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Genome-Wide Identification and Expression Analysis of the *PpYUCCA* Gene Family in Weeping Peach Trees (*Prunus persica* 'Pendula')

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Abstract: Auxin is an important endogenous plant hormone that is usually present as indole-3-acetic acid (IAA). The flavin monooxygenase YUCCA is the rate-limiting enzyme of IAA biosynthesis and plays an important regulatory role in plant growth and development. To further investigate the function of the YUCCA gene family in weeping peach trees, members of the YUCCA gene family were identified via bioinformatics analysis. The gene structure and conserved domains of the weeping peach YUCCA genes were investigated, and phylogenetic analysis and gene annotation were carried out. Fourteen *PpYUCCAs* were identified in the weeping peach variety 'Hongchuizhi' and were found to be randomly located on five different chromosomes. Moreover, the prediction of subcellular localization showed that most of the YUCCA proteins were localized in the cytoplasm. Based on our transcriptome analysis, only nine *PpYUCCAs*, including *PpYUCCA1*, *PpYUCCA3/4/5/6*, *PpYUCCA9*, and *PpYUCCA12/13/14*, were expressed in the weeping peach branches, which could result in the accumulation of auxin. *PpYUCCA6/12* may play a critical role in the appearance of the weeping trait, as indicated by the higher expression levels found in the Hongchuizhi variety compared with the Xiahui 6 variety. The results of this study provide a foundation for further research on the biological functions of *PpYUCCAs* in weeping peach trees.

Keywords: peach; weeping; flavin monooxygenase; auxin; gene family

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1. Introduction

The phytohormone auxin plays a vital role in plant growth and development and has been shown to be involved in processes such as vascular bundle differentiation, embryonic polarization, apical dominance, and senescence [1–3]. In one study, auxin content was markedly enhanced under shade conditions, and expressions of flavin monooxygenase (YUCCA) genes were upregulated to enable the shade avoidance induced by increases in auxin [4]. Liu et al. [5] found that aluminum stress in *Arabidopsis* could affect local auxin biosynthesis in the root apex transition zone by regulating YUCCA, resulting in the inhibition of root growth. However, the phytohormone auxin mainly exists in the form of indole-3-acetic acid (IAA). The synthesis of IAA uses tryptophan as a substrate through the indole-3-pyruvate (IPA) synthesis pathway. The IPA pathway contains two steps: (1) Tryptophan is converted into IPA by tryptophan aminotransferase; (2) IPA is then catalyzed to IAA by YUCCA. The second step is the rate-limiting step of IAA synthesis; therefore, YUCCA genes play a crucial role in the biosynthesis of IAA [6].

There is increasing evidence that suggests that auxin is involved in a variety of stress responses in plants, including responses to low temperature, high salt, drought, and wounds [7,8]. Indeed, auxin can induce the transient and rapid expression of auxin response genes, which activates the plant's defense system. The findings of Wang et al. [8] indicate that the *GhYUCCA22* gene plays a key role in modulating ABA homeostasis and regulating the ability of cotton to withstand drought. Cha et al. [9] also confirmed that the improvement of drought tolerance in *Arabidopsis* lines overexpressing *AtYUCCA6* was

not caused by elevated IAA levels, but rather by increased activity of peroxidase and upregulated expression of genes involved in redox homeostasis. IAA has been reported to act as a strong accelerator of branch growth in many species, and the high content of IAA in weeping branches may lead to longer branches in weeping progeny [10]. Using a comparative transcriptome analysis, Mao et al. [11] identified 10 candidate genes associated with IAA biosynthesis in *Prunus mume* that contributed to the weeping trait, including two IAA biosynthesis genes (*Pm013243* and *Pm030202*). The weeping trait appears to be a complex process regulated by a series of metabolic pathways. Genes that control the weeping trait have been found in a few species [11–14]. In peach and mulberry (*Morus alba*), the weeping trait is likely controlled by a single recessive gene, according to early cross-breeding results [15].

At present, 11 YUCCA genes have been identified in *Arabidopsis*, offering some evidence for the function of *AtYUCCA1/2/4/5/6/7* in the biosynthesis of IAA [16–20]. In one study, an *AtYUCCA6* activation mutant *Arabidopsis* presented higher IAA levels and the typical high-auxin phenotype, with epinastic cotyledons, elongated petioles, and strong apical dominance [21]. Using transcriptome and qRT-PCR analysis, Yang et al. [22] found that the expression level of *TaYUCCA7-A* was significantly upregulated in powdery mildew-induced wheat. Studies on YUCCA gene loss-of-function mutants have further demonstrated the important role that the YUCCA gene family plays in auxin synthesis and plant growth and development. It is worth noting that the inactivation of a single YUCCA gene in *Arabidopsis* does not cause significant developmental defects, whereas the overexpression of any YUCCA gene promotes the biosynthesis of auxin. However, the loss of function of multiple YUCCA genes in *Arabidopsis* has been shown to cause developmental defects. In particular, *AtYUCCA1* and *AtYUCCA4* double mutants were shown to have reduced vascular tissue and failed to produce normal inflorescences, whereas the phenotypes of quadruple *AtYUCCA1/2/4/6* mutants were more severe [16]. Previous studies on wheat [22], rice [23], tomato [24,25], and strawberry [26] have shown that members of the YUCCA gene family have highly conserved motifs, including the nicotinamide adenine dinucleotide phosphate (NADPH)-binding motif (GxGxxGME), FAD-binding motif (GxGPxGLA), GC motif (ERxxxxASL), etc., which may be key sites for the biological function of YUCCA genes [6]. In addition, the FAD-binding motif and NADPH-binding motif were shown to be identical in *FaYUC1* and *FaYUC2* and their corresponding *Arabidopsis* homologues [26].

Peaches (*Prunus persica* L.) are a popular fruit—cultivated worldwide—and a consumer favorite due to their sweet taste, pleasant aroma, phytonutrients, and attractive color. In addition to the edible fruit, peach blossoms also have high ornamental value. The weeping peach (*Prunus persica* var. pendula) variant has high ornamental value because of its weeping branches, which are similar to those of willows. Compared with studies on model plants, such as *Arabidopsis*, and other plants, such as rice and strawberry [16,26,27], there have been few studies focusing on peach YUCCA genes. In this study, with the accomplishment of peach genome sequencing and improvements in the peach genome database [28], a total of 14 peach YUCCA genes were comprehensively identified. In addition, the detailed characteristics, structures, functions, and expression levels of these genes were investigated. These analyses provided useful information for further investigations into the functions of the YUCCA gene family in the weeping peach. This study establishes a foundation for future exploration into the cloning and biological functions of weeping peach; in addition, these genes may be useful for the improvement of weeping peach trees.

