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Genome-wide identification and expression analysis of *Aux/IAA* gene family in strawberry (*Fragaria vesca*)

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Abstract: Auxin signaling and its components (Auxin/Indole-3-Acetic Acid (Aux/IAA)) are critical for plant growth and development. Here, we performed a genome-wide annotation and identified twenty-one Aux/IAA genes in strawberry (*Fragaria vesca*). Most *FveIAAs* were located on chromosomes 1, 2, 4, 5, and 6, while no *FveIAAs* were found in chromosomes 3 and 7. Phylogenetic analysis divided these genes into nine subfamilies. Most FveIAAs contained the DNA-binding and Aux/IAA domains, as well as motifs I–IV. There were 2–6 exons in the *FveIAA* genes based on the gene structure analysis. Also, we found that four pairs of *FveIAA* genes underwent segment duplications. Moreover, four pairs of orthologous genes were observed between strawberry and *Arabidopsis*. Cis-element analysis in the promoter region indicated that *FveIAAs* may be involved in light, phytohormones, stress responses, and growth processes. Prediction of protein-protein interaction revealed that 17 of 21 FveIAA proteins were involved in the auxin-related signaling pathways. Additionally, *FveIAAs* showed tissue-specific expression and responded to IAA treatment. Thus, this systematic annotation of the *FveIAA* family would provide a fundamental basis for further functional and evolutionary analysis and to understanding the role of *FveIAAs* in strawberry growth and development.

Introduction

Auxin, a plant hormone, modulates plant growth and development by regulating the expression of Gretchen Hagen 3 (GH3), Auxin Response Factor (ARF), Indole-3-acetic Acid (Aux/IAA), and Small Auxin Up RNA (SAUR) gene families (Abbas *et al.*, 2016; Aloni *et al.*, 2006; Esmon *et al.*, 2006; Mattsson *et al.*, 2003; Mishra *et al.*, 2009; Tiryaki, 2009). In the presence of cycloheximide, a translational inhibitor, auxin induces the expression of *Aux/IAA* genes. The degradation of Aux/IAA protein through the 26S proteasome pathway is induced by the auxin transport inhibitor response 1 (TIR1), which regulates the expression of auxin-responsive genes by releasing ARFs (Farcot *et al.*, 2015; Hu *et al.*, 2015b).

There are four conserved domains present in *Aux/IAA* genes with domain I containing a leucine repeat motif (LXLXLX) as a potent transcriptional repressor; domain II inducing Aux/IAA protein degradation; domain III constituting a βαα-DNA recognition motif; domain IV representing an acidic region (Liscum and Reed, 2002). Domains III and IV are also known to induce the homodimerization and heterodimerization between the ARFs and the Aux/IAA proteins (Mano and Nemoto, 2012). ARFs modulate the expression of auxin- responsive genes by specifically binding to the AuxRE (TGTCTC) sequence in their promoter region (Kim *et al.*, 1997; Ulmasov *et al.*, 1997). Aux/IAA proteins suppress the activity of ARF by interacting with the DNA-bound ARF partner protein through domains III and IV. Additionally, Aux/IAA proteins are directed towards the nucleus via two localization signals (Retzer *et al.*, 2014; Wu *et al.*, 2012). Genomic analyses have identified Aux/IAA gene family in the following plants: 29 in *Arabidopsis*, 31 in rice (*Oryza sativa*), 26 in tomato (*Solanum lycopersicon*), 27 in cucumber (*Cucumis sativus*), and 34 in maize (*Zea mays*) and other species, including *Medicago truncatula*, *Populus trichocarpa*, *Vitis vinifera*, etc. (Audran-Delalande *et al.*, 2012; Cakir *et al.*, 2013; Dreher *et al.*, 2006; Gan *et al.*, 2013; Jain *et al.*, 2006; Kalluri *et al.*, 2007; Wang *et al.*, 2010;

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Wu *et al.*, 2012). Studies on the functional analysis of IAAs in plant growth and development revealed the role of *iaa3/shy2*, *iaa7/axr2*, *iaa14/slr*, *iaa17/axr3*, *iaa28* in lateral root formation in Arabidopsis (Fukaki *et al.*, 2002; Knox *et al.*, 2003; Mai *et al.*, 2011; Timpte *et al.*, 1994). In rice, three Aux/IAA members: *OsIAA1*, *OsIAA11*, and *OsIAA23*, were involved in regulating root development (Ni *et al.*, 2011; Thakur *et al.*, 2001; Zhu *et al.*, 2012). In tomato, the under-expression of *Sl-IAA9* affected leaf morphogenesis and the fruit set process (Wang *et al.*, 2005). The downregulation of *Sl-IAA15* resulted in reduced apical dominance, a lower trichome number, dark green leaves, and increased lateral root formation (Deng *et al.*, 2012). Also, reduced fertilization and altered fruit development were observed due to the silencing of the *Sl-IAA27* gene (Bassa *et al.*, 2012). Another study revealed the role of the *Sl-IAA17* transcriptional repressor in controlling fruit size by regulating endoreduplication-related cell expansion (Su *et al.*, 2014).

Strawberry, a delicious and healthy food, constitutes an important fruit crop worldwide. *Fragaria vesca* is diploid (2n = 14), has a small genome size (<240 Mb), a relatively short reproductive cycle (14–15 weeks), and its genome sequence is available; thus, it is considered a model plant for studying transformation (Shulaev *et al.*, 2011). Strawberry fruits are non-climacteric as they do not undergo ethylene-induced ripening. Previous studies have confirmed the role of auxin in the fruit set, development, and ripening in strawberry (Kang *et al.*, 2013; Nitsch, 1950); however, its molecular regulation mechanisms remain unclear.

Past studies on plant genome sequences have enabled the genome-wide analyses of several multigenic protein families. Here, we used the public databases to conduct the genome-wide analyses of the strawberry Aux/IAA family, including genomic organization, the conserved protein domains, comparative phylogenetic analyses, prediction of protein structural motifs, putative *cis*-regulatory elements within promoters, subcellular location, and protein-protein interactions (PPI). We studied the expression of the Aux/IAA members in different organs at different developmental stages of the fruit and the expression of *FveIAAs* post-IAA treatment. Thus, this systematic annotation of the *FveIAA* family would provide a fundamental basis for the functional and evolutionary analysis, and to understand the role of the *FveIAAs* in strawberry growth and development.

Materials and Methods

Aux/IAA genes in Fragaria vesca

The strawberry genome and proteome were downloaded from Phytozome V12 (Goodstein *et al.*, 2012) to perform exhaustive data mining of the *FveIAA* family. The IAA protein sets from *A. thaliana* were obtained from The *Arabidopsis* Information Resource (TAIR) (Swarbreck *et al.*, 2008). Default parameters and cutoff value 0.01 was used for the hidden Markov Model (HMM) profiles to identify the *Aux/IAA* genes from the *F. vesca* genome (Eddy, 1998). The presence of conserved domains in the candidate Aux/IAA genes was evaluated using PFAM V32 and SMART tools (Finn *et al.*, 2016; Schultz *et al.*, 1998). NCBI CDD was used to examine the uniqueness of the obtained sequences for the Aux/IAA domains. ProtParam software was used to determine the molecular weight (MW) and the isoelectric point (pI) and of the FveIAA proteins. The annotated Aux/IAAs of strawberry were labeled as '*FveIAA*' followed by a number representing their chromosomal orders.

