



Genome-wide investigation of the *WRKY* transcription factor gene family in weeping forsythia: expression profile and cold and drought stress responses

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Abstract

Weeping forsythia is a wide-spread shrub in China with important ornamental, medicinal and ecological values. It is widely distributed in China's warm temperate zone. In plants, *WRKY* transcription factors play important regulatory roles in seed germination, flower development, fruit ripening and coloring, and biotic and abiotic stress response. To date, *WRKY* transcription factors have not been systematically studied in weeping forsythia. In this study, we identified 79 *WRKY* genes in weeping forsythia and classified them according to their naming rules in *Arabidopsis thaliana*. Phylogenetic tree analysis showed that, except for IIe subfamily, whose clustering was inconsistent with *A. thaliana* clustering, other subfamily clustering groups were consistent. Cis-element analysis showed that *WRKY* genes related to pathogen resistance in weeping forsythia might be related to methyl jasmonate and salicylic acid-mediated signaling pathways. Combining cis-element and expression pattern analyses of *WRKY* genes showed that more than half of *WRKY* genes were involved in light-dependent development and morphogenesis in different tissues. The gene expression results showed that 13 *WRKY* genes were involved in drought response, most of which might be related to the abscisic acid signaling pathway, and a few of which might be regulated by *MYB* transcription factors. The gene expression results under cold stress showed that 17 *WRKY* genes were involved in low temperature response, and 9 of them had low temperature responsiveness cis-elements. Our study of *WRKY* family in weeping forsythia provided useful resources for molecular breeding and important clues for their functional verification.

Keywords Cold stress · Drought stress · *WRKY* transcription factor · Gene expression

Introduction

Ornamental plants play an important role in urban landscape design and ecological construction, contributing to the improvement of the environment and landscape (Wang et al. 2014). Weeping forsythia (*Forsythia suspensa* (Thunb.) Vahl) is an Oleaceae plant, and is native to in Henan, Hebei, Shandong, Shanxi, Hubei, Shaanxi and other provinces in China (Yang et al. 2017). Weeping forsythia is a widely used garden ornamental plant, and is known for its excellent ornamental characteristics of early spring flowering, with flowers emerging spectacularly before spring leaves, and a large number of flowers.

Weeping forsythia also has important medicinal values. Its fruit contains forsythin, forsythoside A, α -pinene, β -pinene, terpinen-4-ol and other volatile oil components, which are widely used in traditional Chinese medicines for

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treating colds, such as Shuanghuanglian Oral Liquid and Lianhua Qingwen Oral Liquid (Xiang et al. 2021). Recent studies showed that weeping forsythia could relieve the symptoms of COVID-19 (Hu et al., 2021; Zhao et al., 2021). In recent years, weeping forsythia has been extensively planted artificially due to the dramatic reduction of natural resources. Therefore, studies of the horticulture, genetics and medicinal use of weeping forsythia are increasing. High-quality genome and whole genome sequencing data of weeping forsythia were published (Li et al. 2022a). Large-scale of transcriptome data of weeping forsythia provide new opportunities to analyze gene expression patterns in different tissues and different environmental stresses.

In horticultural plants whose genome data have been published recently, many gene families have been identified and analyzed, such as *bZip* family in *Prunus mume* (Li et al. 2022b), *GDSL* family in *Pyrus* spp. (Zhang et al. 2022a), *HD-Zip* and *MdCLE* peptide families in *Malus × domestica* (Zhang et al. 2022b, c). The studies on the distribution, gene structure, expression patterns and functional differentiation of these gene families in the genome provide useful resources for analyzing the evolutionary mechanisms of the gene families and for utilizing this information to guide for molecular breeding.

To date, no study on any gene family of weeping forsythia at the genome level has been described. *WRKY* gene family is one of the largest transcription factor families in higher plants. In 1994, the first *WRKY* protein was cloned from sweet potato (Ishiguro and Nakamura 1994). In recent years, with the publication of more plant genomes, a large number of *WRKY* members from different species have been cloned and identified (Wani et al. 2021). *WRKY* gene is named because of its N-terminal containing 7 highly conserved amino acid sequences composed of WRKYGQK (Song et al. 2018). *WRKY* gene is more likely to bind to the upstream W-box region of some gene promoters, which contains a core DNA sequence (T)(T)TGAC(C/T) sequence (Cheng et al. 2019).

WRKY gene is not constitutively expressed in plants. The expression of *WRKY* gene is induced by various environments, such as biotic and abiotic stress and different development stages of plants, and its expression is tissue-specific and participates in various physiological processes in plants (Rinerson et al. 2015). In cold stress, *WRKY* genes improve cold tolerance by regulating CBF signaling pathway (Huang et al. 2022). Recent studies showed that *WRKY* genes were involved into increasing the tolerance to adversity stress (Jiang et al. 2017), improving the immunity against pathogens (Pandey and Somssich 2009), participating in somatic embryogenesis (Yang et al., 2020), promoting the synthesis of secondary metabolites (Chen et al. 2017), regulating the

flowering of plants, accelerating fruit ripening (Wang et al. 2017), and promoting fruit coloring (Cheng et al. 2017).

The number of *WRKY* gene family members varies greatly in different species, while its family members are large in most higher plants (Chen et al. 2019). The sequence homology of other amino acids in the *WRKY* gene family is not high except the conservative region. The diversity of gene structures of the *WRKY* family implies the diversity of their functions. To date, the *WRKY* family has been functionally and physiologically characterized in several ornamental plants, such as *Dendrobium officinale* (Wang et al. 2018), *Acer truncatum* (Li et al. 2021a), *Prunus mume* (Bao et al. 2019). However, *WRKY* family has not been described in weeping forsythia. To better understand the characteristics, evolution and functions of the *WRKY* genes in weeping forsythia, we provide a detailed overview of the number and classification, gene structure and expression of *WRKY* family at the genome level. Thus, our study improves the understanding of the *WRKY* family and provides key candidate genes related to cold and drought stresses in weeping forsythia.