2. Materials and Methods

2.1. Identification of PpYUCCA Genes in the Weeping Peach Genome

In order to identify YUCCA family genes in the weeping peach, we downloaded the nucleotide sequences of the YUCCA genes from the model species, *Arabidopsis* and *Solanum lycopersicum*, from TAIR (The Arabidopsis Information Resource: http://www.arabidopsis.org/) (accessed on 10 May 2022) and NCBI (https://www.ncbi.nlm.nih.gov/genome/?term=tomato) (accessed on 10 May 2022). As *Pyrus bretschneideri*, *Malus domestica*,

and *Prunus persica* belong to the Rosaceae family, and they all have the weeping trait, the nucleotide sequences of the YUCCA genes of *Pyrus bretschneideri* and *Malus domestica* were also downloaded from NCBI (https://www.ncbi.nlm.nih.gov/genome/12793) (accessed on 10 May 2022) and the Genome Database for Rosaceae (https://www.rosaceae.org/species/malus/all) (accessed on 10 May 2022). The predicted sequences were acquired using the BLASTp program (a given nucleic acid sequence was aligned with the database), with a threshold of 1×10^{-20}, according to peach genome data. Then, the encoded sequences of the PpYUCCA proteins were confirmed using pfamScan (v1.6) and Pfam A (v33.1) software. Subcellular localization was predicted using Softberry (http://linux1.softberry.com/berry.phtml?topic=protcomppl&group=programs&subgroup=proloc) (accessed on 10 May 2022). The chemical and physical properties (i.e., molecular weight, amino acid number, isoelectric point (pI), GRAVY, instability index, and aliphatic index) of the PpYUCCA proteins were calculated with the help of the ExPASy website (http://web.expasy.org/protparam/) (accessed on 10 May 2022). A user-friendly online tool (http://mg2c.iask.in/mg2c_v2.1/ (accessed on 10 May 2022), Chinese Academy of Agricultural Sciences, Qingdao, China) was used to explore the chromosome locations of the *PpYUCCA* members.

2.2. Classification and Structural Analysis of PpYUCCA Genes

An analysis of YUCCA gene structures was carried out with the aid of the Gene Structure Display Server (http://gsds.gao-lab.org/ (accessed on 10 May 2022), Peking University, Beijing, China). The common motifs were found using the MEME software (v5.0.5, http://meme.nbcr.net/meme (accessed on 10 May 2022), University of Nevada, Reno, NV, USA), where the maximum number of motifs was set to fifteen and the optimum width of the motifs was set from six to twenty. The protein sequences of YUCCA family genes from five species (peach, *Arabidopsis*, apple, pear, and tomato) were aligned with the help of MAFFT software (v7.427, University of Illinois at Urbana-Champaign, Urbana-Champaign, IL, USA), under the default parameters.

2.3. Phylogenetic Analysis of Weeping Peach YUCCA Genes

The full-length amino acid sequences of YUCCA proteins from five species (peach, *Arabidopsis*, apple, pear, and tomato) were downloaded from their respective genomic databases. A phylogenetic tree was created with the aid of the MEGA 7.0 software (Mega Limited, Auckland, New Zealand), using the neighbor-joining method and verified with one thousand bootstraps.

2.4. Plant Materials and Treatments

In this study, we selected two varieties of trees: the weeping peach 'Hongchuizhi' (herein termed 'HCZ') and the upright peach 'Xiahui 6' (herein termed 'XH', control group). The shoot tips from 6-year-old peach trees were sampled. These trees were grown in the Peach Experimental Garden at the Jiangsu Academy of Agricultural Sciences, Nanjing City, Jiangsu Province, China, and were trained and managed using standard horticultural practices. The shoot tips were harvested during the budding growth period (herein termed 'the early stage of branches growth', S1), and at the end of the growth period (herein termed 'the late stage of branches growth', S2). The shoot tips were immediately frozen in liquid nitrogen and stored at −80 °C prior to RNA extraction and gene expression analysis. To provide three replicates, each group consisted of three subgroups of three trees each.

2.5. Gene Expression Analysis

The transcriptomes of the two varieties were determined as described below. RNA quantification and qualification and cDNA library construction and sequencing were performed. Total RNA was extracted from the shoot tips using an RNAprep Pure Plant Kit (DP441, Tiangent, Beijing, China), following the manufacturer's instructions. The purity and concentration of the obtained RNA were measured using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity was evaluated using an Agilent

2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) with the help of an RNA 6000 Nano Assay Kit (Agilent Technologies, Palo Alto, CA, USA).

A 1 μg RNA sample was used for each examination. Sequencing libraries were generated with the help of Genepioneer Biotechnologies Co. Ltd. (Nanjing, China), and the NEBNext^{R} Ultra^{TM} Directional RNA Library Prep Kit for Illumina^{R} (NEB, Ipswich, MA, USA), according to the manufacturer's recommendations.

Reads containing the adapter and low-quality sequences were removed prior to analysis of the sequencing data. The resulting high-quality clean data were aligned against the peach reference genome of *Prunus persica* Genome V2.0.a1 [29] using HISAT2 software tools. Gene expression levels were calculated using the fragments per kilobase of exon model per million mapped fragments (FPKM) method. A differential gene expression analysis of the two varieties was conducted using the DESeq2 R package (1.26.0). The resulting *p* values were adjusted to control for the false discovery rate using the Benjamini–Hochberg method. Genes with an adjusted *p* value of <0.05 and an absolute $\log_2$(fold change) value of >1, found by DESeq2, were considered to be differentially expressed. KOBAS software was used to test the statistical enrichment of differentially expressed genes (DEGs) in KEGG pathways. The clusterProfiler R package was used to find the KEGG pathways that were significantly enriched compared with the entire genome background. Gene Ontology (GO) enrichment analysis of the DEGs was performed using the clusterProfiler R package.

