5 introns.

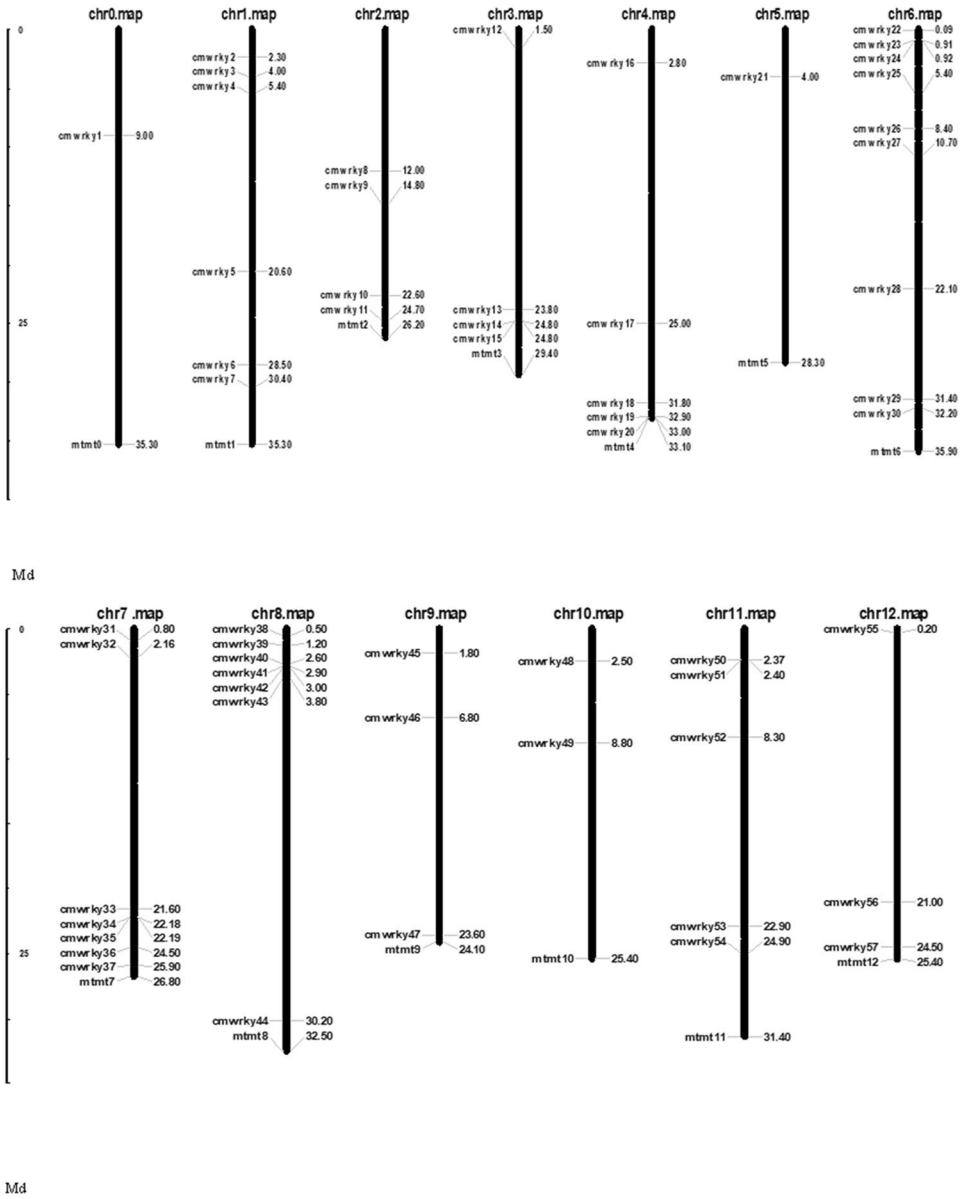

Fig. 1 Localization of the WRKY gene family on the chromosome of *Cucumis melo.* The size of a chromosome is represented by its relative length. The relative length of the chromosome and the location of the gene were obtained from the website Cucurbit Genomics Database. According to the position of the gene on the chromosome, its names range from *CmWRKY1* to *CmWRKY57*

The number of introns in different groups is different, but the number of introns in the same group is similar. For example, Group II a has 3 or 4 introns; Group II e and group III have 1 or 2 introns; Group II d that all has only 2 introns. Intron phase refers to the position of the intron relative to the three nucleotides of the codon in the gene; i.e., an intron is located between two complete codons or after the 1st and 2nd nucleotides in the codons; then the intron phase is 0, 1, 2. According to Xie et al. (2018). 48 out of 57 genes have Phase 0, and only 9 genes do not have phase 0. Group II c has the largest distribution of phase 0, while almost all genes in Group II a and group II b (*CmWRKY11* has only one Phase1) contain only Phase 0.