Materials and methods

Data sources and sequence searches

The weeping forsythia genome was obtained from National Center for Biotechnology Information (accession no. JAHHPY000000000; Li et al., 2022). The keyword “*WRKY*” was used to search *WRKY* genes in the annotation file, and blasted the candidate genes in NCBI (<https://blast.ncbi.nlm.nih.gov/>; Altschul et al. 1990) to identify the *WRKY* domain. The genes with conserved *WRKY* domain were considered the true *WRKY* genes. ExpASY online tool (<https://web.expasy.org/cgi-bin/protparam/protparam>; Artimo et al., 2012) was used to predict and analyze the physicochemical properties of *WRKY* protein in weeping forsythia, including molecular weight, isoelectric point, amino acid number, fat index, instability index, and hydrophobicity. The subcellular localization of weeping forsythia *WRKY* genes was predicted by Plant-mPLoc online software (<http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/>, Chou and Shen, 2010), and the online software SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/secpred_sopma.pl; Geourjon and Deleage, 1995) was used to predict the secondary structure of *WRKY* protein.

According to the identified *WRKY* gene ID of weeping forsythia and the genome sequence of weeping forsythia, the chromosome position information of *WRKY* gene family members was obtained. The position of *WRKY* gene of weeping forsythia on chromosome was visualized by

TBtools software (Chen et al. 2020a). The genome and protein sequence data of *Arabidopsis thaliana* is from the Arabidopsis information resource (<https://www.arabidopsis.org/>; Eulgem et al., 2000).

Phylogenetic relationship and gene structure

A phylogenetic tree using the neighbor-joining (NJ) method (Jones et al. 1992) was constructed using the obtained amino acid sequences. The NJ tree was constructed using MEGA 7.0 (Kumar et al., 2016) with the Poisson model, the pairwise deletion option, and 1,000 bootstrap resampling times. Finally, the phylogenetic tree was visualized using FigTree v1.4.4 (Rambaut 2009). TBtools (Chen et al. 2020a) was used to visualize the introns and exons of all *WRKY* genes of weeping forsythia. The online database of MEME (<https://meme-suite.org/meme/tools/meme>, Bailey et al., 2009) was used to analyze the protein domains and conserved motifs of all *WRKY* genes in weeping forsythia. The analysis value of conserved motifs was set to 10, and the protein domains of *WRKY* family in weeping forsythia were visualized by TBtools (Chen et al. 2020a). TBtools (Chen et al. 2020a) was further used to extract the upstream 2 Kb sequence information of *WRKY* genes in weeping forsythia, and the online software PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>; Thijs et al., 2002) was predicted the possible cis-acting elements of *WRKY* genes, and the predicted results were visualized by TBtools (Chen et al. 2020a).

Expression patterns of *WRKY* gene in different tissues and under cold and drought stresses

The RNA-seq data of 79 weeping forsythia *WRKY* genes were downloaded from NCBI, including data from fruit, stem, leaf (accession no. SRR17386487-SRR17386495), and flower tissues (accession no., SRX11342985, SRX11342993 and SRX11342994). RNA-seq data from fruit, stem, leaf was obtained at harvest time (July) of the weeping forsythia fruit (Li et al., 2022). RNA-seq data of flower were obtained at the flower bud period (March). The gene expression data of weeping forsythia under drought stress were from Wuzhishan populations under 80% and 20% soil water content (accession no. SRX7503009, SRX7503010, SRX7503012-SRX7503015; Li et al. 2021b). The gene expression data of weeping forsythia under cold stress were from Wuzhishan populations at 25 °C and 4 °C (accession no. SRX7440183-SRX7440188; Li et al. 2021c).

Low-quality reads with more than 10% anonymous nucleotides (N) and more than 50% of bases possessing a value $Q \leq 10$ were removed from raw sequencing data. The gene expression level of all genes in these samples was

estimated by fragments per kilobase of transcript per million fragments mapped (FPKM) using StringTie (Pertea et al. 2015). The expression patterns in different tissues and in response to drought and cold stresses were analyzed using the R package Heatmap. $\log_2\text{FPKM} \geq 1$ was used as the threshold to screen the *WRKY* genes that were effectively expressed in different tissues of weeping forsythia. Under drought and cold stresses, $\text{FC} \geq 2$ and $\text{FDR} \leq 0.05$ were used as the thresholds to screen *WRKY* genes involved in stress response.

Results

Gene identification and sequence characteristics of the *WRKY* gene family

A total of 80 possible *WRKY* genes were identified from the weeping forsythia genome through genome annotation files. After further domain identification, 79 of them contained the WRKYGQK conserved domain, and these genes were identified as real *WRKY* genes. *WRKY* proteins varied greatly in weeping forsythia, amino acid length ranged from 146 (FsWRKY21) to 728 aa (FsWRKY27 and FsWRKY63), protein molecular weight ranged from 16.33 (FsWRKY21) to 80.32 Da (FsWRKY63), and isoelectric point ranged from 4.56 (FsWRKY65) to 9.84 (FsWRKY63). All these proteins were located in the nucleus and were hydrophilic proteins (Table S1). Among 79 *WRKY* proteins, 5 proteins (FsWRKY33, FsWRKY50, FsWRKY55, FsWRKY61, FsWRKY74) were stable proteins, and the rest were unstable proteins. The results of subcellular localization prediction showed that all 79 proteins were located in the nucleus (Table S1). The *WRKY* genes were randomly distributed on the chromosomes of weeping forsythia. Among them, the *WRKY* genes in Chr1 (8.9%), Chr4(8.9%), Chr5(8.9%), Chr12(15.2%) and Chr14(10.1%) were the most, and one gene was distributed in the unmounted fragments Scaffold397 and Scaffold413 respectively (Fig. 1).