2.6. Statistical Analysis

A completely randomized design was adopted in the experiments. The data were represented as the mean ± standard error (SE) of three replicates. Next, a one-way ANOVA was performed to establish the differences between the two varieties using Dunnett's post *t*-test with a *p* value of < 0.05 in SPSS 23.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. PpYUCCA Gene Family Analysis in the Weeping Peach Genome

A total of 14 YUCCA genes were identified in *Prunus persica* var. pendula (Table 1); the genes were named from *PpYUCCA1* to *PpYUCCA14*. The amino acid lengths of these *PpYUCCAs* were determined to be between 224 and 532 aa. The pIs were between 4.68 and 9.17, and the molecular weights ranged from 24644.57 to 60781.52 Da. Among these values, the pI of *PpYUCCA7* was the lowest, whereas the pI of *PpYUCCA3* was the highest. In addition, we predicted the subcellular localization of YUCCA proteins in peach cells, and the results showed *PpYUCCA5/6* was localized to the plasma membrane, *PpYUCCA2/8/11/12* in the extracellular space, and the remaining YUCCA proteins in the cytoplasm. The characteristics of the *PpYUCCA* gene family are displayed in Table 1, which contains the gene identifier in the genome database, chromosomal location, and some basic physical and chemical properties.

The genetic mapping of *PpYUCCA* genes to the chromosomes was carried out according to the genome data (Figure 1). These 14 *PpYUCCAs* were randomly located on five different chromosomes. Chromosome 01 had four *PpYUCCAs*; chromosomes 05 and 07 had two *PpYUCCAs* each; chromosome 06 contained only one *PpYUCCA*; and chromosome 08 contained five *PpYUCCAs*. It should be noted that *PpYUCCA5* and *PpYUCCA6* (located on Chr05) and *PpYUCCA11* and *PpYUCCA12* (located on Chr08) were very close, indicating that the two sets of genes were a pair of tandem duplication genes (Figures 1–4).

Table 1. Characteristics of the *PpYUCCA* gene family in peach trees.

Gene Name	Gene ID	Strand	Position	Transcript No.	CDS No.	Amino Acid No.	Molecular Weight (Da)	pI	Instability Index	Aliphatic Index	GRAVY	Location
PpYUCCA1	Prupe.1G054300	+	Pp01:3818775-3820670	1	3	424	47245.45	9	47.25	89.62	−0.158	Cytoplasmic
PpYUCCA2	Prupe.1G401400	–	Pp01:35432515-35435187	1	5	532	60781.52	6.45	40.37	78.97	−0.261	Extracellular
PpYUCCA3	Prupe.1G453400	–	Pp01:38104726-38108612	2	7	477	52240.22	9.17	63.13	81.55	−0.211	Cytoplasmic
PpYUCCA4	Prupe.1G468500	–	Pp01:39008965-39011716	1	4	409	45248.33	8.84	35.61	84.38	−0.163	Cytoplasmic
PpYUCCA5	Prupe.5G074800	+	Pp05:8900638-8903781	1	7	467	52998.92	5.64	43.01	79.44	−0.344	Plasma membrane
PpYUCCA6	Prupe.5G074900	+	Pp05:8904055-8907232	1	7	455	51384.34	6.15	38.17	80.53	−0.401	Plasma membrane
PpYUCCA7	Prupe.6G157500	–	Pp06:14093728-14095013	1	1	224	24644.57	4.68	28.57	80	−0.235	Cytoplasmic
PpYUCCA8	Prupe.7G193500	–	Pp07:18401078-18404874	1	5	521	59740.42	6.97	43.75	80.81	−0.311	Extracellular
PpYUCCA9	Prupe.7G231200	+	Pp07:20262969-20266244	1	4	432	48257.2	8.52	37.57	90.3	−0.053	Cytoplasmic
PpYUCCA10	Prupe.8G014100	+	Pp08:1154046-1156168	1	4	383	42744.13	8.78	37.17	87.99	−0.191	Cytoplasmic
PpYUCCA11	Prupe.8G177000	–	Pp08:17881032-17883309	2	7	514	57946.66	6.26	47.05	82.84	−0.135	Extracellular
PpYUCCA12	Prupe.8G177100	–	Pp08:17886297-17888935	1	5	528	59623.4	6.87	46.61	82.88	−0.222	Extracellular
PpYUCCA13	Prupe.8G211000	–	Pp08:19516938-19519204	1	3	423	47290.8	9.06	45.81	84.99	−0.145	Cytoplasmic
PpYUCCA14	Prupe.8G252500	–	Pp08:21655932-21658120	2	5	384	42867.25	8.67	38.03	86.02	−0.181	Cytoplasmic

Note: ID—identifier; pI—isoelectric point; GRAVY—grand average of hydropathy.