Table 3 Comparison of WRKY group sizes in different plants

Group	*CmWRKY*	*CsWRKY*	*AtWRKY*	*OsWRKY*
I	11	10	15	34
II	41	39	44	30
III	5	6	14	36
Total	57	55	72	100

Transcriptional Level of the Melon WRKY Gene in Response to Powdery Mildew Infection and Sprayed with SA

According to the previous research data from our laboratory concerning the melon transcriptomics of infected and uninfected powdery mildew in disease-resistant and

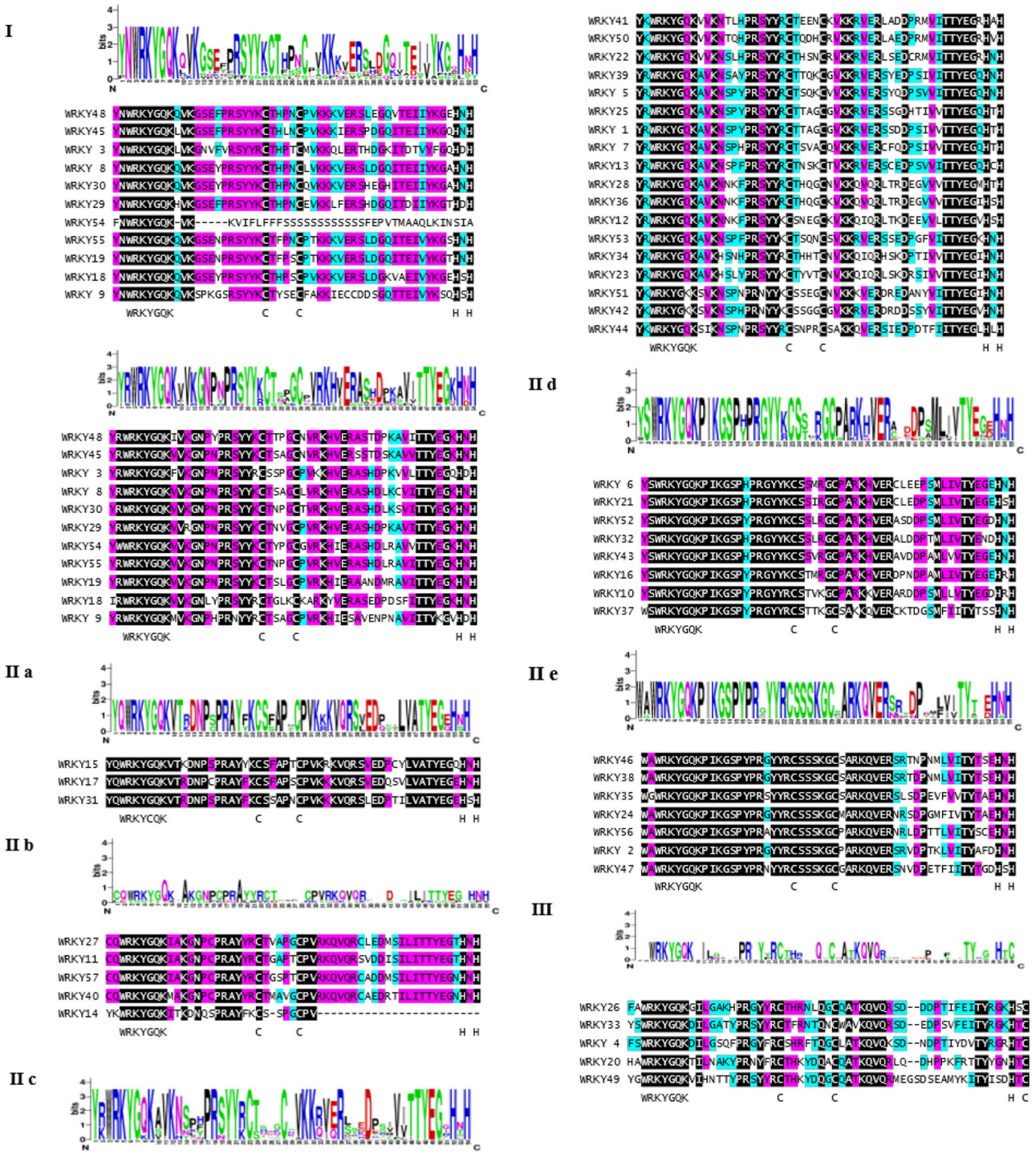

Fig. 2 Multiple sequence alignment of the WRKY domains in melon. The highly conserved WRKYGQK and zinc finger motif sequences are highlighted in different colors. The conserved domain of the CmWRKY protein sequence was analyzed by using WebLogo

susceptible varieties (unpublished), 24 genes affected by powdery mildew were obtained, and remove the eight genes for which no primer was found on the software DNAMAN; then primers of 16 genes were designed by DNAMAN, and primer specificity was detected from the NCBI. Finally, the primers for 16 *CmWRKY* genes are obtained as follows: *MELO3C007470* (*CmWRKY42*), *MELO3C017157* (*CmWRKY11*), *MELO3C024787* (*CmWRKY52*), *MELO3C007458* (*CmWRKY41*), *MELO3C002675* (*CmWRKY56*), *MELO3C000030* (*CmWRKY1*),