In silico characterization of FveIAAs

Based on the strawberry genome database, we mapped all *FveIAA* genes to strawberry chromosomes using Circos (An *et al.*, 2015). The chromosomal location of the FveIAA family was visualized using MapChart V2.1 (Voorrips, 2002). The gene structure of *FveIAA* genes was extracted from Phytozome and visualized using GSDS V2.0 (Hu *et al.*, 2015a). MEME V5.1 and PFAM were used to detect the conserved motifs and functional domains, respectively (Bailey *et al.*, 2009; Finn *et al.*, 2016). A Multiple Collinearity Scan toolkit (MCScanX) with default parameters was used to study the gene duplication events (Wang *et al.*, 2013). A syntenic analysis map was built using Dual System Plotter to study the relationship between the orthologous *Aux/IAA* genes obtained from strawberry and other organisms. STRING V11.0 with a threshold of 0.7 was used to construct the PPI network (Szklarczyk *et al.*, 2017). The promotors (1500 to 1 bp before ATG) of the *FveIAA* genes were obtained from Phytozome, and the cis-elements were predicted by PlantCARE (Lescot *et al.*, 2002), and subcellular localization of FveIAAs was predicted by WoLF PSORT (Horton *et al.*, 2007).

Phylogeny of FveIAAs and AtIAAs

ClustalW and DNAMAN were used for multiple sequence alignment of the complete FveIAA and AtIAA protein sequences. The neighbor-joining (NJ) phylogenetic tree was built using MEGA V7.0 (Kumar *et al.*, 2016), with a poison model and 1000 bootstrap replications. FveIAA protein sequences from *Arabidopsis* (Dreher *et al.*, 2006) were obtained from Phytozome (Goodstein *et al.*, 2012).

Plant growth condition and hormonal treatments

The strawberry (*F. vesca f. semperflorens*) plants were procured from Xi'an University, Xi'an, China. The plants were grown in a greenhouse at a temperature of 20°C–25°C with a 14-h light/10-h dark cycle and relative humidity of 70%–85%. During the first week after anthesis, more than 100 small green (SG) fruits on 50 strawberry plants were labeled. Fruits were collected on days 7, 14, 25 days post fertilization, at three different stages: SG, BG (Big green), and R (Red), respectively. At each stage, sampling of uniformly sized fruits (N = 10) was done in triplicates. Small cubes of the receptacle (pulp) (0.5–0.8 cm^3) were flash-frozen in liquid nitrogen and stored at −80°C. The other tissues were collected from healthy strawberry plants (N = 10) at the flowering stage and analyzed in triplicates. The newly growing and fully expanded leaves were used for the IAA treatments. The branch containing three-piece leaves were cut from the strawberry plants and dipped in 10 μM IAA (pH 6.6) solution. The branches treated with H_2O served as the control. The samples were collected 0, 1, 6, and 12 h after treatment. For each treatment, 24 branches were sampled from 12 different plants. All samples were flash-frozen in liquid nitrogen and stored at −80°C for RNA extraction.

RNA isolation and qRT-PCR analysis

TRIZOL Reagent was used to isolate total RNAs from the frozen samples (100–200 mg), followed by treatment with Turbo DNA-free TM kit to eliminate DNA contamination. After reverse transcription into cDNA, qRT-PCR was performed with the reaction mixture (20 µL) containing 1 µL forward/reverse specific primer (10 µM), 1 µL cDNA (30 ng/µL), and 10 µL SYBR Green Master Mix on an ABI Quant Studio tm 6 Flex Real-Time PCR System. The cycle parameters included 95°C for 3 min; 40 cycles at 95°C for 20 s, 58°C for 30 s, and 72°C for 30 s; 71 cycles increasing from 60°C to 95°C at 0.5°C per cycle for 30 s. FvActin (GenBank accession no. AB116565.1) was used as the internal reference to calculate the relative fold differences, and the data were analyzed using a previously described comparative CT method ($2^{-\Delta\Delta Ct}$) (Su *et al.*, 2015). A *P*-value < 0.05 was regarded as statistically significant. NCBI primer blast was used to design the primers for qRT-PCR (Suppl. Tab. S1) using *F. vesca* mRNA as the reference database.

Results

Identification of Aux/IAA family genes in the strawberry genome

We identified and characterized 21 *Aux/IAA* genes in the *F. vesca* genome, which were labeled *FveIAA1-21* based on their chromosomal localization. In-depth analysis of each predicted *FveIAA*, including chromosomal localization, gene length, deduced protein length, MW, pI, and exon numbers, was performed (Tab. 1). The *FveIAA* genes encode 181 (*FveIAA16*) to 370 amino acids (aa) (*FveIAA3*) with corresponding MWs of 20.45 to 39.84 kDa. The pI ranged from 5.23 (*FveIAA5*) to 8.61 (*FveIAA10*), indicating that different Aux/IAA proteins might function under different pH conditions. Sixteen *FveIAAs* were located in the nucleus, except *FveIAA10*, *FveIAA11*, *FveIAA16*, and *FveIAA18*, which were located in the chloroplasts; *FveIAA12* was located in the mitochondria (Suppl. Tab. S2).

Chromosomal location and duplications of FveIAA genes

The 21 identified *FveIAA* genes were distributed across five chromosomes, mainly on chromosomes 1, 2, 4, 5, and 6, but were not found on chromosomes 3 and 7 (Fig. 1). These genes did not show random distribution on the chromosome due to gene clusters and hot regions. The unequal distribution of *FveIAA* genes suggested genetic variation during the evolutionary process (Li *et al.*, 2020a, Li *et al.*, 2019; Li *et al.*, 2015). Thus, the segmental duplication and tandem duplication events were investigated to explore potential gene duplication within the strawberry genome. Four duplicated gene pairs (*FveIAA2* and *FveIAA4*, *FveIAA3* and *FveIAA15*, *FveIAA7* and *FveIAA11*, *FveIAA8* and *FveIAA20*) of *FveIAAs*, all occurring on different chromosomes with a possibility of segmental duplication,

TABLE 1

Aux/IAA gene family in strawberry

Gene name	Phytozome ID	Chr	Localization	Strand	ORF (bp)	Length (aa)	WT (kDa)	PI	Exon No.
FveIAA1	mrna30941.1	1	1791848..1792687	F	699	232	26.22	6.21	5
FveIAA2	mrna31097.1	1	2494206..2494918	R	645	214	24.41	6.65	3
FveIAA3	mrna05555.1	1	15821563..15824821	R	1113	370	39.84	8.09	5
FveIAA4	mrna11624.1	2	9274779..9280831	F	708	235	27.02	6.04	4
FveIAA5	mrna08191.1	2	12260178..12260861	R	570	189	21.07	5.23	2
FveIAA6	mrna08194.1	2	12276543..12278218	F	753	250	27.03	7.55	5
FveIAA7	mrna08336.1	2	13518189..13520450	F	894	297	31.7	6.92	5
FveIAA8	mrna09007.1	2	20679865..20681061	F	873	290	34.24	8.79	4
FveIAA9	mrna32593.1	4	2279826..2280730	R	600	199	22.01	6.44	3
FveIAA10	mrna32595.1	4	2289754..2311425	F	717	238	26.52	8.61	5
FveIAA11	mrna27891.1	4	9607509..9609398	F	903	300	32.23	8.58	5
FveIAA12	mrna22779.1	4	20538272..20539079	F	639	212	23.53	8.44	4
FveIAA13	mrna03675.1	4	24854998..24861311	F	558	185	21.01	5.37	4
FveIAA14	mrna11861.1	5	21765417..21772896	F	906	301	32.14	8.07	5
FveIAA15	mrna26830.1	5	22395720..22397029	F	1068	355	38.85	5.41	5
FveIAA16	mrna16569.1	6	19898725..19899389	R	546	181	20.45	7.58	2
FveIAA17	mrna16571.1	6	19906315..19908136	F	747	248	27.15	7.59	5
FveIAA18	mrna05990.1	6	24020846..24022932	F	588	195	21.59	5.99	3
FveIAA19	mrna05993.1	6	24039962..24041070	F	594	197	21.85	7.56	4
FveIAA20	mrna25723.1	6	25163315..25165808	R	1029	342	37.74	8.4	5
FveIAA21	mrna25817.1	6	25615441..25617695	R	1017	338	37.07	8.57	5

All *FveIAAs* are listed. Abbreviations: pI, isoelectric point; aa, amino acid; Chr, chromosome; MW, molecular weight. In column Strand, R represent the reverse strand and F represents the forward strand.