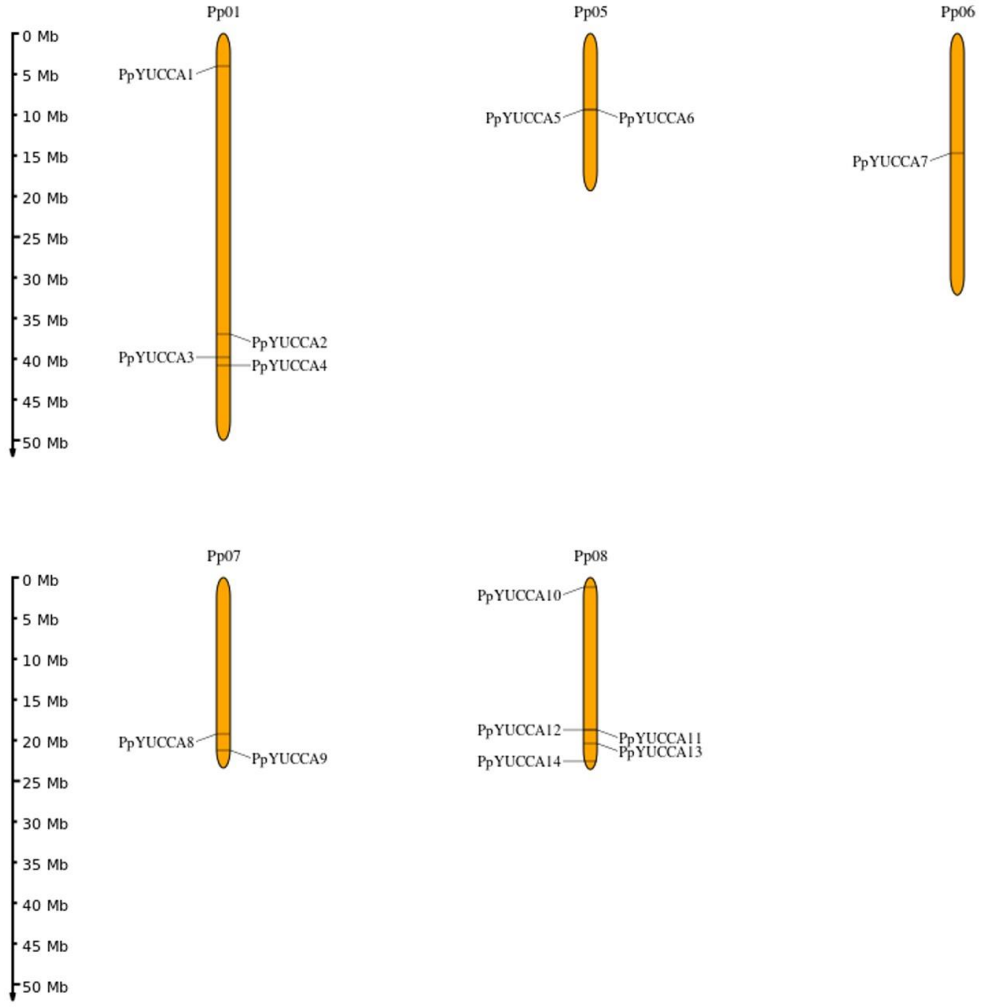

Figure 1. Chromosomal locations of *Prunus persica* var. pendula YUCCA genes. The scale represents a 50 Mb chromosomal distance.

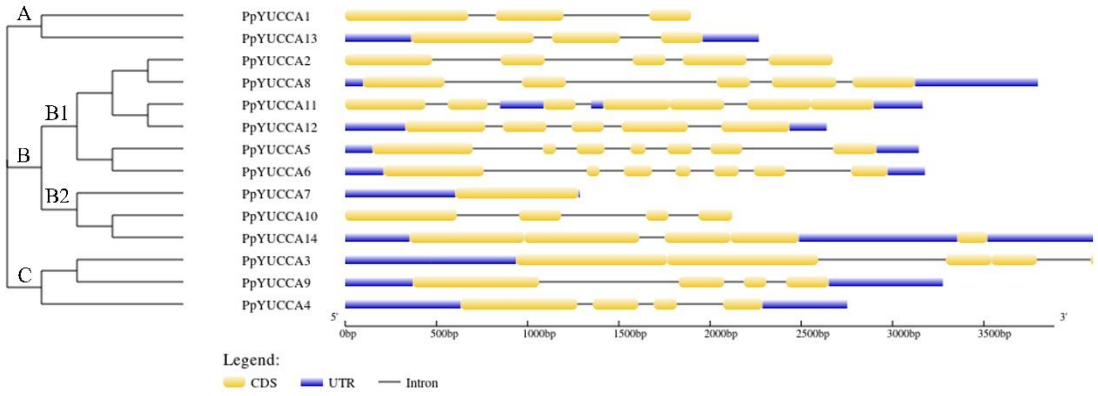

Figure 2. Structure of *Prunus persica* var. pendula YUCCA genes. Introns, exons, and untranslated regions (UTRs) are represented by black lines, deep orange boxes, and blue boxes, respectively.

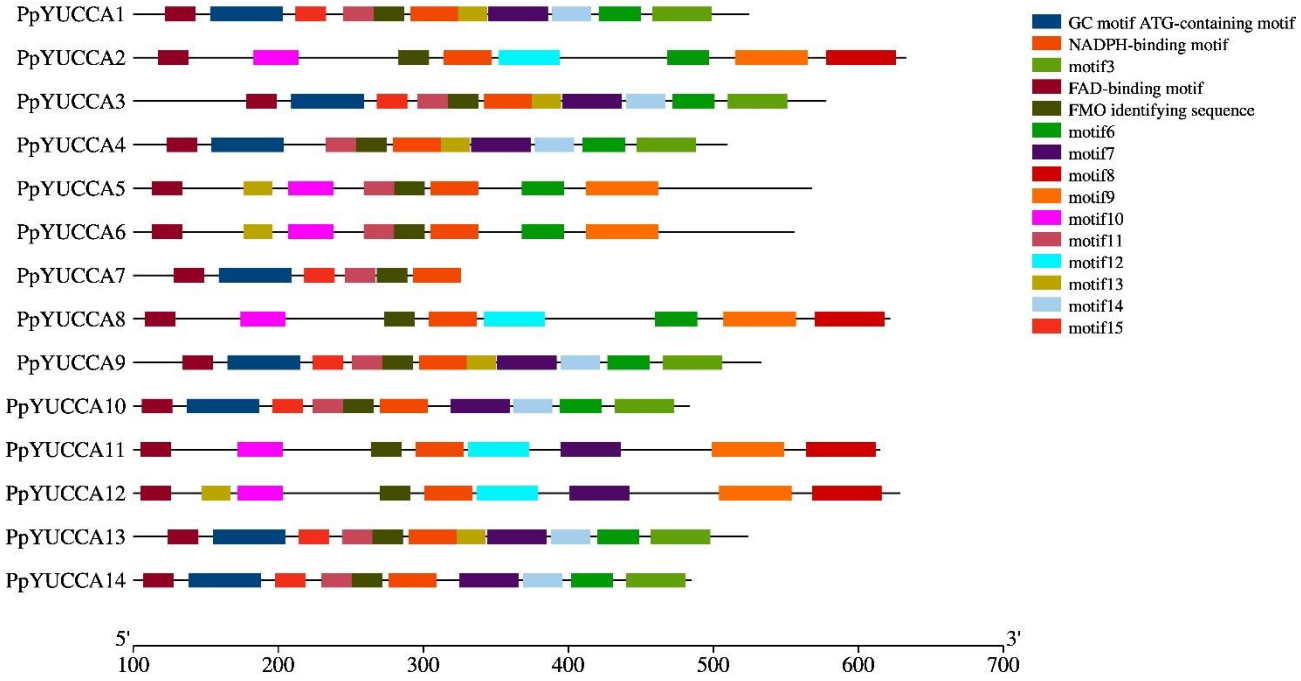

Figure 3. YUCCA gene family motifs in *Prunus persica* var. pendula. The various conserved motifs are labeled with colored boxes.