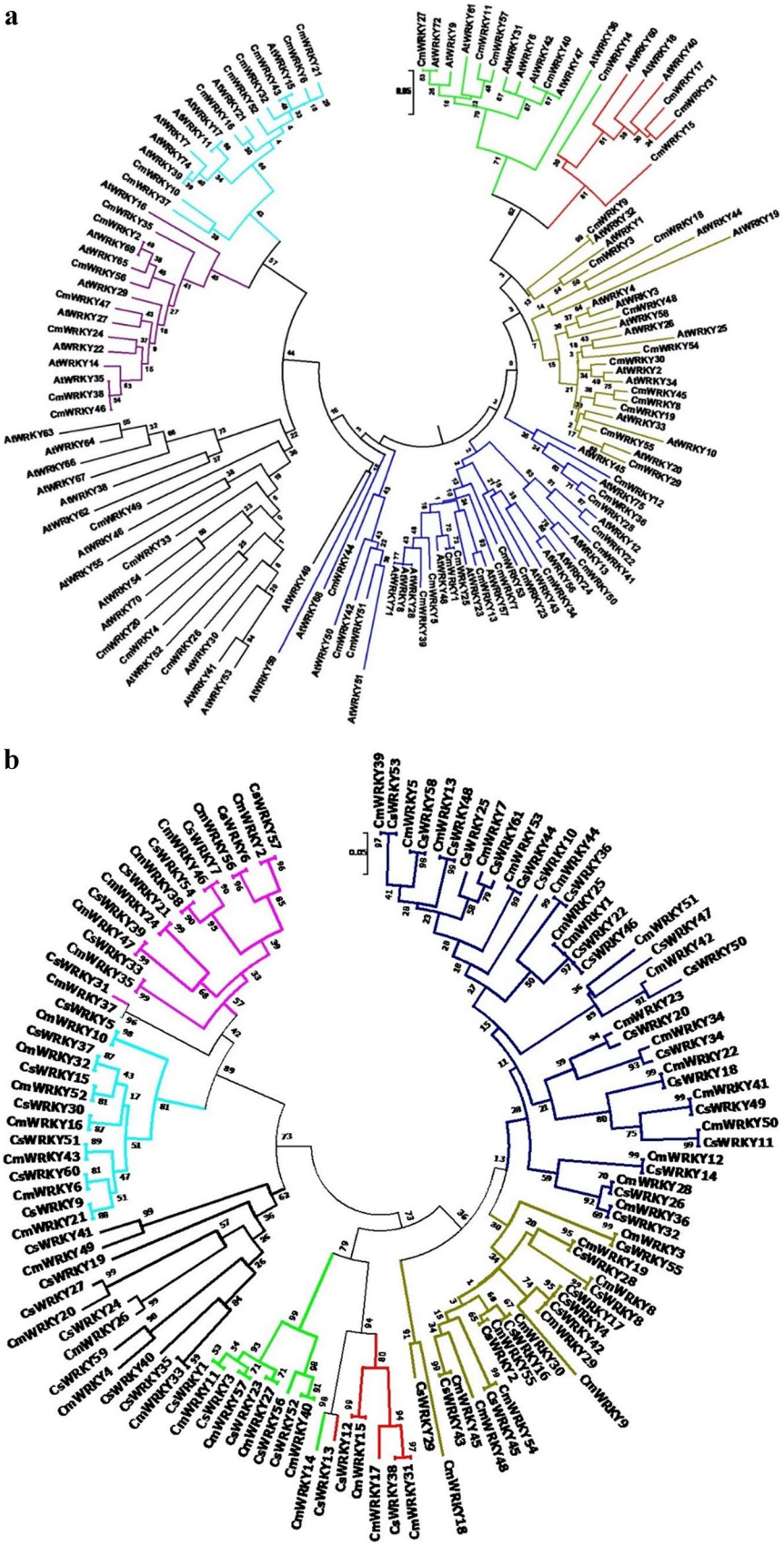

Fig. 3 Phylogenetic trees of WRKY proteins. Fig. 3a is a phylogenetic tree of Arabidopsis and melon, and Fig. 3b is a phylogenetic tree of cucumber and melon. The WRKY family of *Arabidopsis thaliana* and *Cucumis sativus* was used as an alignment. Mapping was carried out by ITOL and MEGA 6.0 software according to classification method of *Arabidopsis thaliana*. Brown represents Group I, red represents Group II a, green represents Group II b, blue represents Group II c, cyan represents Group II d, purple represents Group II e, and black represents Group III

