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ORIGINAL ARTICLE

Genome-Wide Identification of the Aux/IAA Family Genes (*MdIAA*) and Functional Analysis of MdIAA18 for Apple Tree Ideotype

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Abstract

The *Aux/IAA* (auxin/indole-3-acetic acid) gene family is one of the early auxin-responsive gene families, which play a central role in auxin response. Few reports are involved in *Aux/IAA* genes in fruit trees, especially in apple (*Malus* × *domestica* Borkh.). A total of 33 *MdIAA* members were identified, of which 27 members contained four conserved domains, whereas the others lost one or two conserved domains. Several *cis*-elements in promoters of *MdIAAs* were predicted responsive to hormones and abiotic stress. Tissue-specific expression patterns of *MdIAAs* in different apple tree ideotypes were investigated by quantitative real-time PCR. A large number of *MdIAAs* were highly expressed in leaf buds and reproductive organs, and *MdIAAs* clustered in same group showed similar expression profiles. Overexpression of *MdIAA18* in *Arabidopsis* resulted in compact phenotype. These results indicated that *MdIAA* genes may be involved in vegetative and reproductive growth of apple. Taken together, the results provide useful clues to reveal the function of *MdIAAs* in apple and control apple tree architecture by manipulation of *MdIAAs*.

Keywords *Malus* × *domestica* Borkh. · *MdIAA* · Auxin-responsive genes · Plant architecture

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Introduction

The phytohormone auxin triggers extensive transcriptional reprogramming through a very short nuclear cascade (Chapman and Estelle 2009; Winkler et al. 2017) that plays a critical role in many aspects of plant growth and development including embryogenesis, plant organ formation, apical dominance, gravitropic response, and plant architecture (Benkova et al. 2003; Ljung 2013; Wilson 2000). Much of the attention given to lateral roots, floral, and shoot architecture has focused on the auxin/IAA response mechanism (Galli et al. 2015; Lu et al. 2015; Roy et al. 2017; Santner and Estelle 2009).

Auxin induces various distinct developmental responses, partly by regulating gene expression (Catala et al. 2000; Mockaitis and Estelle 2008; Peer 2013). Three major classes of early auxin response genes have been identified: *auxin/indole-3-acetic acid* (*Aux/IAA*) (Liscum and Reed 2002), *small* auxin *unregulated* (*SAUR*) (Hagen and Guilfoyle 2002), and *Gretchen Hagen 3* (*GH3*) (Jain et al. 2006b). The *Aux/IAA* genes are a large gene family found in many woody plants, including 35 in *Populous trichocarpa* (Kalluri et al. 2007), 27 in cucumber (Wu et al. 2014), 26 in grape (Wan et al. 2014), 18 in *Carica papaya* (Liu et al. 2017), and 22 in *Carya cathayensis* (Yuan et al. 2018).

Canonical Aux/IAA proteins share four conserved domains: I, II, III, and IV. Domain I at the N-terminus represented by an ethylene-response-factor-associated (ERF) motif is characterized by transcriptional regulation and shows transcriptional repression activity (Chen et al. 2018; Liscum and Reed 2002). Domain II contains one highly conserved VGWPPV motif that is responsible for the instability of Aux/IAA proteins through the ubiquitin–proteasome degradation pathway (Gray et al. 2001; Kepinski and Leyser 2005; Santner and Estelle 2009; Tan et al. 2007). Mutation of domain II can remarkably prolong protein half-life times and thus induce auxin-related aberrant phenotypes (Ludwig et al. 2014; Tao and Estelle 2018; Uehara et al. 2008). Domains III and IV at the C-terminus are dimerization regions involved in homo- and heterodimerization with other *Aux/IAA*s and auxin response factor (ARF) proteins (Abel and Theologis 1995; Guilfoyle and Hagen 2012). Domain III contains an amphipathic βαα (VKVxM and RK) that is recognized as a DNA-binding domain (DBD) (Zenser et al. 2001). Domains IV contains one conserved motif (GDVPW) and one conserved SV40 type nuclear localization signal (NLS: KRxRxxK) that contributes to dimerization (Wu et al. 2017). Additionally, conservative bipartite NLSs are found between domains I and II that guide the Aux/IAA proteins to the nucleus and are more effective than the SV40 type NLS (Abel and Theologis 1995; Wu et al. 2017).