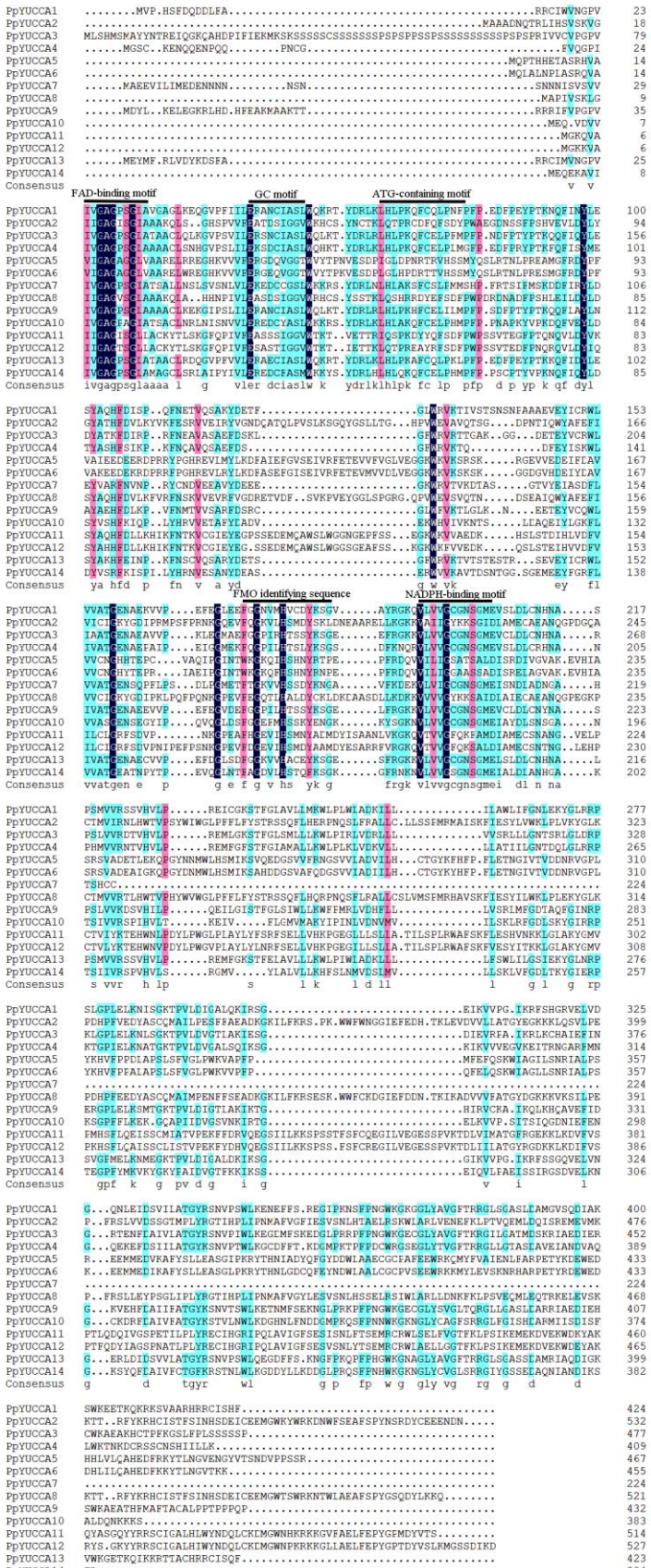

Figure 4. Multiple sequence alignment of *Prunus persica* var. pendula YUCCA gene family.

3.2. Classification and Structural Analysis of PpYUCCA Genes

The peach *PpYUCCAs* were divided into four subfamilies based on their similarities (Figure 2). As shown in Figure 2, *PpYUCCA1* and *PpYUCCA3* were grouped in subfamily A, and *PpYUCCA7/10/14* and *PpYUCCA3/9/4* were placed in subfamily B2 and C, respectively. The remaining genes were grouped in subfamily B1 (Figure 2).

In order to study the gene structure of *PpYUCCAs*, information on the genome and CDS sequence for each YUCCA gene was obtained according to the genome data; the exon–intron gene structure map is shown in Figure 2. The *PpYUCCA* genes contained between one and seven exons. *PpYUCCA7* only contained one exon, whereas *PpYUCCA3/5/6/11* contained seven exons. Moreover, *PpYUCCA1/3* contained three exons, *PpYUCCA4/9/10* contained four exons, and *PpYUCCA2/8/12/14* contained five exons (Figure 2).

To further study the conserved domain of YUCCA proteins in the weeping peach, multiple alignment of amino acid sequences of the YUCCA proteins were used to identify the conserved protein motifs (Figures 3 and 4). A total of 15 distinct motifs were discovered in the experiment; among them, five motifs were identified. These identified conserved motifs were the FAD-binding motif, GC motif, ATG-containing motif, FMO-identifying sequence, and NADPH-binding motif. Of these five conserved motifs, the FAD-binding motif, GC motif, and ATG-containing motif were located close to each other at the N-terminus of the amino acid sequences. The FMO-identifying sequence and NADPH-binding motif were located in the middle region of the amino acid sequences.

3.3. Phylogenetic Analysis of Weeping Peach YUCCA Genes

In order to further analyze the evolutionary relationships between YUCCA proteins among different species, the YUCCA proteins of *Arabidopsis thaliana*, *Malus domestica*, *Pyrus bretschneideri*, *Solanum lycopersicum*, and *Prunus persica* var. pendula were used to construct a phylogenetic tree using MEGA 7.0 software. As shown in Figure 5, 76 YUCCA proteins from five species were grouped into five categories. Among them, Groups I, III, and V each contained two different weeping peach YUCCA genes: *PpYUCCA7* and *PpYUCCA10* (Group I); *PpYUCCA1* and *PpYUCCA13* (Group III); and *PpYUCCA3* and *PpYUCCA9* (Group V). Group IV only contained one YUCCA protein, namely *PpYUCCA4*, whereas the remaining YUCCA proteins were all in Group II. Except for the six YUCCA proteins in Group II (*PpYUCCA2/5/6/8/11/12*), the results of the phylogenetic analysis certified that the YUCCAs of Rosaceae tended to be clustered together, as shown in Figure 5. Therefore, we concluded that the weeping peach YUCCA proteins were highly evolutionarily conserved. These results will provide important support for the further analysis of weeping peach YUCCA genes.

3.4. Gene Expression Analysis

When comparing the DEGs between HCZ and XH at the same point in time based on KEGG pathways, the most enriched pathway at the early stage of branch growth was ‘carbon metabolism’, followed by ‘metabolism of terpenoids and polyketides’ and ‘biosynthesis of other secondary metabolites’ (Figure 6A); the most enriched pathway at the late stage of branch growth was ‘carbon metabolism’, followed by ‘biosynthesis of other secondary metabolites’ and ‘amino acid metabolism’. Three major categories were identified according to the GO annotations and comparison of DEGs between HCZ and XH at the same point in time, including those associated with cellular components, molecular functions, and biological processes (Figure 7). Genes associated with cell, cell parts, and cellular process were the most abundant.