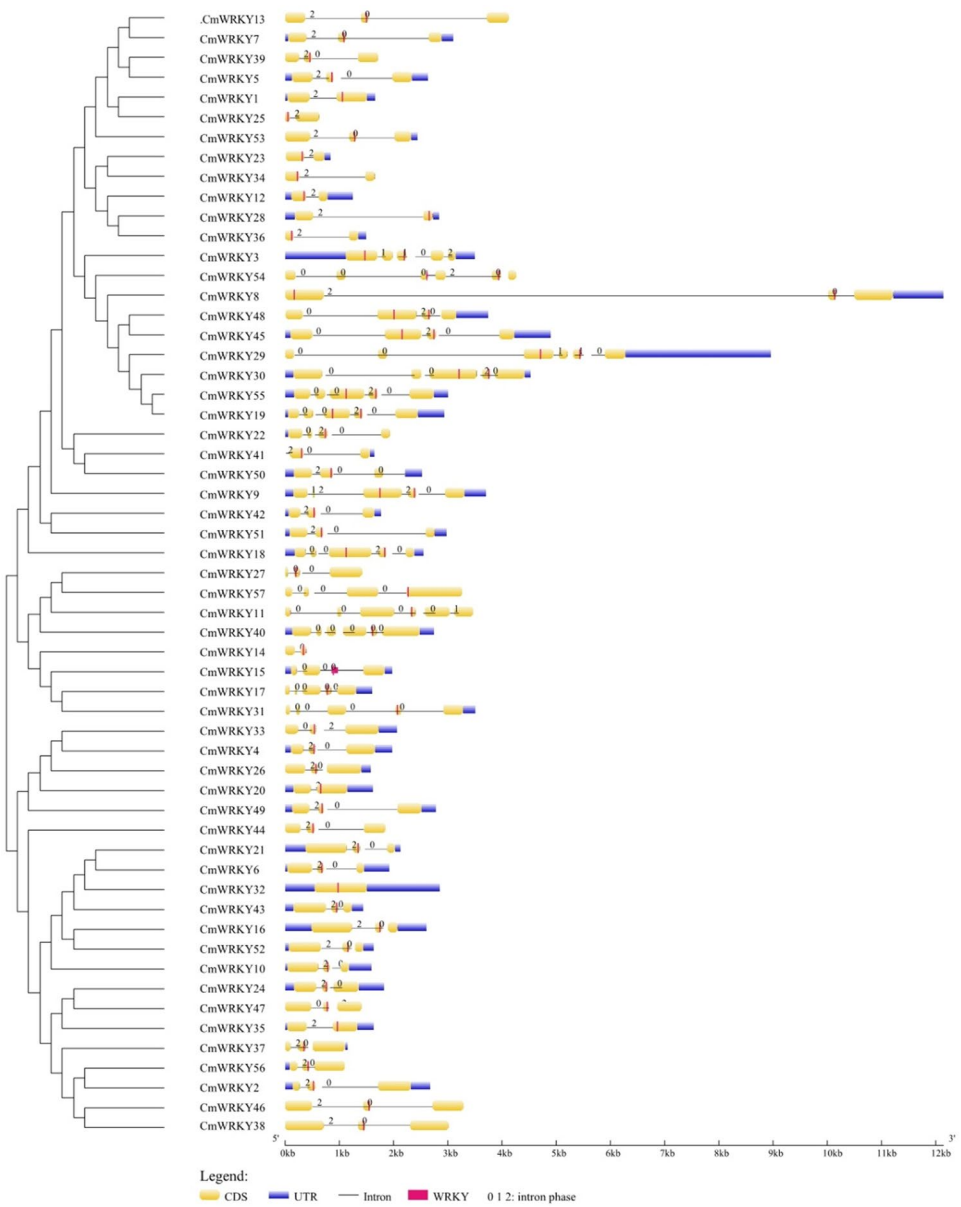

Fig. 4 Schematic representations of the exon–intron compositions of CmWRKY proteins from different groups or subgroups. The phylogenetic tree of *WRKY* genes are placed on the left. The schematic diagram of exon-intron composition is located on the right. Blue boxes indicate untranslated 5′- and 3′-regions; yellow boxes indicate exons; black lines indicate introns. Intron phases 0, 1, and 2 are indicated by numbers 0, 1 and 2, respectively. The WRKY domains are highlighted by red boxes

MELO3C025686 (*CmWRKY54*), *MELO3C016338* (*CmWRKY35*), *MELO3C014305* (*CmWRKY21*), *MELO3C009097* (*CmWRKY20*), *MELO3C007409* (*CmWRKY40*), *MELO3C006725* (*CmWRKY25*), *MELO3C020166* (*CmWRKY49*), *MELO3C026932* (*CmWRKY26*), *MELO3C020963* (*CmWRKY51*), and *MELO3C011295* (*CmWRKY15*) (Fig. 5).