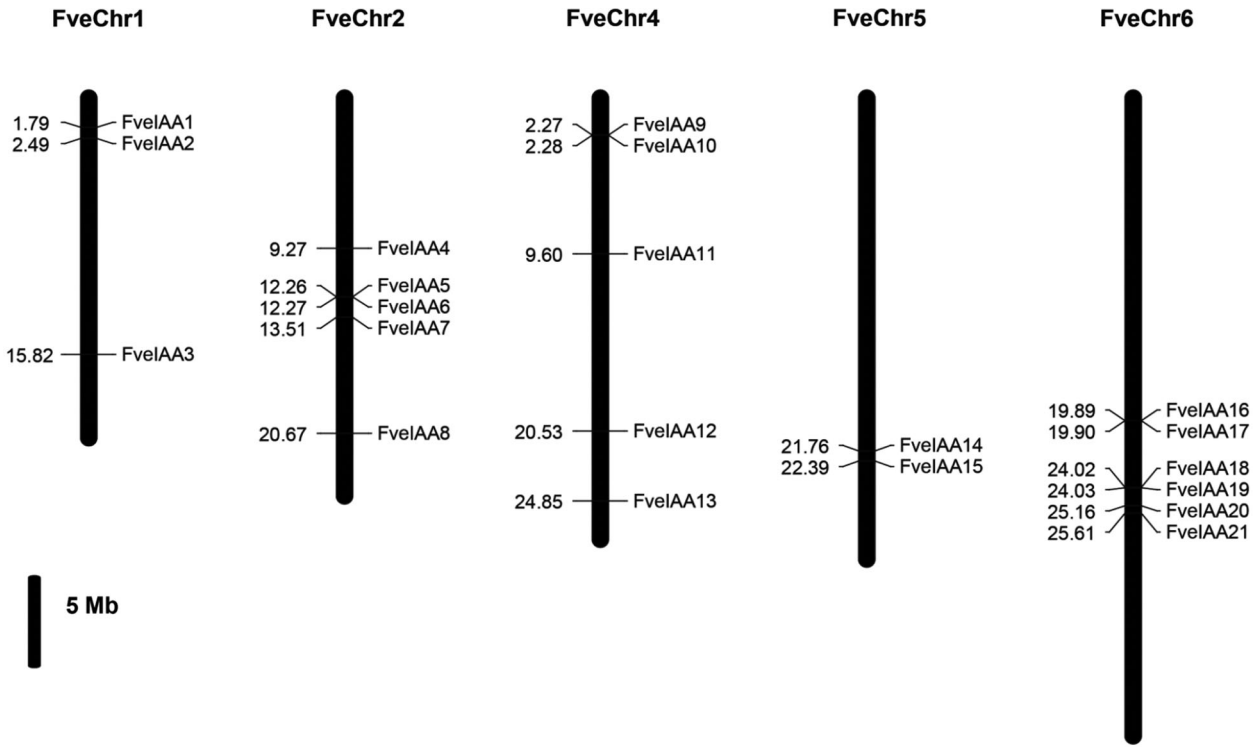

FIGURE 1. Chromosomal localization of *FveIAA* genes.
The serial number of the chromosome is indicated at the head of each chromosome. Twenty-one *FveIAA* genes were unevenly located on five chromosomes and were mapped based on the *F. vesca* genome database using MapChart v2.2. The length of chromosomes is on the scale (Mb). The localization of each gene was written on the left of each position while the name on the right.

were observed in *F. vesca* (Fig. 2A). We hypothesized that apart from expanding the *FveIAA* gene family size, these segmental gene duplications also increased their functional diversity. We constructed a comparative syntenic map of *F. vesca* and *Arabidopsis* to further explore the phylogenetic mechanism of the *F. vesca IAA* gene family. A syntenic relationship was discovered between four *FveIAA* genes and *Arabidopsis* (Fig. 2B), indicating the importance of these genes from the *Aux/IAA* gene family during the evolutionary process.

Multiple sequence alignment and conserved domains

Protein motif analysis by PFAM and sequence alignment revealed that 20 FveIAAs had four characteristic conserved domains: I–III and IX (Fig. 3).The domain I of most FveIAA proteins had a highly conserved LXLXLX motif, which served as a protein transcriptional repressor except FveIAA12, which was a non-canonical Aux/IAA protein missing domain I, also found in tomato and *Medicago truncatula*, indicating that these proteins may play specialized roles while mediating auxin response during plant growth and development. Most FveIAA proteins exhibited two distinct types of nuclear localization signals (NLS): A typical NLS (at the end of domain IV) and a bipartite NLS (between domains I and II).

The phylogeny of FveIAAs and AtIAAs

A phylogenetic NJ tree was built based on the complete sequence alignment of 21 FveIAAs and 29 AtIAAs to explore the phylogenetic relationship between the Aux/IAA proteins of *F. vesca* and *A. thaliana* (Suppl. Tabs. S3 and S4). Based on the phylogenetic distribution, IAA proteins were classified into nine major groups (labeled 1 to 9) with well-supported bootstrap values (Fig. 4). Also, we analyzed the identity of the predicted Aux/IAA protein sequences between *Arabidopsis* and strawberry predicted based on the orthologs between these two species. We found no organism-specific group from the tree. In the common clades, there was an unequal distribution of the IAAs from the two organisms. For instance, Group 4 contained one FveIAA and four AtIAAs. Also, there was an unequal distribution of the FveIAAs in the nine groups. Groups 1–6, 8, and 9 included twenty *FveIAA* members (largest), which were located in the nucleus and the chloroplast, while Group 7 contained one gene (*FveIAA12*), which was located in the mitochondria (Suppl. Tab. S2).

Conserved motifs and exon-intronic structures

We performed phylogenic and conserved motif analysis of the *FveIAAs* to explore the relationship between motifs and evolution and to identify the conserved regions (Fig. 5A). Twenty-one *FveIAAs* containing 2–10 conserved motifs were detected (Fig. 5B and Suppl. Fig. S1). Motifs 1 and 2 were common in all 21 *FveIAAs*, indicating that they were essential for basic functions of *FveIAAs*, while the other eight motifs were more or less missing from the *FveIAAs*. structure analysis showed that the gene structure of most of the *FveIAA* genes was identical to the *AtIAAs*, which included 3–5 exons and 2–4 introns, except for *FveIAA15* (six exons and five introns) and *FveIAA5* and *FveIAA16* (each containing two exons and one intron) (Fig. 5C). Exon-intron structure analysis provides critical insights into