Aux/IAA genes encode short-lived nuclear-localized proteins that function as transcriptional repressors by interacting with ARFs (Tiwari et al. 2003, 2001). At low auxin concentrations, the complex of Aux/IAA proteins and co-repressor TOPLESS (TPL) dimerizing with ARF proteins binds to the promoter regions of auxin-responsive genes, thus repressing auxin responses (Choi et al. 2018; Szemenyei et al. 2008). At high auxin concentrations, auxin directly stimulates

the interaction between Aux/IAA proteins and SCF^{TIR1} E3 ubiquitin-ligase complexes resulting in the degradation of Aux/IAA proteins and the release of ARFs from TPL-mediated repression, thereby promoting the expression of auxin response genes (Dharmasiri et al. 2005; Okushima et al. 2005; Ulmasov et al. 1995; Woodward and Bartel 2005).

In fruit trees, auxin has been implicated in the regulation of plant architecture by maintaining apical dominance and inhibiting outgrowth of axillary buds (Hollender and Dardick 2015). The columnar apple 'Wijcik,' a mutation of 'McIntosh,' is characterized by compaction of the internodes, reduced lateral shoot growth, and increased short spur (Lapins 1969). It has been reported that auxin has a role in determining the columnar habit in terms of reduced lateral branching (Looney and Lane 1984; Watanabe et al. 2008). However, little information of *Aux/IAA* genes is available in apple (*Malus* × *domestica* Borkh.) and the potential correlation between *Aux/IAA* genes and apple architecture has not yet been reported. In this study, a comprehensive analysis of apple *Aux/IAA* genes was conducted. And an *Aux/IAA* gene with four conserved domains, located near the locus of the *Columnar* (*Co*) gene on Chromosome 10, was characterized by ectopic expression in *Arabidopsis thaliana* to elucidate its function in plant growth and development. Potential roles of *Aux/IAA* genes in the development of apple tree structure were also discussed.

Materials and Methods

Plant Materials

Apple cultivars 'Fuji,' 'McIntosh,' and 'Wijcik' were planted at the China Agricultural University Experimental Station in Beijing, China (40.138044°N, 116.185320°E). The Arabidopsis 'Columbia' ecotype was preserved in Fruit Science Laboratory. The shoot tips, unexpanded leaves, bark tissues, and mature leaves were sampled from current shoots, blooming flowers, and young fruit (3 weeks after the anthesis) were collected in the outside of tree canopy at the end of April in 2016; and the roots were sampled at the same time. Flower buds and leaf buds were obtained from one-year woods in March in the same year. The same method was used to harvest the materials of non-columnar apple 'McIntosh' and columnar apple 'Wijcik,' then Keeping all the samples in liquid nitrogen and storing at − 80 °C (Wang et al. 2018; Xu et al. 2017).

Arabidopsis seeds storing in Fruit Science Laboratory were sterilized and sown on MS medium (30 g/L sucrose and 7.0 g/L agar) in Petri dishes and kept at 4 °C for 3 d for vernalization. Seeds were then cultured for 2 w at 22 °C in a 16/8-h light/dark cycle (130 μmol $m^{-2}s^{-1}$). The seedlings were subsequently transplanted as previously described (Shapiro et al. 2015).

Genome-Wide Isolation of *Aux/IAA* Family Genes in Apple

The *AtAux/IAA* family genes and proteins (https://www.arabidopsis.org/) were used as queries to searches against the apple tree genome, SRA and EST

databases in NCBI (GenBank: ACYM00000000), and GDR (https://www.rosaceae.org/) using BLASTN and TBLASTN. The protein domains and DBD were predicted for the obtained sequences in the Pfam (https://pfam.xfam.org/) and InterPro databases (https://en.wikipedia.org/wiki/InterPro) to remove non-*Aux/IAA* family genes. Primer 5.0 was used to design special primers (Table S1). The mixed cDNAs from the root, shoots, young leaves, buds, flowers, and young fruit of 'Fuji' apple tree were used as templates to isolate *Aux/IAA* family genes.