In order to verify the data of the transcriptome, qRT-PCR was performed for analysis the expression level of melon leaves with powdery mildew infection or sprayed with SA. In the qRT-PCR analysis, 16 *CmWRKY* genes exhibited distinct expression patterns after powdery mildew infection, of which 3 (*CmWRKY15*, *CmWRKY40*, *CmWRKY56*) showed significant changes (> two-fold change). *CmWRKY15* was significantly up-regulated at 3 h and reached a maximum at 12 h. *CmWRKY40* and *CmWRKY56* began to up-regulated after infection by powdery mildew, peaked at 3 h, and began to decrease from 3 to 24 h (Fig. 6). Studies have shown that the tomato *WRKY* gene can increase the resistance of plants to powdery mildew (Kissoudis et al. 2016). However, SA can induce the expression of the *WRKY* gene (Lui et al. 2017). Therefore, this experiment also performed qRT-PCR analysis of leaf WRKY after leaves were sprayed with SA. Sixteen *CmWRKY* genes exhibited distinct expression patterns after sprayed with SA, of which four (*CmWRKY35*, *CmWRKY52*, *CmWRKY54*, *CmWRKY15*) showed significant changes (> five-fold change). The gene *CmWRKY52* was down-regulated from 0 to 3 h, significantly up-regulated from 3 to 24 h, and was most significant at 24 h. *CmWRKY15*, *CmWRKY35*, and *CmWRKY54* showed the most significant

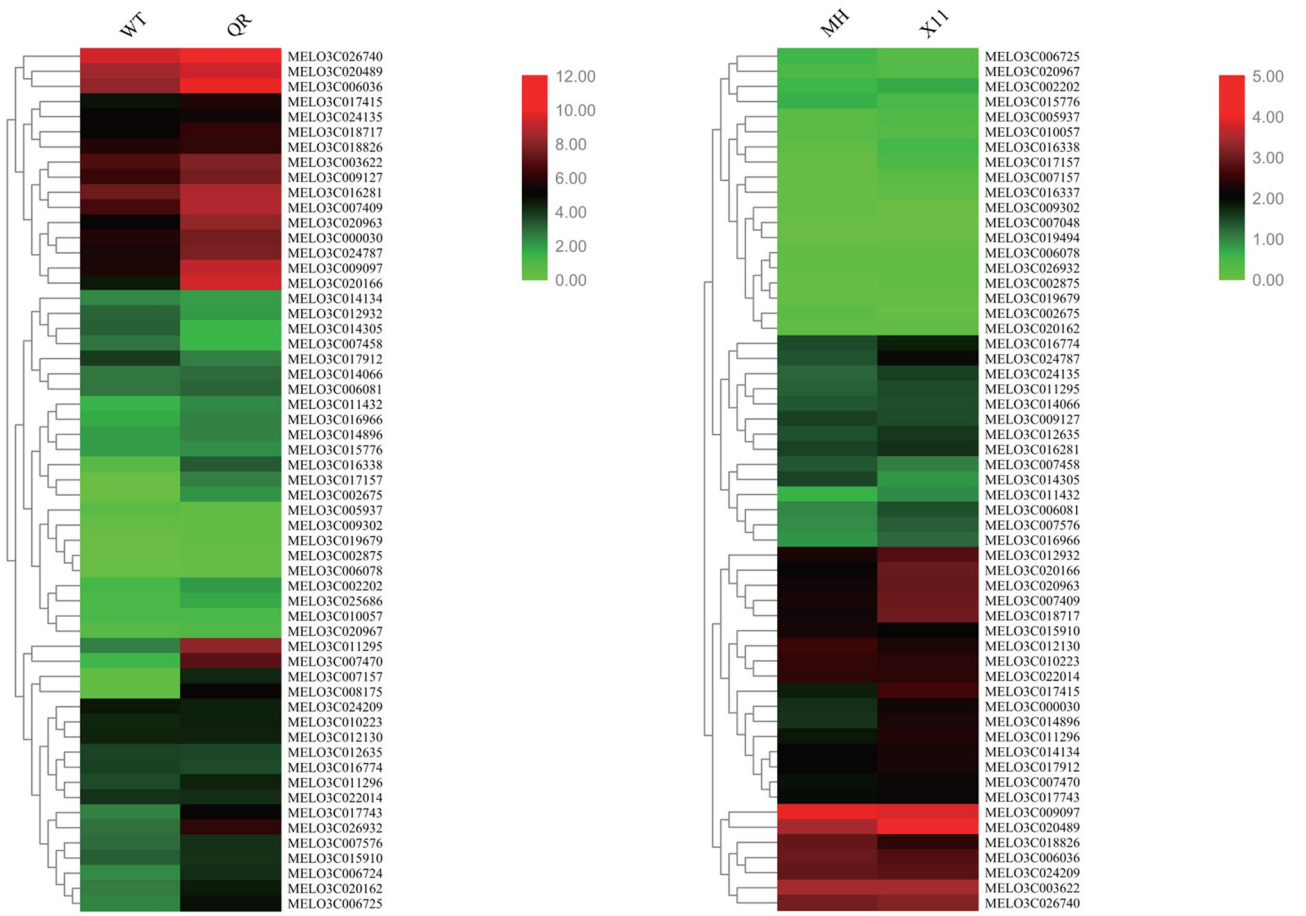

Fig. 5 Heat maps obtained from transcriptomics data of infected and uninfected powdery mildew in disease-resistant and susceptible varieties. QR represents infected by powdery mildew, WT represents uninfected by powdery mildew, and all the varieties used were "Mihe silver melon." MH stands for "Mihe Silver Melon," a common commercial species sensitive to powdery mildew, purchased from Shandong Qingzhou Huanglou Company; X11 stands for "Shannong No.1" and is a disease-resistant variety produced by Shandong Agricultural University. Red represents up-regulated, and green represents down-regulated