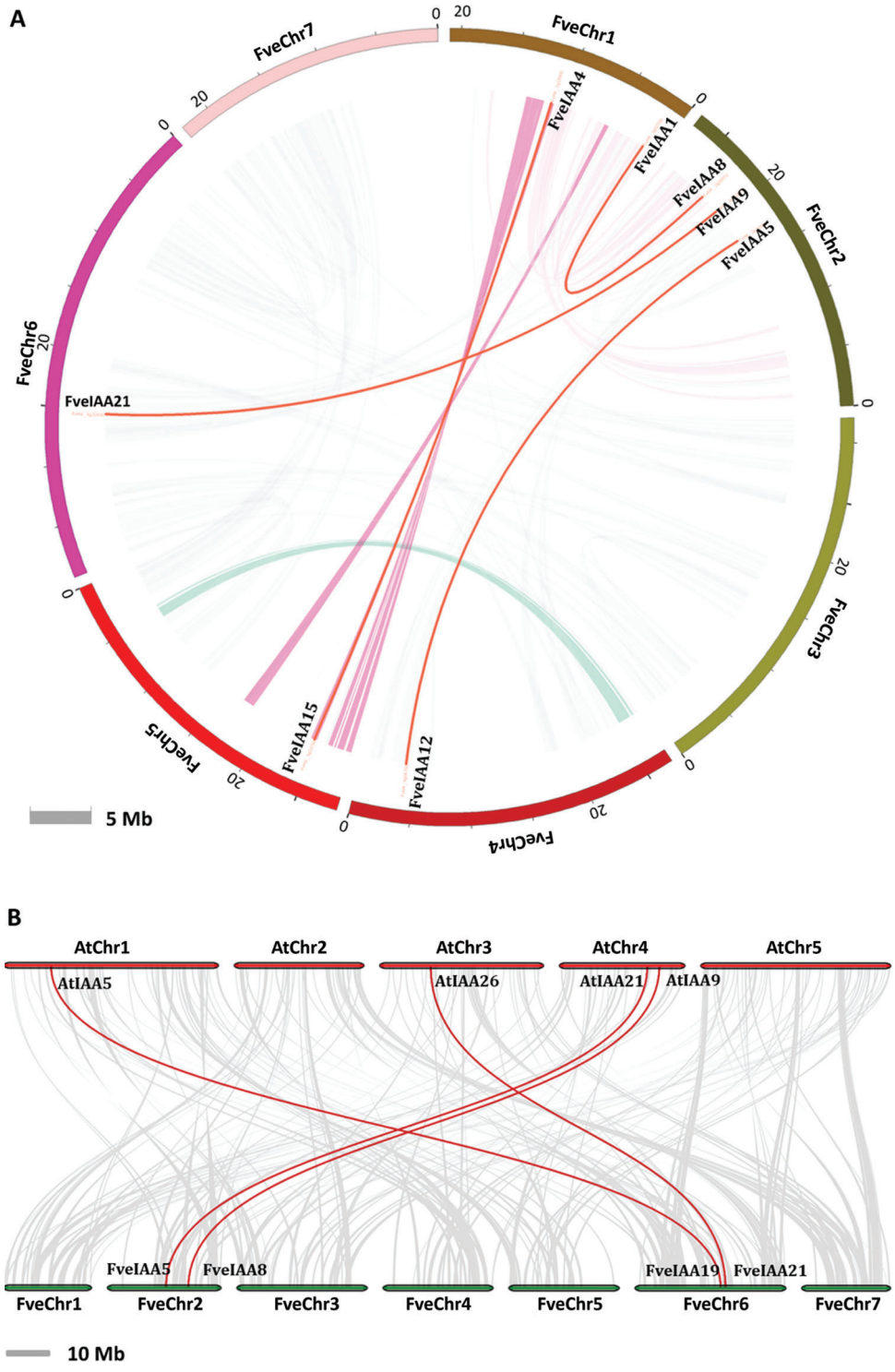

FIGURE 2. Segmentally duplicated gene pairs in *F. vesca*.
(A) The synteny relationships of *FveIAA* genes were displayed using Circos software. The seven chromosomes of *F. vesca* have been drawn as colored bars. There were four segment duplications gene pairs and no tandem duplications. The length of chromosomes is on the scale (Mb). (B) The collinear correlation of the Aux/IAA is displayed between *A. thaliana* and *F. vesca*. The green color represents *F. vesca* chromosomes (Fv01-07)) and the red color represents *A. thaliana* chromosomes (At01–AT05). Gray lines in the background indicate the collinear blocks between *A. thaliana* and *F. vesca*, while the red lines indicate the syntenic Aux/IAA gene pairs between *A. thaliana* and *F. vesca*. The length of chromosomes is on the scale (Mb).

the process of evolution of gene families. Motif composition, arrangements, and gene structures were consistent with the phylogenetic tree (Fig. 5).

Cis-element analysis and subcellular localization prediction
The interaction between *cis*-elements and the corresponding *trans*-acting factors is known to promote gene regulation. PlantCARE was used to determine the probable *cis*-regulatory elements within the promoter region of the *FveIAA* genes to understand possible regulatory patterns of the FveIAAs. We found that all 21 *FveIAAs* promoter sequences contained several light-responsive elements, indicating that *FveIAAs* played a critical role in strawberry morphogenesis. Additionally, we found hormonal response-related *cis*-regulatory elements, such as auxin, methyl jasmonate (MeJA), salicylic acid (SA), abscisic acid (ABA), and gibberellins (GA), as well as stress-responsive elements, including anaerobic induction, defense, drought, and low temperature in the promoter region of most *FveIAA* genes (Fig. 6). Some of the *FveIAA* genes contained tissue-specific elements (endosperm, meristem, and seed-specific activation) and circadian control elements.

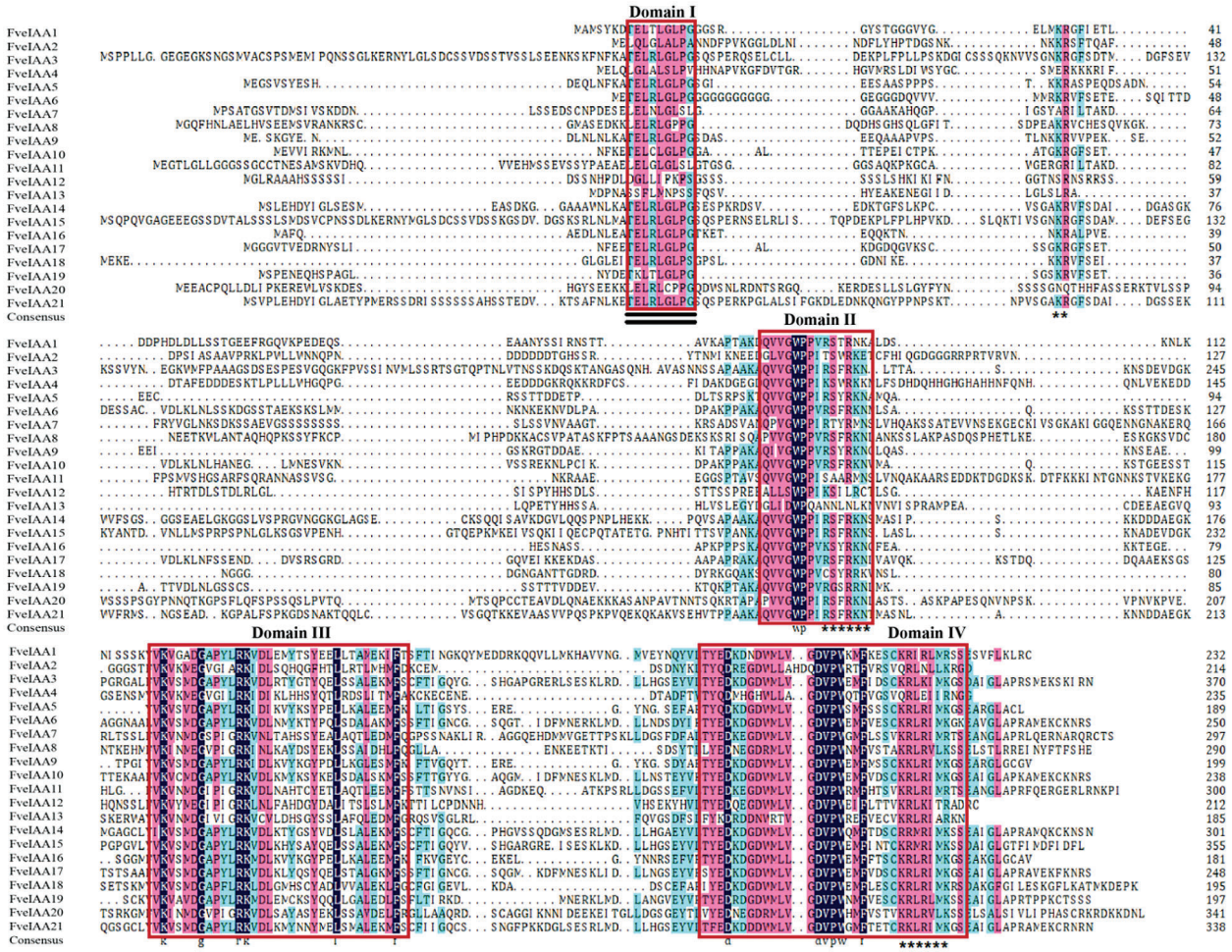

FIGURE 3. Multiple sequence alignment of the *FveIAA* gene family.
Multiple alignments of the FveIAAs were done using ClustalW. The red frame shows domains I–IV of the FveIAA proteins. Colorized shading represents identical and conserved amino acid residues, respectively. The thin black double lines mark the LXLXLX motifs. *marks the two NLSs.