Phylogenetic relationship of *WRKY* protein in weeping forsythia

To better understand the phylogenetic relationship of *WRKY* protein of weeping forsythia, the phylogenetic tree of 79 *WRKY* proteins of weeping forsythia and 74 *WRKY* proteins of *A. thaliana* was constructed using the NJ method. The phylogenetic tree showed that the members of *WRKY* protein I, IIa, IIb, IIc, IId and III of weeping forsythia were grouped together well with the members of this subfamily of *A. thaliana*, but the members of IIe subfamily did not group together in one clade (Fig. 2). FsWRKY5, AtWRKY65 and

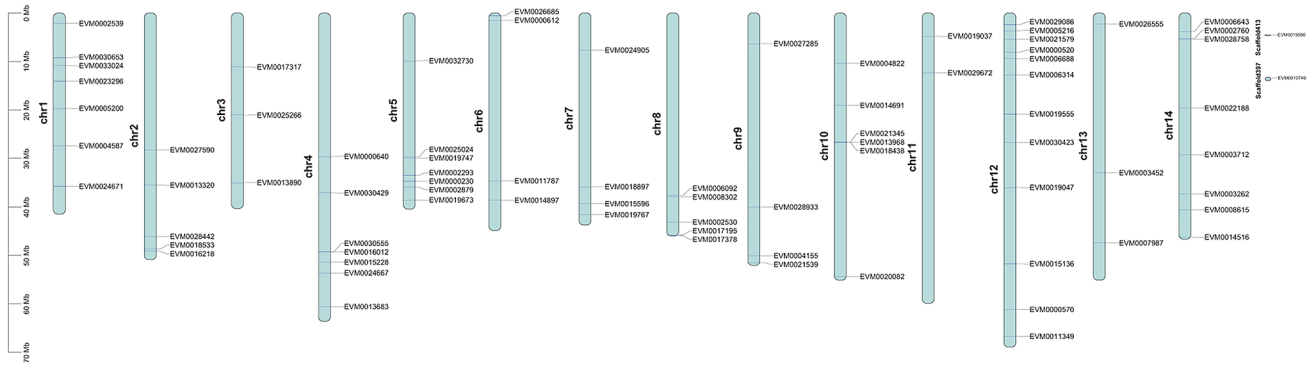


Fig. 1 Distribution map of *WRKY* gene family in the weeping forsythia genome

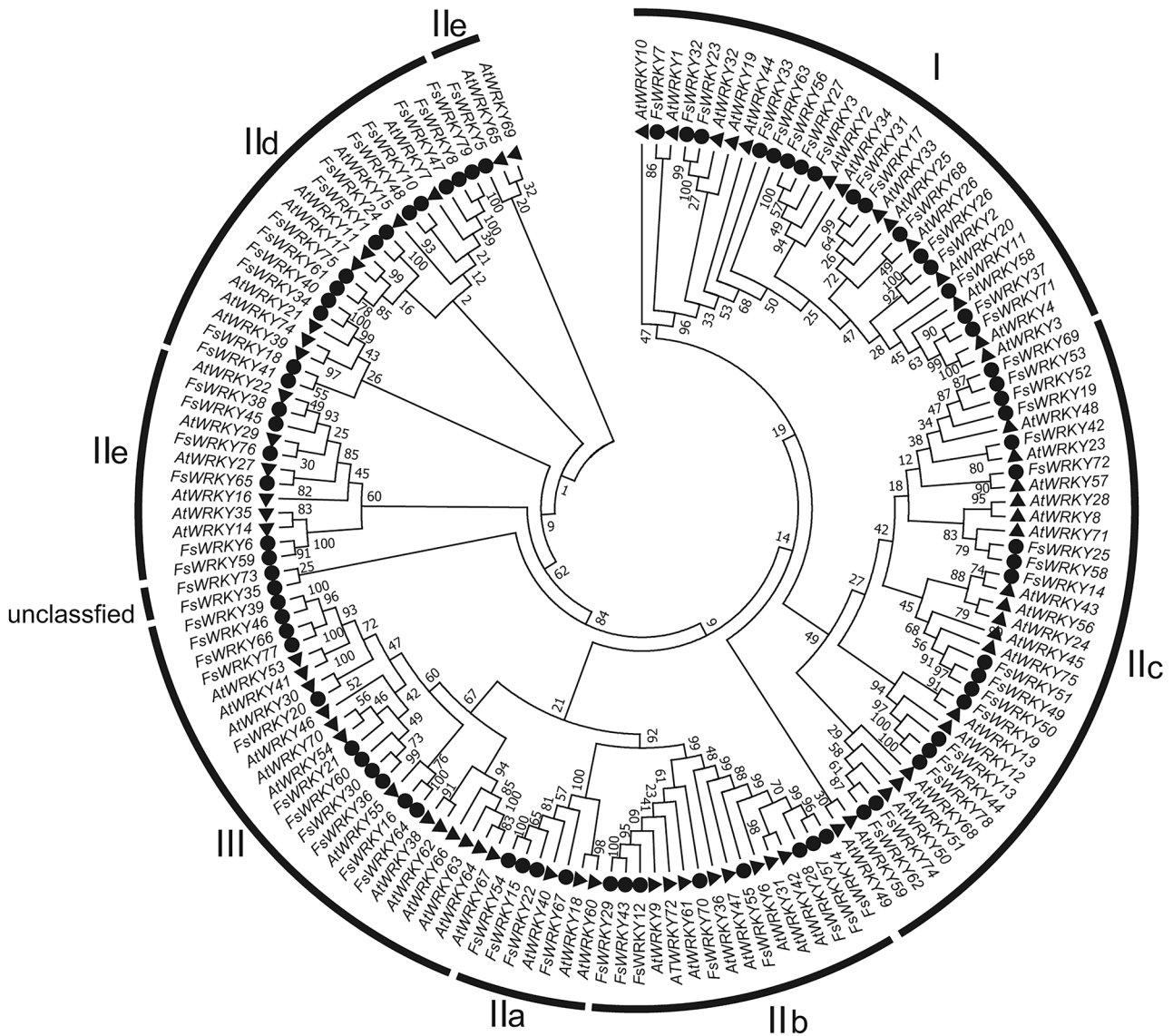


Fig. 2 Phylogenetic tree construction and analysis of *WRKY* proteins in weeping forsythia and *Arabidopsis thaliana*

FsWRKY69 grouped together separately, which was inconsistent with the classification results of WRKY protein in *A. thaliana*. Our results showed that members of IId were clustered into two clades, which indicated that IId subfamily had obvious genetic differentiation in weeping forsythia. Our clustering results were basically consistent with those of *A. thaliana*, except for the IIE subfamily. According to the classification of seven WRKY subfamilies of *A. thaliana*, we counted the number of members of WRKY gene of weeping forsythia in seven subfamilies, including 16 members in I subfamily, 4 members in IIA subfamily, 8 members in IIB subfamily, 18 members in IIC subfamily, 13 members in IID subfamily, 7 members in IIE subfamily, 11 members in type III, and two unclassified members.

Gene structure and protein structure of WRKY family

In a gene family, the gene intron and exon structure can reflect the evolutionary relationship of the members of the gene family. We analyzed the WRKY gene sequences of all weeping forsythia and visualized the WRKY gene structure by using TBtools (Fig. 3). I subfamily has 4 to 6 exons and 3 to 6 introns, and the average gene length is larger than other subfamilies. IIA subfamily had 3 to 4 introns and 5 exons except *FsWRKY22*, which had 4. IIB subfamily had 4 to 6 exons and 4 to 5 introns. IIC subfamily has 2 to 4 exons and 1 to 3 introns. IID subfamily had 3 to 4 exons and 2 to 3 introns. IIE subfamily has 3 to 4 exons and 2 to 3 introns. III subfamily had 2 to 4 exons and 1 to 3 introns. The unclassified *FsWRKY73* had no intron.

To further analyze the structure and function of WRKY protein in weeping forsythia, the conserved domain and conserved motif of WRKY family of weeping forsythia were analyzed (Fig. 4). Conservative domain analysis showed that 16 members of the I subfamily of weeping forsythia contained two WRKY domains, while the other subfamily members contained only one WRKY domain. In addition, among the 13 members of IId subfamily, 8 members had Plant_zn_clust conserved domain, which might indicate that they were zinc-dependent transcription