changes from the period of 12 to 24 h, showing a significant up-regulation. As shown in Fig. 6, SA treatment was positively correlated with powdery mildew infection in a certain period of time, for example, after treatment for 3 h, both of them simultaneously inhibited the expression of 13 genes (*CmWRKY1*, *11*, *15*, *20*, *25*, *26*, *35*, *41*, *42*, *49*, *51*, *52*, *54*). After 12 h of treatment, both of them simultaneously inhibited the expression of 10 genes (*CmWRKY1*, *11*, *20*, *21*, *25*, *35*, *41*, *49*, *51*, *56*), and 4 genes (*CmWRKY15*, *26*, *42*, *52*) were induced. After 24 h of treatment, both of them inhibited the expression of 11 genes (*CmWRKY1*, *11*, *20*, *21*, *25*, *26*, *40*, *41*, *49*, *51*, *56*) and induced the expression of *CmWRKY52*. SA treatment and powdery mildew infection will also have a negative correlation in a certain period of time. For example, after 3 h of SA treatment, *CmWRKY21*, *CmWRKY40*, and *CmWRKY56* were inhibited, while these three genes were induced to express after being infected with powdery mildew. After 12 h of SA treatment, *CmWRKY40* and *CmWRKY54* were inhibited, while *CmWRKY40* and *CmWRKY54* were induced to express after infection with powdery mildew. After 24 h of SA treatment, *CmWRKY15*, *CmWRKY35*, and *CmWRKY54* were induced to express and *CmWRKY42* was inhibited to express, whereas the powdery mildew infection showed the opposite effects.

The 16 genes were constructed into a phylogenetic tree. *CmWRKY1* and *CmWRKY25*, *CmWRKY42* and *CmWRKY51*, *CmWRKY52* and *CmWRKY21*, *CmWRKY40* and *CmWRKY11*, *CmWRKY35* and *CmWRKY56*, and *CmWRKY26* and *CmWRKY20* have very close genetic distances, and *CmWRKY1* and *CmWRKY25* were down-regulated simultaneously after powdery mildew infection or sprayed with SA, *CmWRKY42* and *CmWRKY51*, *CmWRKY40* and *CmWRKY11*, and *CmWRKY26* and *CmWRKY20* were simultaneously down-regulated at different time periods after powdery mildew infection or sprayed with SA. *CmWRKY35* and *CmWRKY56* had high