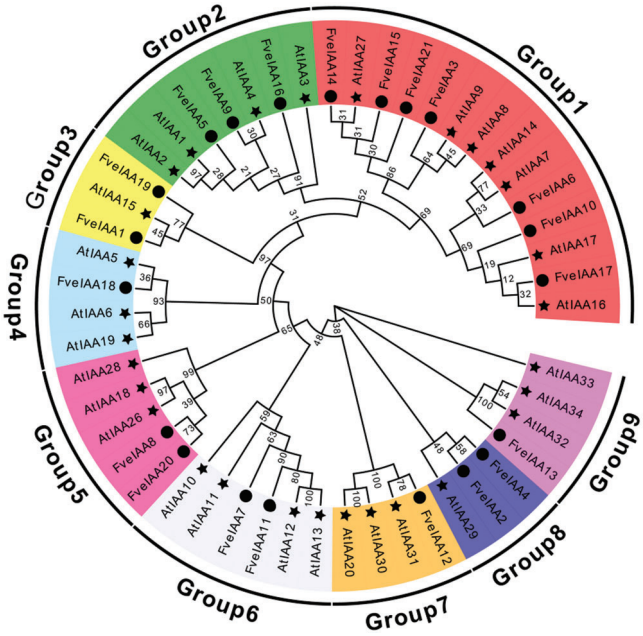

FIGURE 4. Phylogenetic relationships of IAA proteins between *A. thaliana* and *F. vesca*.
MEGA 7.0 software was used to build this NJ phylogenetic tree with 1000 bootstrap replicates. Different colors represent different groups of FveIAAs and AtIAAs. Black colored dots and stars indicate *F. vesca* and *A. thaliana*.

Prediction of PPI networks of FveIAA family

A complete interaction network of the FveIAA family and its interacting proteins were predicted using the STRING tool to investigate the functions of the FveIAAs. Seventeen FveIAAs were identified in the interaction network except FveIAA4, FveIAA5, FveIAA6, and FveIAA20 (Fig. 7). Protein-protein relationship analysis revealed that most FveIAAs interacted with each other indicating collaborative functioning, such as in FveIAA1, FveIAA13, and FveIAA19. Also, the direct interaction between FveIAAs and the nodal proteins suggested the possibility of occasional indirect interaction between the FveIAAs, such as XP_004293901.1, a common core protein, induced indirect interaction between FveIAA19, FveIAA1, FveIAA15, FveIAA17, and FveIAA7 (Fig. 7). Functional annotation revealed that most of the common interacting proteins in the PPI network were involved in auxin-related signaling pathways (Suppl. Tab. S5).

Expression analyses of the Aux/IAA genes in F. vesca organs

We investigated the spatial-specific expression pattern of the 21 FveIAAs in different organs, including stems, roots, flowers, leaves, and fruits, to investigate the physiological function of FveIAAs (Fig. 8). All the FveIAAs were detected in different organs, except for *FveIAA21*, whose expression was downregulated in all organs. All the *FveIAAs* showed

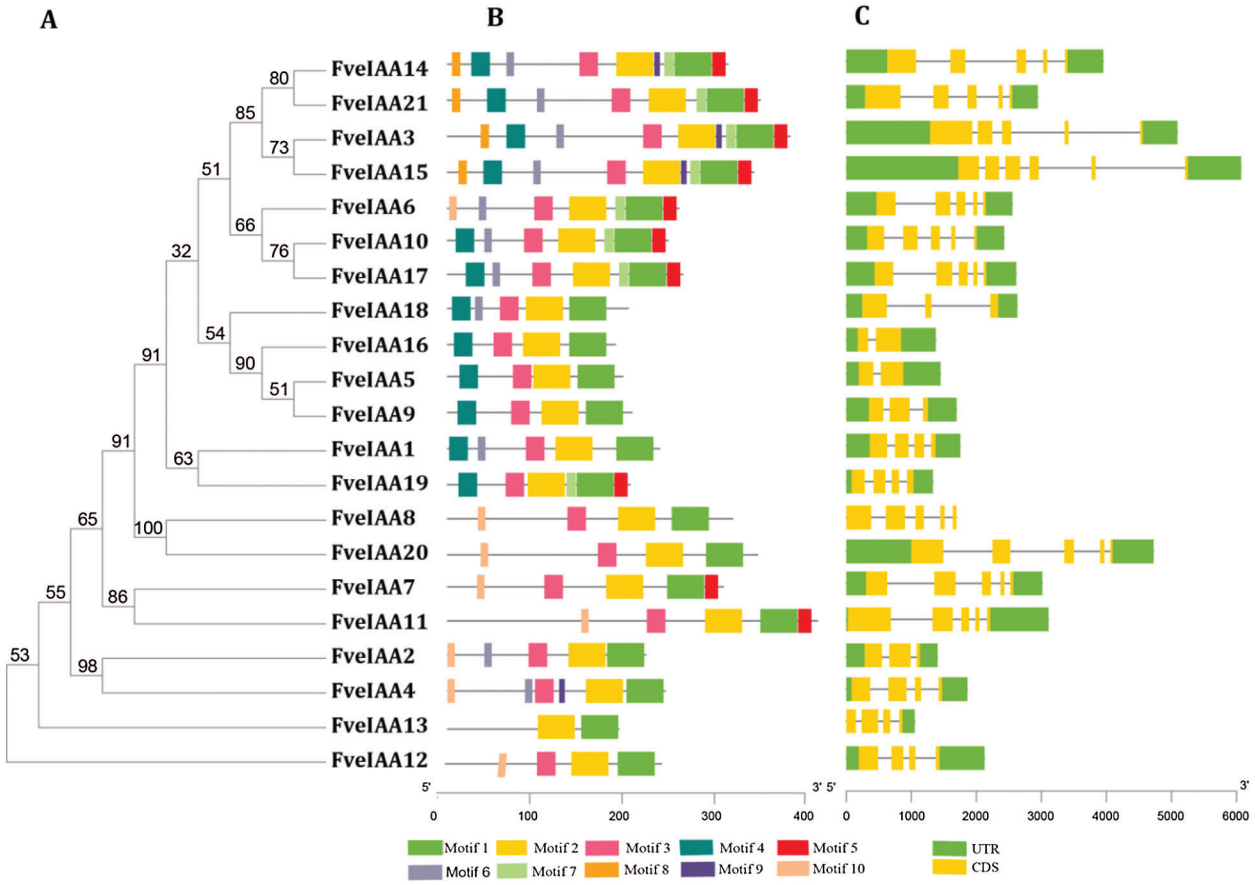

FIGURE 5. Phylogeny, motifs, and exon-intronic structures of *FveIAAs*.
(A) Unrooted NJ phylogeny of FveIAAs. The phylogeny was constructed based on full-length FveIAAs amino acid sequences with MEGA V7.0 (bootstrap = 1000, Poisson model) with a length corresponding to the number of substitutions per site. (B) MEME was used to determine the conserved motifs of FveIAAs. Variable color codes, labeled 1–10 at the bottom, represent different motifs and their positions. The scale bar represents the length of the respective amino acid sequence (Suppl. Fig. S1). (C) The structures of FveIAA genes generated from the GSDS. Exons (yellow box); UTR (green); introns (black lines); scale bar represents the length of the respective DNA sequences.

tissue-specific expression patterns in *F. vesca*. Most *FveIAAs* showed high stem-specific mRNA expression compared with other organs, except *FveIAA4*, *FveIAA5*, *FveIAA13*, and *FveIAA18* whose expression level were upregulated in the fruit; *FveIAA2*, *FveIAA9*, *FveIAA10*, and *FveIAA17* which showed root-specific expressions; *FveIAA3*, *FveIAA11*, and *FveIAA16* also showed flower-specific expression; *FveIAA1*, *FveIAA19*, and *FveIAA14*, which showed leaf-specific expression. Transcriptional analysis of the *FveIAAs* showed tissue-specific expression in *F. versa*, suggesting that *FveIAA* genes might play distinct roles in different organs during strawberry development.