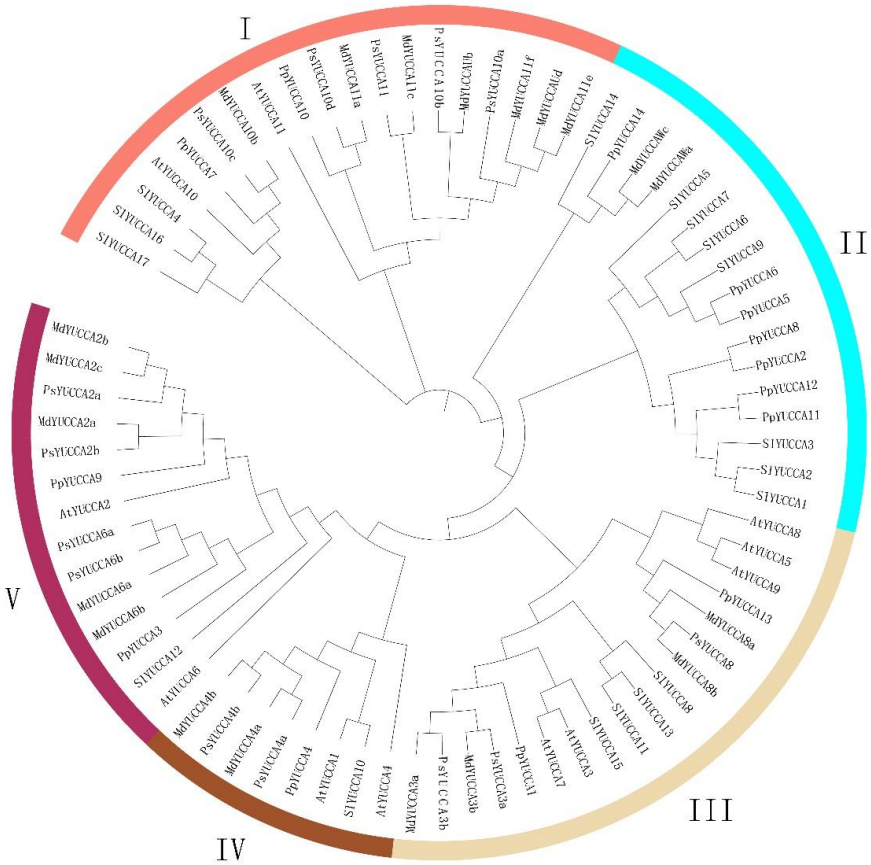

Figure 5. Phylogenetic tree of YUCCA genes in Prunus persica var. pendula. At—*Arabidopsis thaliana*; Md—*Malus domestica*; Ps—*Pyrus bretschneideri*; Sl—*Solanum lycopersicum*; Pp—*Prunus persica* var. pendula.

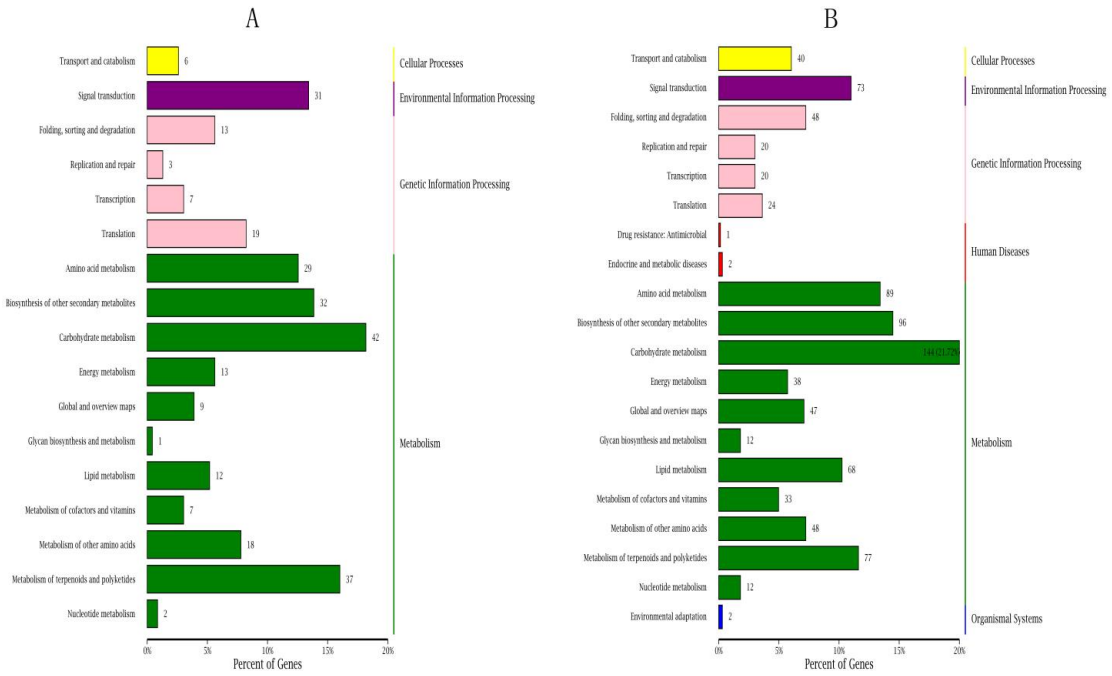

Figure 6. KEGG pathway classification of DEGs during the growth period of weeping peach branches: (**A**) comparison of HCZ and XH at the early stage of branch growth (HCZS1 vs. XHS1); (**B**) comparison of HCZ and XH at the late stage of branch growth (HCZS2 vs. XHS2). HCZ—weeping peach 'Hongchuizhi' variety; XH—standard peach 'Xiahui 6' variety.