factors. Conservative motif analysis showed that the WRKY domains were mostly composed of three combinations of conservative motif4, motif1, and motif2, motif9, motif1, and motif2, or motif 3 and motif10, among which the WRKY domain composed of motif 3 and motif10 was unique to I subfamily. The prediction of protein secondary structure of WRKY family showed that the proportion of alpha helix range from 7.47% (FsWRKY26) to 35.52% (FsWRKY67), beta turn range from 1.13% (FsWRKY64) to 10.78% (FsWRKY49), extended strand range from 5.35% (FsWRKY45) to 21.98% (FsWRKY13), and random coil range from 44.91%

(FsWRKY49) to 77.97% (FsWRKY72) (Table S2). The results showed that the secondary structure of WRKY protein in weeping forsythia was mainly composed of alpha helix and random coil.

Cis-acting elements of WRKY gene family

A total of 1,910 possible cis-acting regulatory elements were identified in the upstream 2 Kb range of 79 WRKY genes (Table S3; Fig. 5). The results showed that there were many cis-acting elements in the promoter region of WRKY gene of weeping forsythia. In addition to a large number of light-responsive elements, cis-acting elements related to plant hormones, such as methyl jasmonate (MeJA), abscisic acid (ABA), gibberellin (GA), auxin, salicylic acid (SA) and cis-acting elements related to low temperature, such as drought, anaerobic, and defense and stress, were also found in WRKY genes of weeping forsythia. Among them, 42 cis-elements related to low temperature stress were distributed among 28 WRKY genes, and 52 cis-elements related to drought involving MYB transcription factors were distributed among 38 genes.

Expression patterns of WRKY gene in different tissues, cold and drought stresses

The expression of WRKY genes in fruits, stems, leaves and flowers of weeping forsythia were investigated. The results showed that 52 WRKY genes were effectively expressed in fruits and stems, 53 in leaves and 46 in flowers (Table S4; Fig. 6). Our results indicated that a large number of WRKY genes might be involved in the development and morphogenesis of fruit, stem, leaf and flower tissues in weeping forsythia.

The expression patterns of WRKY genes were further investigated under cold and drought stresses. We found that 17 WRKY genes responded significantly to cold stress (Table S5; Fig. 7), of which 9 (*FsWRKY22*, *FsWRKY36*, *FsWRKY38*, *FsWRKY42*, *FsWRKY45*, *FsWRKY47*, *FsWRKY56*, *FsWRKY70*, and *FsWRKY75*) had cis-elements related to low temperature stress, and 13 WRKY genes were significantly differentially expressed under drought stress (Table S6; Fig. 8), in which 4 genes had drought-related cis-elements involved in MYB transcription factors (*FsWRKY19*, *FsWRKY54*, *FsWRKY56*, and *FsWRKY67*), and 10 cis-elements with ABA responsiveness (*FsWRKY4*, *FsWRKY8*, *FsWRKY17*, *FsWRKY19*, *FsWRKY27*, *FsWRKY28*, *FsWRKY34*, *FsWRKY54*, *FsWRKY74*, and *FsWRKY76*), and two of them (*FsWRKY19* and *FsWRKY54*) both had two kind of cis-elements.