Auxin inducibility of FveIAAs

We detected the expression pattern of FveIAAs in leaves at 1, 6, and 12 h of post-IAA treatment using qRT-PCR to test the responsiveness to exogenous auxin stimuli (Fig. 9). We found that except for *FveIAA21*, which was downregulated in all organs, all other genes were auxin-responsive. Exogenous auxin upregulated the expression of most *FveIAA* genes after 1 h and 6 h; however, the expression was restored to near pre-stress levels after 12 h in *FveIAA2*, *FveIAA3*, *FveIAA5*, *FveIAA7*, *FveIAA8*, *FveIAA14*, *FveIAA16* and *FveIAA18*. Also, elevated expression of *FveIAA4*, *FveIAA10*, *FveIAA13*, *FveIAA17* and *FveIAA20* was observed at all time points, including 12 h after treatment. In contrast, the expression of *FveIAA9*, *FveIAA11*, and *FveIAA15* were downregulated by auxin at every time point. Thus, the complexity of auxin-regulated gene expression was reflected in the diverse pattern of expression of the *FveIAA* genes post-treatment with auxin.

Discussion

Auxin, a plant hormone, is critical for plant growth and development (Liu *et al.*, 2017; Mano and Nemoto, 2012; Tiryaki, 2009). Aux/IAAs regulate the transcription of auxin-responsive genes that are involved in variable aspects of plant growth and development (Golan *et al.*, 2013; Liscum and Reed, 2002; Luo *et al.*, 2018). Therefore, to elucidate the function of strawberry IAAs in stimulating specific auxin responses, we performed a genome-wide comprehensive survey of the Aux/IAA gene family in strawberry. In this study, 21 strawberry IAA genes were identified and labeled based on their chromosomal location. Fewer duplication resulted in fewer *FveIAA* genes compared with other species, such as *Arabidopsis*, tomato, rice, and maize (Audran-Delalande *et al.*, 2012; Dreher *et al.*, 2006; Jain *et al.*, 2006; Wang *et al.*, 2010) (Suppl. Tab. S6). Next, we assessed the conserved structural domains of the

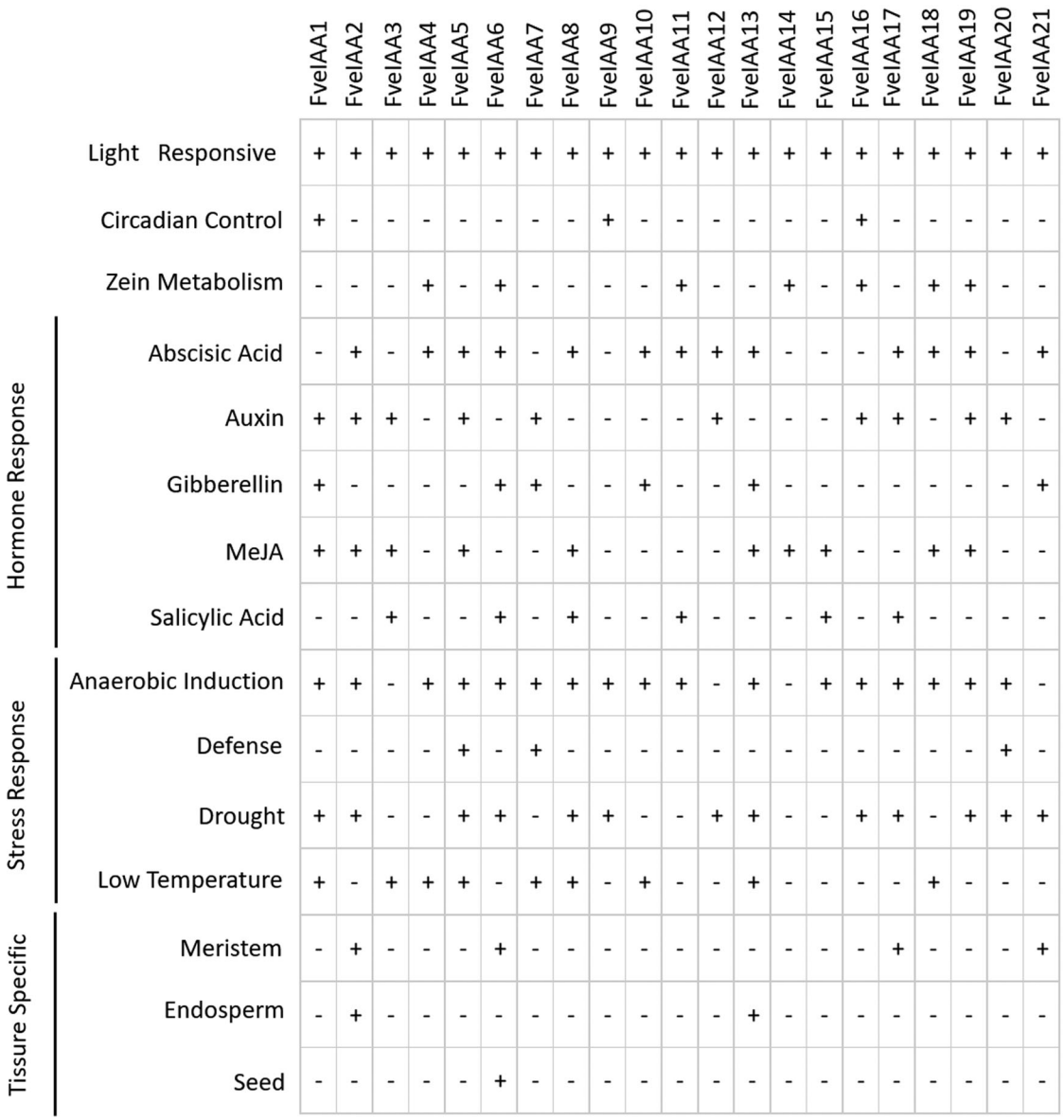

FIGURE 6. Putative cis-regulatory elements in the promoter region of *FveIAA* genes.
The promotors (1500 to 1 bp prior to the start codon ATG) of the *FveIAA* genes were obtained from Phytozome, and the *cis*-elements were predicted by PlantCARE. +: The presence of the *cis*-element; −: The absence of the *cis*-element.

strawberry Aux/IAA proteins. The amino acid sequence analysis revealed four highly conserved domains between the *FveIAA* gene family and *Arabidopsis*, suggesting the possibility of similar functions.

Previous studies have suggested that phylogenetic analysis not only helps elucidate phylogenetic relationships but also predicts putative functions of various genes, which helps in the selection of candidate genes (Horton *et al.*, 2007; Xu *et al.*, 2018; Zhai *et al.*, 2014). Here, the *F. vesca Aux/IAA* gene family members were divided into nine groups based on their sequence similarity. The phylogenetic tree between strawberry and *Arabidopsis* showed that all the FveIAAs had orthologs in *Arabidopsis* (Fig. 2). Comparative genome analysis of the *Aux/IAA* genes in *F. vesca* and *Arabidopsis* confirmed gene duplication (segmental duplication) in at least four pairs of *FveIAA* genes and the absence of tandem duplications. Segmental and tandem duplication events are critical for the expansion of the gene families (He *et al.*, 2019; Kramer *et al.*, 2004; Li *et al.*, 2020b; Zhu *et al.*, 2014). Typically, angiosperm evolution is associated with the whole-genome duplication events leading to the expansion of gene families (Kawai *et al.*, 2014; Yamada *et al.*, 2019). Also, the *cis*-element analysis confirmed the presence of *cis*-regulatory elements associated with hormone response, tissue-specific, and stress response on most of the strawberry *Aux/IAA* gene promoter sequences.