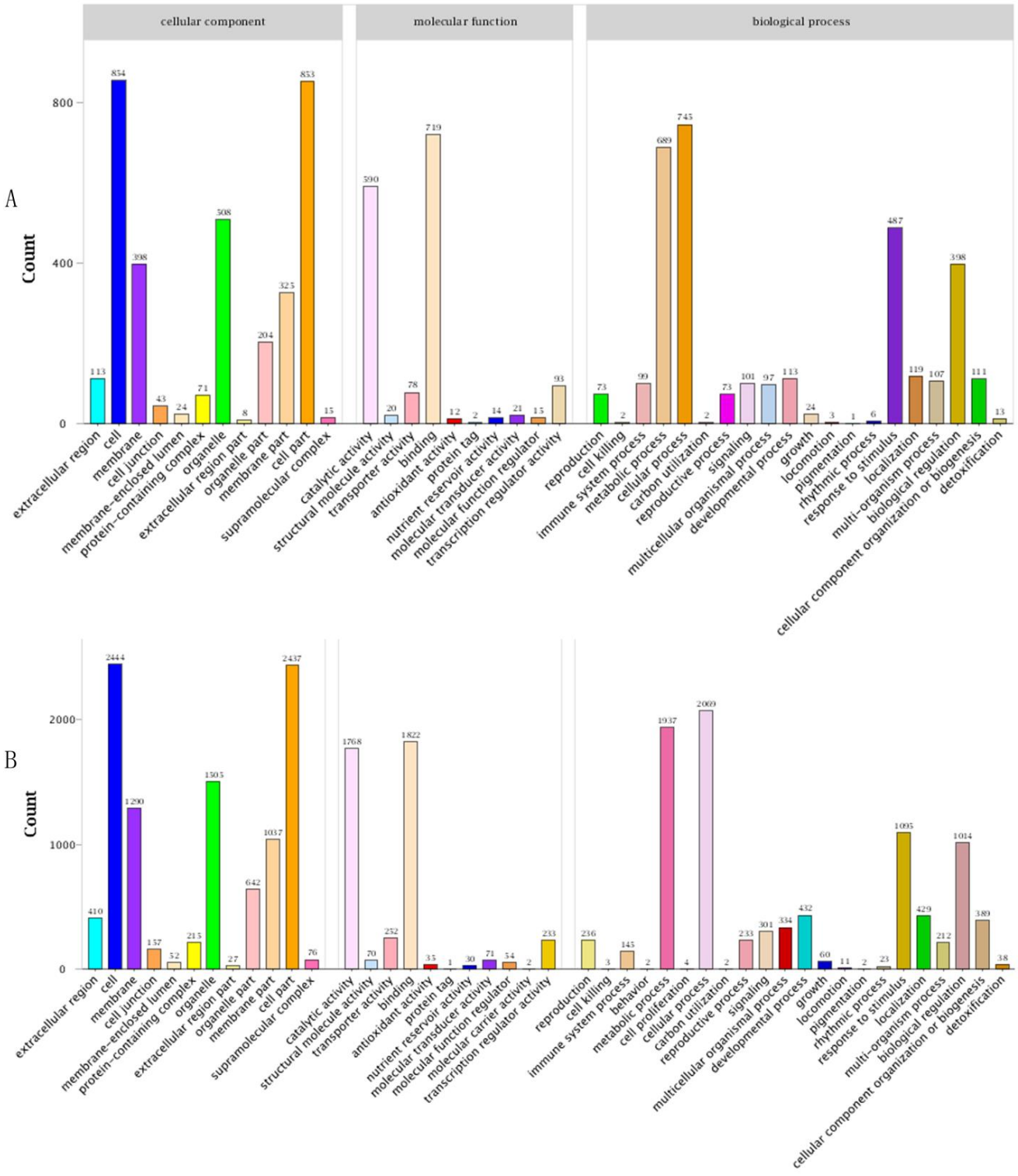

Figure 7. GO classification of DEGs during the growth period of weeping peach branches: (**A**) comparison of HCZ and XH at the early stage of branch growth (HCZS1 vs. XHS1); (**B**) comparison of HCZ and XH at the late stage of branch growth (HCZS2 vs. XHS2). HCZ—weeping peach 'Hongchuizhi' variety; XH—standard peach 'Xiahui 6' variety.

In this experiment, nine *PpYUCCAs* were found to be expressed in weeping peach branches (Figure 8), including *PpYUCCA1*, *PpYUCCA3/4/5/6*, *PpYUCCA9*, and *PpYUCCA12/13/14*. To explore the roles of these genes, the expression patterns of the

different varieties of *PpYUCCAs* during different developmental periods were carried out using a heatmap.

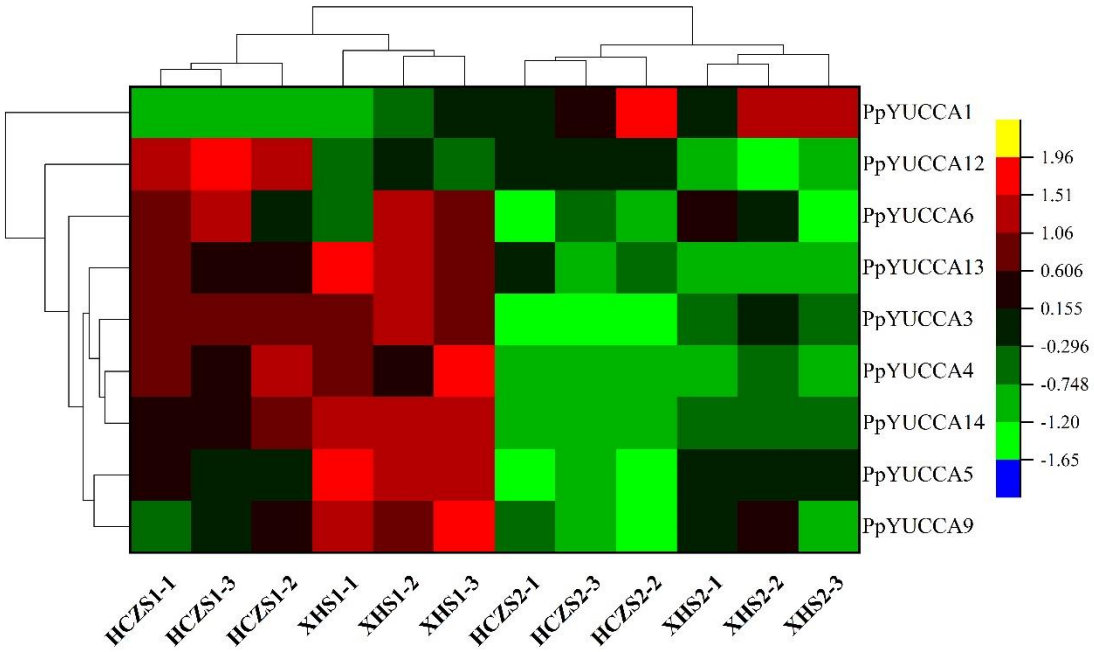

Figure 8. Heatmap cluster analysis of the FPKM of YUCCA genes during the growth period of weeping peach branches. HCZ—weeping peach 'Hongchuizhi' variety; XH—standard peach 'Xiahui 6' variety; S1—early stage of branch growth; S2—late stage of branch growth.