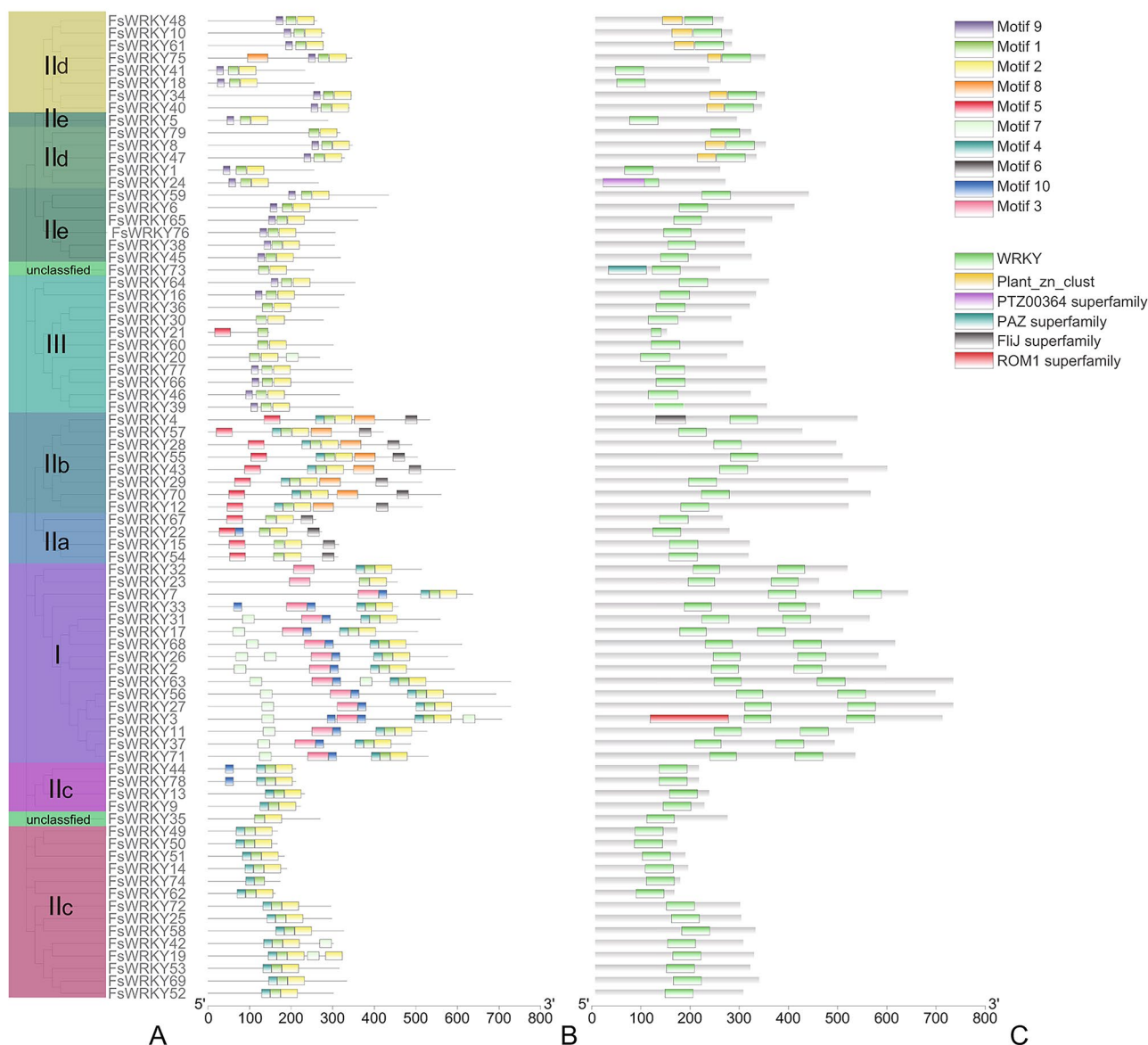


Fig. 4 The protein motifs and protein domain analysis of *WRKY* genes of weeping forsythia. A, Phylogenetic tree of *WRKY* genes; B, Protein motif of *WRKY* genes; C, Protein domain of of *WRKY* genes

which is more than the number of *WRKY* genes (74 *bZIP* genes) identified in *A. thaliana* (Eulgem et al. 2000), *Hippophae rhamnoides* (48) (Wang et al. 2019a), *Cucumis sativus* (61) (Chen et al. 2020b), but less than the 109 members identified in *Oryza sativa* (Hwang et al. 2016), or the 126 members in *Raphanus sativus* (Karanja et al. 2017), 171 members in *Triticum aestivum* (Ning et al. 2017), and 81 members in *Lycopersicon esculentum* (Huang et al. 2012).

All *WRKY* genes of weeping forsythia were consistently clustered with the reference genome of *A. thaliana*, except for the IIe subfamily which was differently clustered from *A. thaliana*. These results suggest that the IId subfamily of

weeping forsythia has undergone genetic differentiation, and it was divided into two genetic clades.

In the two unclassified members, *FsWRKY73* was special, being the only member without introns and having the shortest gene length. *FsWRKY73* did not effective express in our investigated tissues of weeping forsythia, and it was not differentially expressed under drought and cold stresses. A special PAZ domain was found in *FsWRKY73*. At present, it is usually considered that PAZ domain is related to the binding of single-stranded RNA, which was likely to lead to gene silencing (Yan et al. 2003).