Plants are frequently exposed to abiotic and biological stresses, such as cold, desiccation, salinity, and hormones during the developmental stages (Kim *et al.*, 2015; Ku *et al.*, 2018; Mishra and Richa, 2016). One study reported that Aux/IAA as transcriptional regulators might promote Auxin signal transposition directly (Mano and Nemoto, 2012). We analyzed the promoter *cis*-elements of the *FveIAA* genes family and found that several hormone-responsive stress elements were present in the promoter region. Thus, we analyzed the expression of the *FveIAA* genes in the

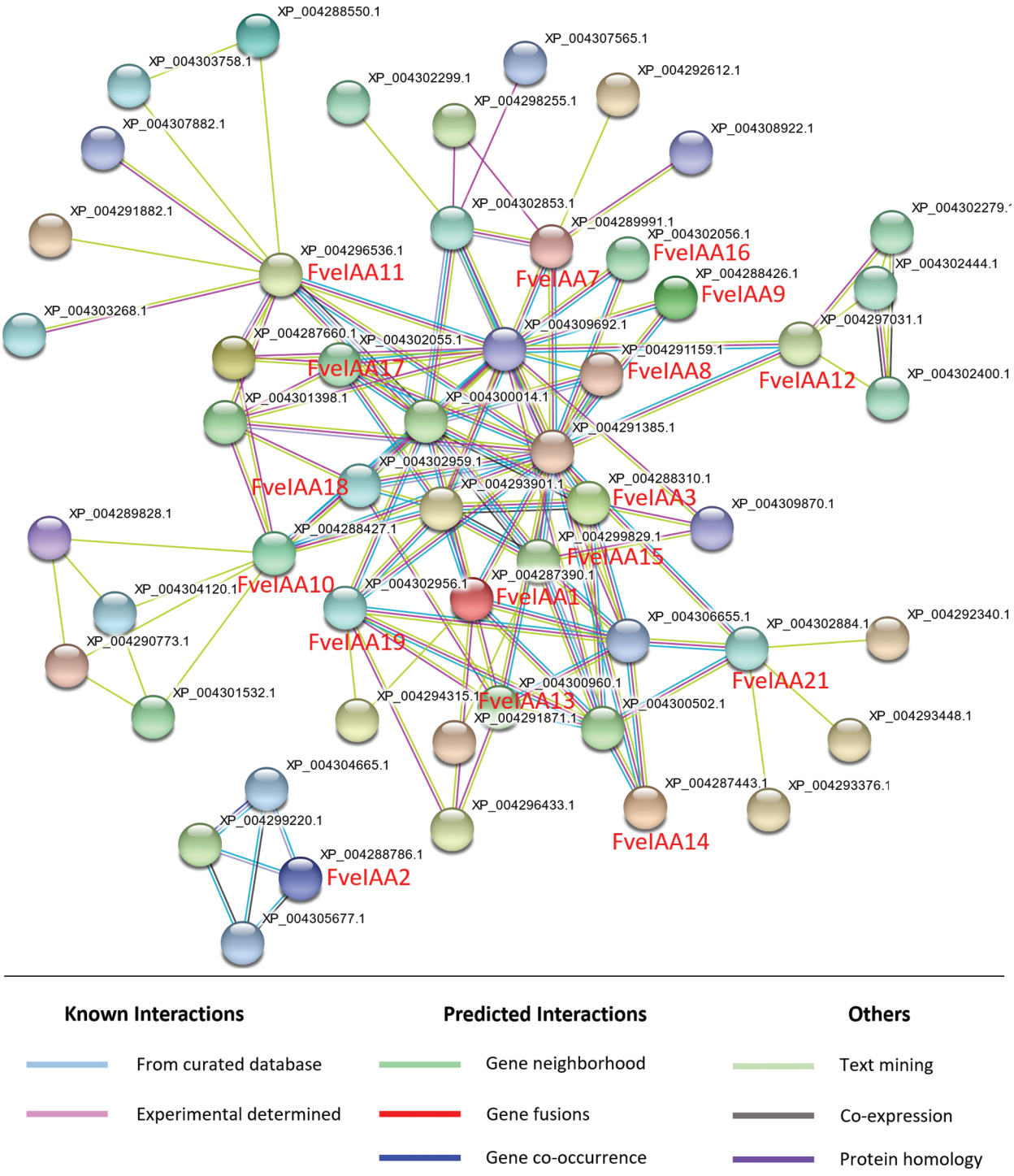

FIGURE 7. Putative interaction network of FveIAA proteins in *F. vesca*.
PPI networks of all the FveIAAs and their interacting proteins. Edge confidence was taken >0.7. Network nodes indicate proteins; colored nodes indicate query proteins and first shell of interactors. In the network, interacting proteins were termed in black, while FveIAAs were termed in red.

strawberry seedlings post-IAA treatment and found that most of the *FveIAA* genes were responsive to IAA treatment, despite the absence of corresponding *cis*-elements in some of the genes, indicating the possibility of indirect regulation of these genes. The results also substantiated the involvement of FveIAA in auxin signaling pathways.

Expression patterns of FveIAAs were investigated in different organs using real-time PCR to study their physiological functions, especially fruit development (Fig. 8). Some *FveIAA* genes showed organ-specific expression patterns, indicating their differential roles during strawberry development. *FveIAA3*, *FveIAA4*, *FveIAA5*, *FveIAA11*, *FveIAA13*, *FveIAA16*, and *FveIAA18* showed preferential expression in flower and/or fruit, suggesting their importance in improving fruit-related agronomic traits in strawberry. Interestingly, during the fruit development and ripening stage, we observed a rapid increase in the transcription levels of *FveIAA4* and *FveIAA5* from the SG to the BG stage and maintained a high expression throughout the fruit ripening. While *FveIAA13* and *FveIAA18* showed

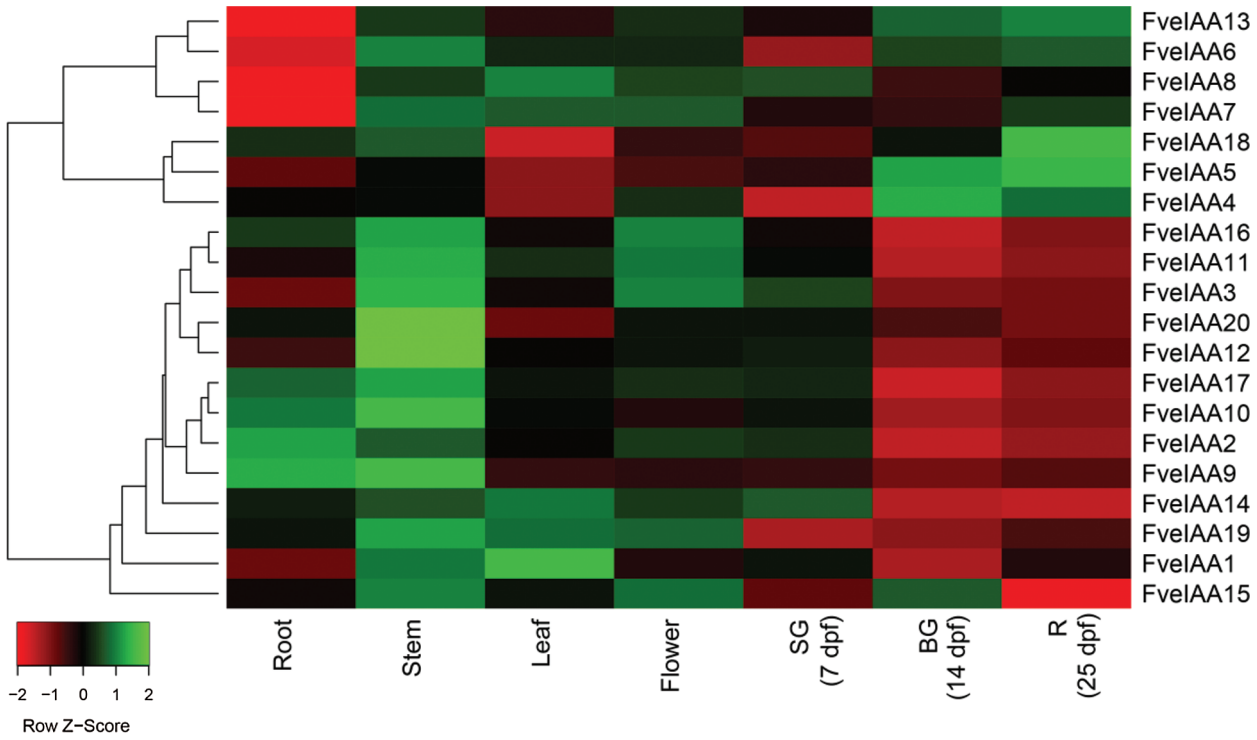

FIGURE 8. Heatmap of transcription profiling individual *FveIAA* genes in different organs.
The Omicshare website provided the log2 transformation of the relative expression data. The relative mRNA level of individual *FveIAAs* was normalized to *FvActin* in different tissues, including stem, root, flower, leaf, small green fruit (SG), big green fruit (BG), and red fruit (R). Blue blocks and red blocks represent downregulated and upregulated transcription levels, respectively.