As shown in Figure 8, in comparison to the budding growth period, only *PpYUCCA1* exhibited higher expression at the end of the growth period in both varieties. The expressions of *PpYUCCA3/4/5/6*, *PpYUCCA9*, and *PpYUCCA12/13/14* in the two varieties were higher during the budding growth period than at the end of the growth period. In addition, the expressions of *PpYUCCA6/12* in HCZ were higher than those in XH during the budding growth period. These results suggest that *PpYUCCA6/12* may have greater effects on the weeping branches of peach trees.

4. Discussion

Weeping branches, which act as a special morphological structure of trees, have become a research hotspot, as they are widely used in greenery and garden landscapes worldwide due to their beautiful shape. An increasing number of studies have indicated that auxins, including IAA, play a critical role in the weeping trait of branches [30,31]; however, the molecular information underlying weeping peach trees is still unclear. YUCCA, which converts IPA into IAA, is the rate-limiting enzyme in the IPA pathway [6]. In this paper, to gain insight into peach YUCCA genes, the gene structure and conserved domains of the YUCCA gene family were investigated, and a phylogenetic analysis and gene annotation were carried out. Based on our transcriptome analysis, a total of 14 YUCCA genes were identified in peach trees, only nine of which were expressed in the 'HCZ' weeping peach variety.

The structure of proteins determines their biological function, and proteins with similar structures have similar functions. From the subcellular localization analysis, it was found that most of the weeping peach *PpYUCCA* proteins were localized in the cytoplasm, indicating that the synthesis of auxin by these genes was conducted in the cytoplasm; this is consistent with the results of Yang et al. [22]. The analysis of the conserved motifs of the weeping peach YUCCA proteins showed that the number of motifs in the 14 proteins ranged from 6 to 11, indicating that the functions of the weeping peach YUCCA gene family are diverse. As shown in Figures 2 and 3, the closely related proteins clearly share

the same motif profiles, indicating that the weeping peach YUCCA proteins within one branch may have the same conserved function [8]. Furthermore, the conserved motifs were almost identical in number and type. In a way, the specific motifs may result in functional divergences [8,32]. Although the presence or lack of specific motifs may lead to functional variation, this needs to be experimentally demonstrated with precision. Moreover, the specific biological functions of the remaining motifs remain to be further investigated. Similar to the results of studies using wheat and strawberry [22,26], the conserved motifs—including the FAD-binding motif, GC motif, ATG-containing motif, FMO-identifying sequence, and NADPH-binding motif—were also found in the 14 weeping peach YUCCA proteins (Figure 4); thus, these conserved motifs might be critical sites for the biological function of YUCCA genes [6,26].

Phylogenetic analysis showed that the *PpYUCCAs* could be divided into five subfamilies. Most of the Rosaceae YUCCA proteins were clustered together (Figure 5), indicating that peach YUCCA genes are highly evolutionarily conserved compared with those of *Malus domestica* and *Pyrus bretschneideri* [33,34]. In barley and *Arabidopsis*, high temperature conditions were shown to lead to male sterility via the repression of *YUCCA2/6* expression, which decreased the auxin level in the developing anthers [35]. *PpYUCCA3/9* were most closely related to *AtYUCCA2*/6 of *Arabidopsis thaliana* (Figure 5); it was speculated that they may mediate peach growth and early pollen development by regulating auxin signal transduction [36]. The higher expressions of *PpYUCCA3/9* in the two varieties during the budding growth period compared with the end of the growth period also verified the above results (Figure 8). In one study, *MdYUCCA11d* appeared to be specifically expressed in the apple pistil, and it was found to be responsible for auxin synthesis there [33]. *PpYUCCA2/10* appeared to be closely related to *MdYUCCA11* in the phylogenetic analysis (Figure 5), indicating specific roles during the development of flower and fruit [33]. This may be the reason why we did not observe the expression of *PpYUCCA2/10* in weeping peach branches. The overexpression of *MdYUCCA8a* in *Arabidopsis* was shown to increase the level of auxins and produce expected auxin overproduction phenotypes, such as taller plant height and enhanced apical dominance [33]. An increase in the expression of the homologous gene *PpYUCCA13* was also successfully detected in weeping peach branches during the budding growth period (Figure 5), indicating that *PpYUCCA13* may be a critical gene for IAA biosynthesis in weeping peach trees [37].

Interestingly, the *PpYUCCA2/5/6/8/11/12* proteins were not clustered together with the proteins of Rosaceae and *Arabidopsis thaliana*; instead, they were placed in Group II with the YUCCA proteins of *Solanum lycopersicum* (Figure 5). These results suggest that the weeping peach YUCCA gene family has been amplified along with whole-genome duplication [38]. The *PpYUCCA5/PpYUCCA6* genes and *PpYUCCA11/PpYUCCA12* genes were determined to be a pair of tandem duplication genes, indicating that tandem duplication is also a method of gene amplification in the weeping peach YUCCA gene family [39]. As genes can be amplified in a variety of ways, including whole-genome duplication, tandem duplication, retrotransposition, etc., the specific gene amplification mode of the members of the weeping peach YUCCA gene family should be further explored. However, the functions of the Group II genes have not yet been validated in the weeping peach or *Solanum lycopersicum*. Our transcriptome data suggest that the expression of *PpYUCCA6/12* in HCZ was higher than that in XH during the budding growth period (Figure 8); thus, we speculate that *PpYUCCA6/12* may contribute to the weeping trait of peach branches.

5. Conclusions

In conclusion, 14 *PpYUCCAs* were systematically identified in weeping peach branches. In this study, the gene structure and conserved domains of the weeping peach YUCCA gene family were determined, and phylogenetic analysis and gene annotation were carried out. Based on our transcriptome analysis, a total of nine *PpYUCCAs*, including *PpYUCCA1*, *PpYUCCA3/4/5/6*, *PpYUCCA9,* and *PpYUCCA12/13/14*, were expressed in the weeping peach branches, which may result in the accumulation of auxin. Among them,

PpYUCCA6/12 were more likely to play major roles in the appearance of the weeping shape, as indicated by the higher expression levels in HCZ. However, the functions of these genes require further verification to determine what controls the weeping trait of peach trees more precisely. The useful genetic information presented in this study will be beneficial to the improvement of weeping peach trees.

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