In the *WRKY* gene family, I subfamily contains two *WRKY* domains, which is considered as the oldest clade

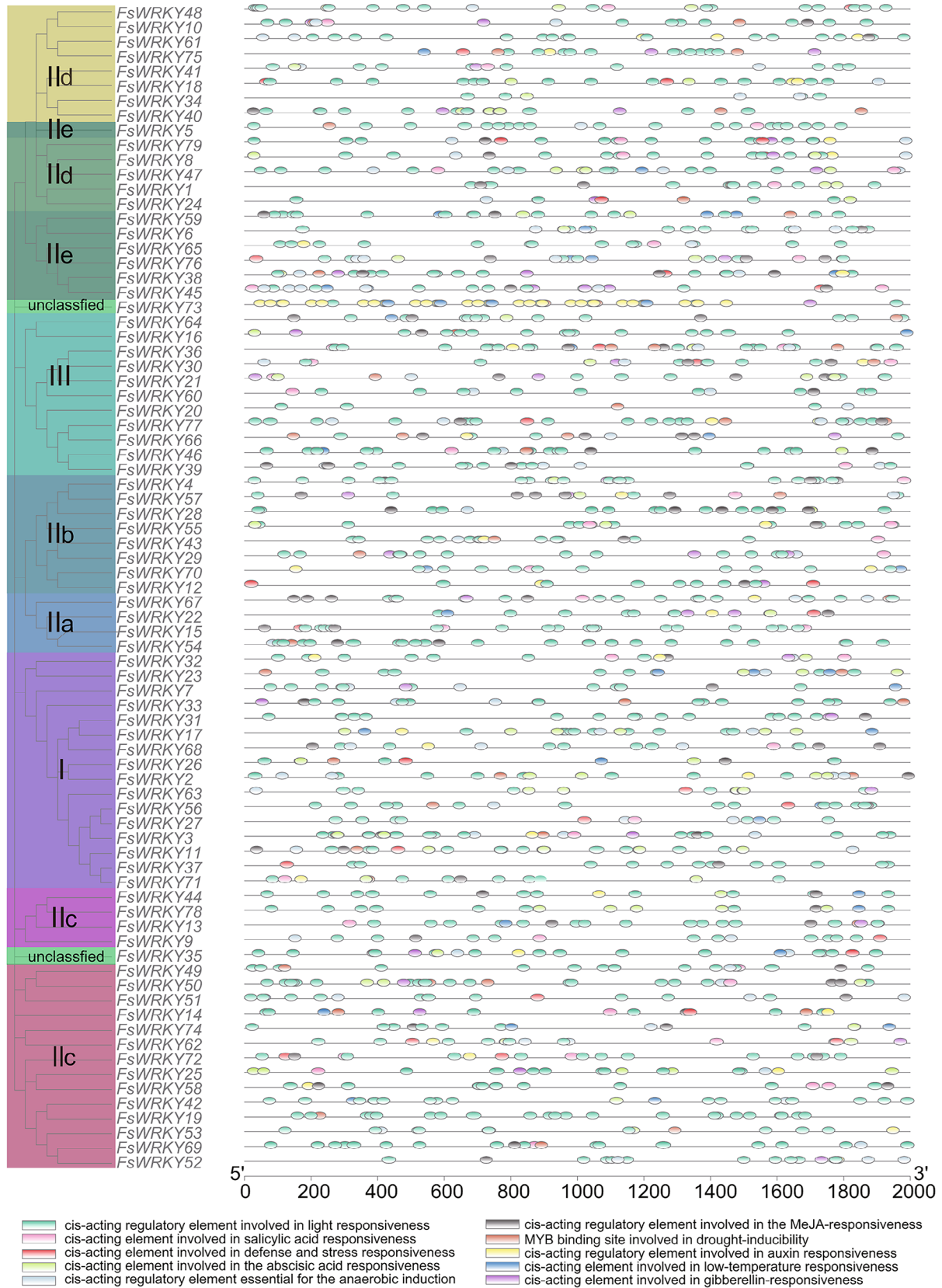


Fig. 5 Cis-acting elements analysis of the promoters of *WRKY* genes of weeping forsythia