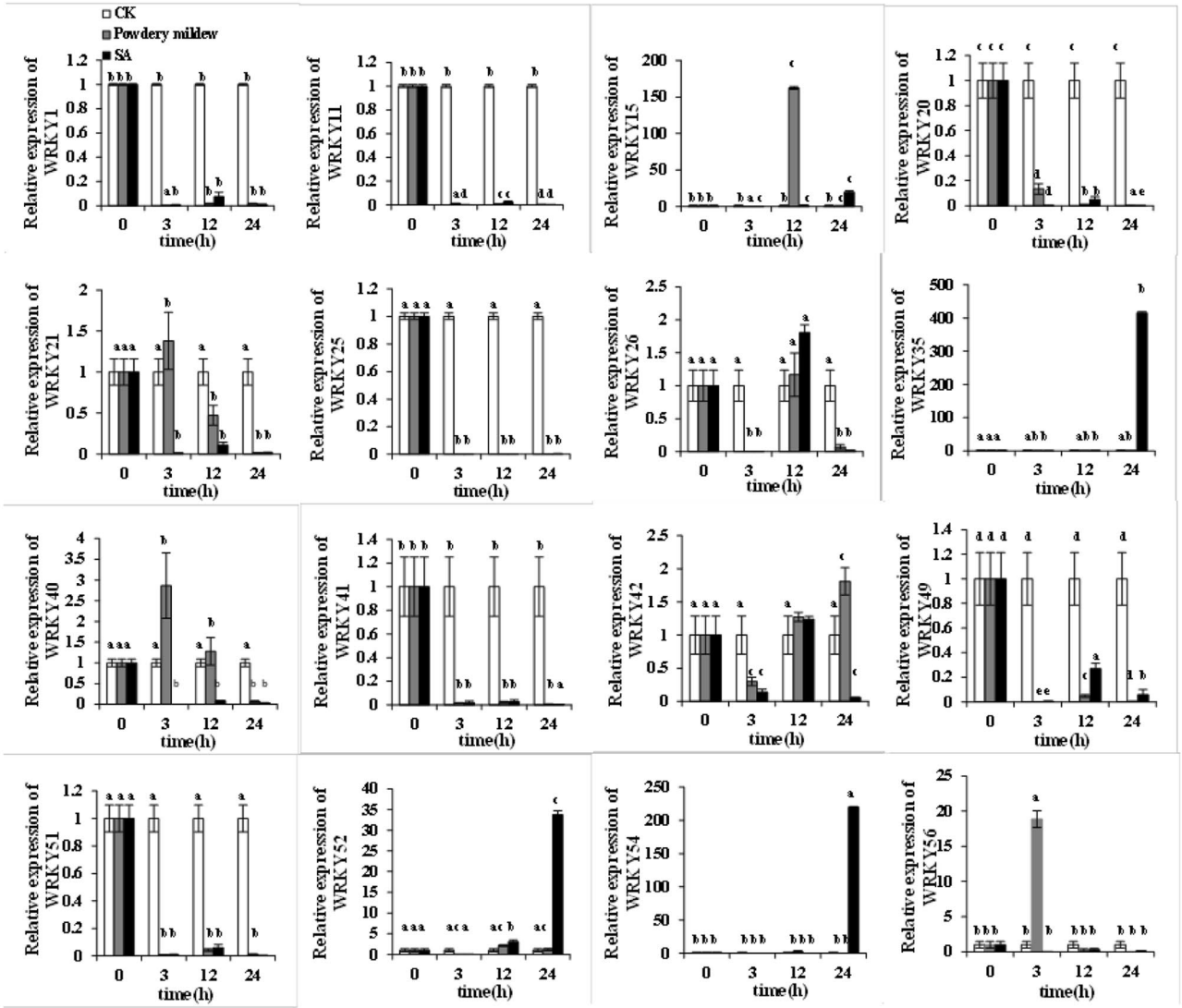

Fig. 6 The expression of 16 *WRKY* genes in melon leaves after powdery mildew infection or sprayed with SA. The *y*-axis indicates the relative expression level; vertical coordinates (0, 3, 12, and 24 h) indicate the hours of powdery mildew and SA treatment. The horizontal coordinates are plotted using vertical lines. a, b, and c indicate a significant difference

expression levels at certain time periods after powdery mildew infection or sprayed with SA, respectively (Supplementary Figure S1). These genes may have more research value in powdery mildew resistance.

Discussion

As a TF family in plants, *WRKY* genes are widely involved in the regulation of biotic and abiotic stress (Guo et al. 2018). At present, the cloning, isolation, and functional analysis of WRKY TFs have become popular research topics in the field of plant molecular biology, with more research in model crops Arabidopsis, rice, and tomato, but relatively less research in Cucurbitaceae crops. For example, in *A. thaliana*, a three-way amplification cycle, consisting of *AtWRKY75*, SA, and reactive oxygen species, can regulate leaf senescence (Guo et al. 2017); *AtWRKY33* of Arabidopsis and *OsWRKY13* of rice are both related to disease resistance (Zheng et al. 2006; Qiu et al. 2007); Tobacco *GhWRKY27a* is related to drought stress and resistance to *Rhizoctonia solani* infection (Yan et al. 2015). Therefore, the study of WRKY TFs in melon is conducive to further understanding the physiological and biochemical reactions of Cucurbitaceae crops under stress conditions.

In this study, a total of 57 melon *WRKY* genes were identified. The number of WRKY family members in melon is approximately equal to that of cucumber (55) (Ling et al.

2011), but lower than that of Arabidopsis (72) and rice (100) (Song and Gao 2014). Compared with Arabidopsis and rice, the genome of Cucurbitaceae is much smaller, which may be related to the fact that the Cucurbitaceae crop has not experienced a genome-wide repetitive event. In fact, previous studies have shown that Arabidopsis and rice have undergone genome-wide repetitive events that promote rapid expansion of the gene family (Jiao et al. 2018). Garcia-Mas et al. (2012) observed a lack of recent genome-wide duplication in the cucumber and melon genomes. According to the analysis of transcription data, 16 WRKY genes were found to be responsive to powdery mildew. Twelve (*CmWRKY1*, *11*, *15*, *21*, *25*, *35*, *40*, *41*, *42*, *51*, *52*, *56*) of these 16 genes belong to group II, among which *CmWRKY15*, *42*, and *52* were induced to express at the same time after spraying SA and powdery mildew infection. Three members (*CmWRKY20*, *26*, *49*) belong to Group III, among which *CmWRKY26* was induced to express at the same time after spraying SA and powdery mildew infection. And one gene (*CmWRKY54*) belongs to Group I, which may indicate that group II plays a crucial role in biotic and abiotic stress in melon. For example, *VvWRKY30* plays an active regulatory role under salt stress (Zhu et al. 2019); soybean WRKY TF genes *GmWRKY13* and *GmWRKY54* endow transgenic Arabidopsis plants with different tolerance to abiotic stress (Yu et al. 2016; Zhou et al. 2008). The above genes belong to group II. These results indicate that *WRKY* genes with close evolutionary relationships may have similar biological functions in plants (Lu et al. 2015). The information above can be used to explore potential stress-related *WRKY* genes in plants and lay a foundation for functional analysis of melon *WRKY* genes in the future.