the highest expression levels in the R stage (fruit maturity period). Previous studies have demonstrated the involvement of several *Aux/IAA* genes in the fruit developmental processes (Pattison *et al.*, 2014); however, there is a scarcity of information on the regulation of fruit ripening regulation by Aux/IAA proteins, which needs to be further studied (Luo *et al.*, 2018; Ori, 2019). Here, fruit-specific expression pattern and response to auxin indicated a novel role of these genes in regulating fruit development and ripening in strawberry. *FveIAA21* exhibited a downregulated expression, which indicated it might have a different role during plant growth and development. Most *FveIAA* family genes showed higher expression in stem compared with other organs, indicating that Aux/IAAs could be vital for stem development.

Thus, we identified twenty-one putative candidate *Aux/IAA* genes in *F. vesca* in this study. FveIAAs were localized across five chromosomes of *F. vesca* and were divided into nine groups. All members had high homology and conserved domains, but they were different in a way. The study of the synteny analysis and phylogenetic relationships between *F. vesca* and *Arabidopsis* provided valuable information about the evolutionary characteristics of *FveIAA* genes. Protein motif architecture and PPI analysis indicated that *FveIAA* genes played a role in gene regulation and protein interaction net. Thus, *FveIAA* genes probably played an important role during strawberry development via the auxin signal transduction pathway. Based on tissue-specific expression and IAA treatment response, *FveIAA4*, *FveIAA5*, *FveIAA13*, and *FveIAA18* were involved in fruit formation and ripening. These results would help decipher the biological roles of the Aux/IAA family in *F. vesca*.

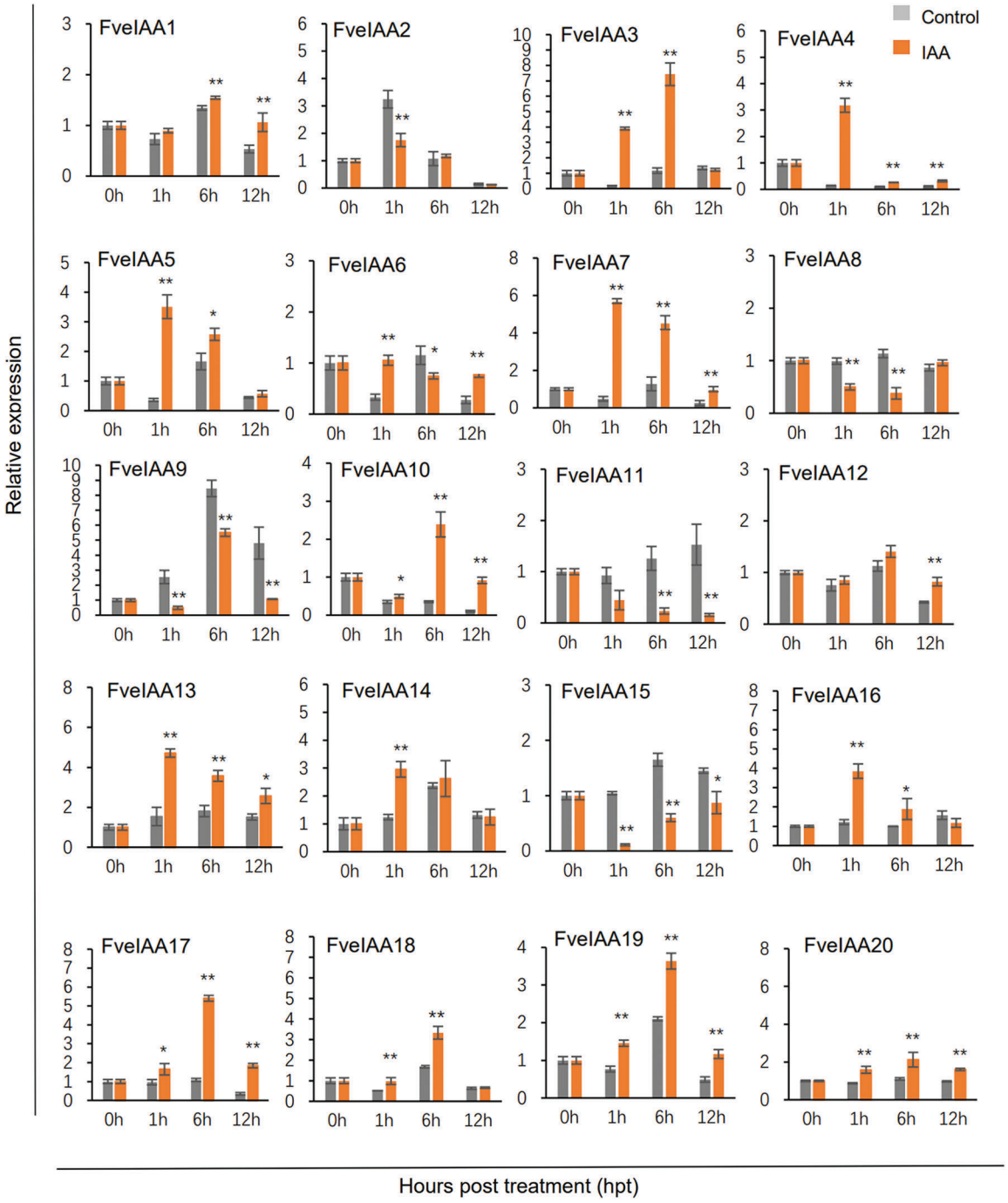

FIGURE 9. Relative expression of FveIAAs under auxin treatment.
Relative mRNA abundance of each gene was calculated using transcription levels at 0 h (untreated plants) as the internal control (mean value = 1). The FvActin gene was used as the internal standard. FveIAA21 could not be detected. The experiments were performed in three biological replicates, and data points represent the mean of three biological replicates. Error bars indicated the standard error (SE) of mean expression values from three independent experiments. A significant difference among the phenotype is represented by asterisks at the top of each column ($^{*}P < 0.05$, $^{**}P < 0.01$).

Author Contribution: The authors confirm contribution to the paper as follows: Conceptualization: QL and LS; methodology: XZ and XL; software: Formal analysis, LS, HY and JZ; data curation: QL; writing—original draft preparation: LS and QL; writing—review and editing: QL; project administration: QL. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: All data generated or analysed during this study are included in this published article and its supplementary information files.

Ethics Approval: The experiments involved in this manuscript have no ethics related issues.

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Supplementary materials

Figure S1. The motif weblog visualization of FveIAAs. MEME was used to elucidate and visualize the conserved motifs of FveIAAs. The different colors of amino acids are classified according to the chemical properties of amino acids.

Table S1. The primers used in this study.

Table S2. Subcellular localization analysis of FveIAAs.

Table S3. List and sequences of FveIAAs annotated in this study.

Table S4. List and sequences of AtIAAs for the phylogenic analysis.

Table S5. List of interacting proteins of FveIAAs.

Table S6. Summary of IAA numbers in some species.