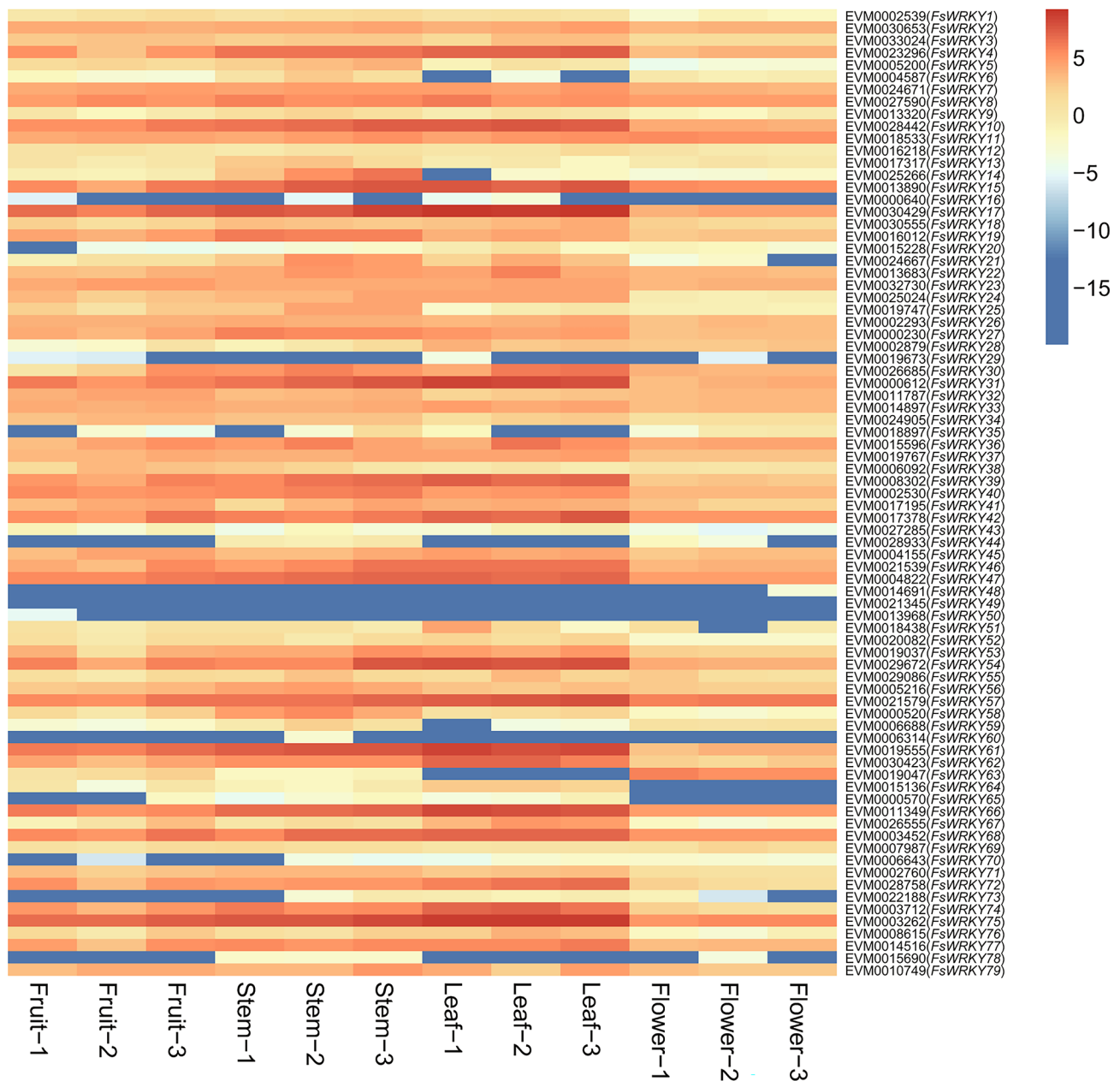


Fig. 6 Heat map of *WRKY* gene expression (Log_2FPKM) in different tissues of weeping forsythia

(Zhang and Wang, 2005). We identified 16 members in this subfamily in weeping forsythia, the same number as *A. thaliana* in this subfamily. The other six subfamilies are differentiated from I subfamily. In these newly differentiated subfamilies, except IIb subfamily, which had the same number of members as *A. thaliana*, the number of other subfamilies was slightly different. Not all II subfamilies of *WRKY* had zinc finger structure, only some members of IId subfamily had the conserved domain of Plant_zn_clust. This result indicated that the function of these members of IId subfamily might require the participation of zinc ions.

Except for the conserved *WRKY* domain, the *WRKY* gene varied greatly in length and structure. According to the number of members and clustering results, the members of each subfamily of *WRKY* gene in weeping forsythia did not have significant family expansion and contraction compared with that of *WRKY* gene in *A. thaliana*, and they might have more similar functions with that of *A. thaliana*.

At present, many studies had been conducted on the function of the *WRKY* genes. According to the existing research, *WRKY* gene functions mainly fall into the following three categories. First, it could increase the resistance to biotic

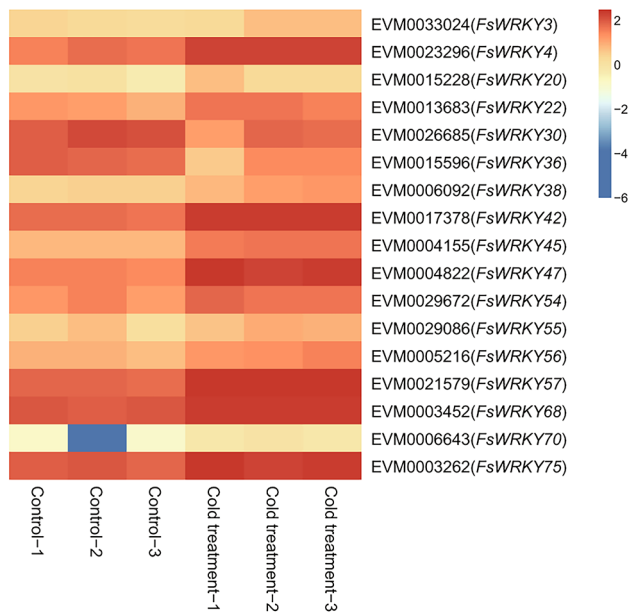


Fig. 7 Heat map of *WRKY* gene expression ($\text{Log}_{10}\text{FPKM}$) in response to cold stress of weeping forsythia