Powdery mildew is a common disease of Cucurbitaceae vegetables worldwide, causing serious damage to cucumbers and melons (Huang et al. 2012). Studies have shown that the *WRKY* gene can regulate plant resistance to powdery mildew; for example, *HvWRKY10*, *HvWRKY19*, and *HvWRKY28* can trigger the basic defense of barley against powdery mildew (Meng and Wise 2012). In order to confirm whether the expression of the *CmWRKY* gene was affected by powdery mildew, 16 *CmWRKY* members were selected from 57 melon *WRKY* genes. Further, qRT-PCR was performed to analyze their expression patterns in response to powdery mildew (Fig. 6). The results show that different genes were only induced or inhibited at specific treatment time points, indicating that the expression of most genes may be regulated by time. For example, *CmWRKY15* was significantly induced only at 12 h, and the expression of *CmWRKY56* increased significantly at 3 h. In contrast, the expression of *CmWRKY42* decreased only at 3 h. These findings are consistent with the disease-resistant function of *AtWRKY50* and *AtWRKY51* (Hussain et al. 2012). These gene expression changes may be related to induction or inhibition of powdery mildew. Research has shown that SA and H_2O_2 strongly induce *WRKY* gene expression in several plant species (Xie et al. 2005). *Bacillus subtilis* UMAF6639 can alleviate melon powdery mildew by activating JA- and SA-dependent defenses in the rhizosphere (García-Gutiérrez et al. 2013). Therefore, the *WRKY* gene can alleviate the harm of powdery mildew through the SA route. In this study, 16 representative *CmWRKY* genes were selected to study their expression profiling under powdery mildew infection and SA treatment. The results showed that *CmWRKY35*, *CmWRKY52*, and *CmWRKY54* were significantly induced at 24 h. *CmWRKY15* was significantly induced at 12 h, and this trend was consistent with the expression profile of *AtWRKY18* under *Pseudomonas syringae* DC3000 infection (Chen and Chen 2002). This shows that *CmWRKY15* has a role in stress resistance. The expression of *CmWRKY35* was inhibited after powdery mildew infection, while the expression level was increased after sprayed with SA; on the contrary, the expression of *CmWRKY40* and *CmWRKY56* were increased after powdery mildew infection, while the expression level was inhibited after sprayed with SA. These results suggest that these genes may be involved in two signaling pathways, and that the same gene may play different roles in different signaling pathways. Although we found that the expression levels of many genes were different after spraying SA and infecting powdery mildew, but we also found that some genes were valuable. For example, the expression of *CmWRKY15*, *CmWRKY26*, *CmWRKY42*, and *CmWRKY52* were induced when spraying SA and infecting powdery mildew for 3–12 h. The expression of *CmWRKY1* and *CmWRKY25* were inhibited at 0–3 h and 12–24 h, which was consistent with the response of *AtWRKY48* to jasmonic acid (Schluttenhofer et al. 2014), while *AtWRKY48* had the closest genetic distance to *CmWRKY1* and *CmWRKY25*. Studies have shown that *AtWRKY30* can improve the tolerance of Arabidopsis to oxidative stress and salt stress (Scarpeci et al. 2013), and the genetic distance between *AtWRKY30* and *CmWRKY26* was the closest. These findings indicate that these *CmWRKY* genes may play a role in a certain pathway and have high research value, which lays a foundation for the future research of melon *WRKY* gene.

Plant hormone signaling pathways are not independent, but interact with various complex regulatory networks and various defense signaling pathways. Through plant signal transmission, plants can realize the growth and development process and react to biotic and abiotic stresses. However, *WRKY* gene is not constitutively expressed in plants, but is induced and expressed by various environmental factors (such as pathogens, fungal elicitors, signal molecule SA and its functional analogues, various abiotic stresses and biotic stresses such as drought, low temperature, and mechanical stress), and its expression has tissue specificity. Therefore, it

is of great significance to understand how plants coordinate various hormones to trigger the expression of the *WRKY* gene, thereby further activating various defense responses.

In order to better understand the structure and function of melon WRKY TF, this study carried out bioinformatics analysis of melon WRKY TF, which laid a foundation for studying its function. The way in which the melon WRKY TF responds to the hormonal signal regulation of melon resistance as well as the participation in the signal transduction of stress response will be the emphasis of future research.

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Authors' Contributions Qinghua Shi and Yuanyuan Chen conducted the experiment and wrote the paper; Xin Jing and Jianquan Wang participated in making the Phylogenetic Tree; Shuoshuo Wang and Xin Jing provided transcriptome data; Shizhong Zhang provided the method of analytical method; all the authors participated in the discussion of the article.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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