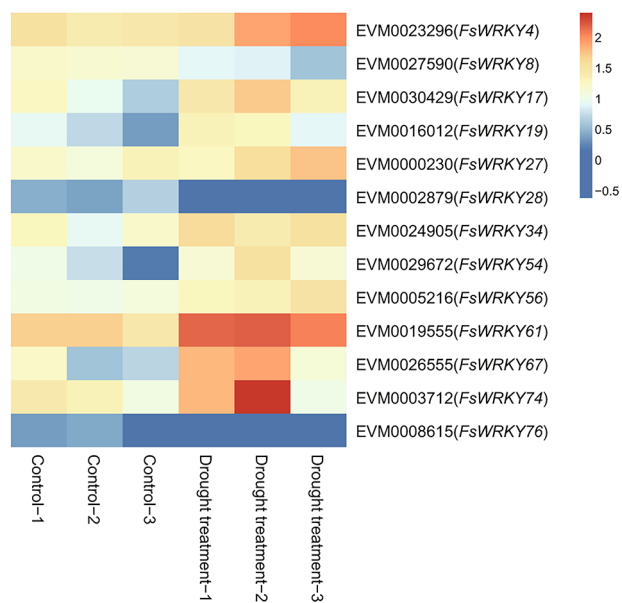


Fig. 8 Heat map of *WRKY* gene expression ($\text{Log}_{10}\text{FPKM}$) in response to drought stress of weeping forsythia

stress, such as the resistance to fungi (Fan et al. 2017), bacteria (Qiu et al. 2007), and viruses (Zou et al. 2019). Second, it could improve the abiotic stress resistance. For example, *WRKY* genes have been shown to participate in response regulation in drought (He et al. 2016), temperature (Wang et al. 2019b), salt (Fang et al. 2021) and nutrition (Wang et al. 2019c) stresses. Third, *WRKY* genes are likely involved into plant development regulation. Specifically, *WRKY* genes were suggested to participate in somatic embryo genesis

(Yang et al., 2020), promoted the synthesis of secondary metabolites in plants (Chen et al. 2017), regulated the flowering of plants (Lei et al., 2020), accelerated fruit ripening (Wang et al. 2017), and promoted fruit coloring (Cheng et al. 2017).

In *WRKY* genes of weeping forsythia, many cis-responsive elements (Fig. 5) of MeJA and SA were found in the upstream of the *WRKY* family members. The signal pathways mediated by MeJA and SA were usually associated with plant pathogen resistance (Halim et al. 2006; Findling et al. 2014). The expression patterns of *WRKY* gene in leaves, stems, fruits, flowers of weeping forsythia showed that more than half of *WRKY* genes were effectively expressed in these tissues. The *WRKY* genes might be involved in the development process and morphogenesis of leaf, stem, fruit, and flower tissues. In addition, the results of cis-element analysis showed that a large number of light responsive cis-regulatory elements in the upstream of these *WRKY* genes (Fig. 5). The development process and morphogenesis of leaves, stems, fruits, and flowers were usually high light-dependent (Franklin 2009). The effective expression of the *WRKY* genes in these tissues might be activated by light through light responsive cis-regulatory elements.

Two abiotic stresses, drought and cold stresses, are often encountered in the production and cultivation of weeping forsythia. Here, we investigated the expression patterns of genes in weeping forsythia leaves under drought and cold stresses. Under drought stress, 13 *WRKY* genes in weeping forsythia showed significant response expression, among which 4 genes had drought-related cis-elements with *MYB* binding sites, indicating that the responses of these 4 genes might be regulated by *MYB* transcription factors. Except for the drought-related cis-elements with *MYB* binding sites, 10 of these 13 genes had the ABA responsiveness cis-elements, indicating that most of the responding genes might be involved in ABA regulation pathways. In wheat, *WRKY* genes have been shown to participate in ABA-dependent way in response to abiotic stress (Budak et al. 2013). ABA signaling pathways is an important way to improve drought tolerance in plants, and *WRKY* genes play important roles in ABA signaling pathways (Wei et al. 2019). Under cold stress, 17 *WRKY* genes showed significant response expression, and 9 of the 17 genes had low temperature responsiveness cis-elements, which further confirmed that these genes were indeed related to cold stress. Previous studies have also confirmed that *WRKY* genes play important roles in improving cold tolerance in plants (Kim et al. 2016; Fei et al. 2022). Other genes which did not have low temperature responsiveness cis-elements might be activated by other signal pathways. On the whole, the genes involved in drought and cold stresses response in the *WRKY* gene family were

significantly fewer than those involved in light-dependent development and morphogenesis of different tissues.

Conclusion

In this study, we identified 79 *WRKY* genes in weeping forsythia and classified them according to their naming rules in *A. thaliana*. A phylogenetic tree showed that, except for the IIe subfamily, clustering was inconsistent with *A. thaliana* clustering. Cis-element analysis showed that *WRKY* genes related to pathogen resistance in weeping forsythia might be related to Me-JA and SA-mediated signaling pathways. Combining cis-element and expression pattern analyses of *WRKY* genes showed that more than half of *WRKY* genes were involved in light-dependent development and morphogenesis in different tissues. The gene expression results showed that 13 *WRKY* genes were involved in drought response, most of which might be related to the ABA signaling pathway, and a few of which might be regulated by *MYB* transcription factors. The gene expression results under cold stress showed that 17 *WRKY* genes were involved in low temperature response, and 9 of them had low temperature responsiveness cis-elements. Our study of the *WRKY* family in weeping forsythia provided useful resources for molecular breeding and important clues for their functional verification.

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Data Availability The datasets generated and/or analysed during the current study are available in the NCBI repository, <https://www.ncbi.nlm.nih.gov/> and accession no. RNA-seq of fruits, stems, and leaves; SRR17386487-SRR17386495; RNA-seq of flowers, SRX11342985, SRX11342993 and SRX11342994; RNA-seq of drought stress, SRX7503009, SRX7503010, SRX7503012-SRX7503015;

RNA-seq of cold stress, SRX7440183-SRX7440188; Genome data, JAHHPY000000000. Material samples are available from authors.

Declarations

Ethical approval and Consent to participate Not applicable.

Human and Animal Ethics Not applicable.

Consent for publication Not applicable.

Competing Interest The authors declare no competing interests.

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