Bioinformatics Analysis

Chromosome localization was conducted using MapInspect software (Zhang et al. 2018) based on BLAST-retrieved results. The exon–intron structures of *Aux/IAA* family genes were confirmed by aligning with the genomic sequences. GSDS 2.0 (https://gsds.cbi.pku.edu.cn/) was used to build the structure diagram (Hu et al. 2015). The isoelectric point (pI) and molecular weight (MW) of *Aux/IAA* family proteins were calculated using ExPASy (https://web.expasy.org/compute_pi/). ClustalW (https://www.megasoftware.net/) was used to perform multiple sequence alignments of the proteins (Kumar et al. 2016). Neighbor-joining method was used to conduct a phylogenetic tree analysis (Tamura et al. 2004) in MEGA 7 (Robert and Gouet 2014).

Promoter Structure Analysis

To investigate the *cis*-elements in the promoter sequences of *Aux/IAA* family members, 2 kb upstream of the initiation codon (ATG) was isolated for each gene. The promoter 2.0 prediction server and the plantcare tool (https://www.cbs.dtu.dk/services/Promoter and https://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Knudsen 1999; Lescot et al. 2002) were adopted to identify putative *cis*-elements in promoters.

RNA Extraction and Quantitative Real-Time PCR Analysis

Total RNA was extracted using CTAB method (Asif et al. 2006) and purified using an RNeasy Mini Elute Cleanup kit (Qiagen, Dusseldorf, Germany). A PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China) was used to synthesize complementary DNA. A SYBR Premix Ex Taq™ II kit (TaKaRa, Dalian, China) was used for each qRT-PCR reaction using the Applied Biosystems 7500, running in triplicate. *MdActin* expression level regarded as internal reference. The following PCR conditions were used as previously described (Xu et al. 2017). The $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression levels (Livak and Schmittgen 2001).

Plasmid Construction and Genetic Transformation

The coding sequence of *MdIAA18* and the binary vector pCAMBIA1305.1 were digested at common restriction enzyme sites (*Spe*I and *BstE*II), and then combined and ligated to generate the *MdIAA18* overexpression vector; an empty vector control was also generated. The resulting overexpression construct was introduced into *Agrobacterium tumefaciens* strain EHA105 using the freeze–thaw method (Hofgen and Willmitzer 1988). For *Arabidopsis* transformation, an overnight culture of *A. tumefaciens* was introduced into wild-type *Arabidopsis* using the floral-dip method (Clough and Bent 1998). T_0 transformants were confirmed by RT-PCR using specific primers for *MdIAA18* and the 35S promoter. Putatively transformed regeneration plants were obtained by selection medium supplemented with 20 mg/L hygromycin (Hyg).

Seeds from three dwarf *MdIAA18*-overexpressing *Arabidopsis* T_0-generation lines, TS1, TS2, and TS3, were harvested and stored at 4 °C for at least 1 w. After storage, positive seeds selecting from the upper selection medium were sowed and transplanted into a climate-controlled chamber as same as the upper description. Repeating the above steps, T3 generations were obtained (Shapiro et al. 2015).

Statistical Analysis

Samples were analyzed in triplicates, and the data are presented as the mean ± standard error unless noted otherwise. SPSS 17 was used to determine the statistical significance (Wang et al. 2014). A difference at $P < 0.05$ was considered significant, and $P < 0.01$ for extremely significant.

Results

Identification of *Aux/IAA* Family Genes in the Apple Genome

To identify the *Aux/IAA* genes in apple, the *AtAux/IAA* genes were used as templates to search against the apple genome with BLASTN and BLASTP. Thirty-three predicted *MdIAA* genes were identified. They were named *MdIAAs* from *MdIAA1* to *MdIAA33* based on their chromosomal locations (Fig. 1 and Table 1). *MdIAA16*, *MdIAA17, MdIAA18,* and *MdIAA19* were located on chromosome 10 and *MdIAA18* was closest to the *Co* locus conferring the columnar growth phenotype in apple. The MdIAA proteins ranged from 158 to 373 amino acids in length with a protein mass from 17.18 to 40.24 kD; the protein pIs ranged from 5.51 to 9.08.

Gene Structure and Phylogenetic Analysis of MdIAAs

Structural analyses were intended to provide valuable information concerning duplication events within gene families (Figs. 2, S1). *MdIAA12* and *MdIAA28* had the

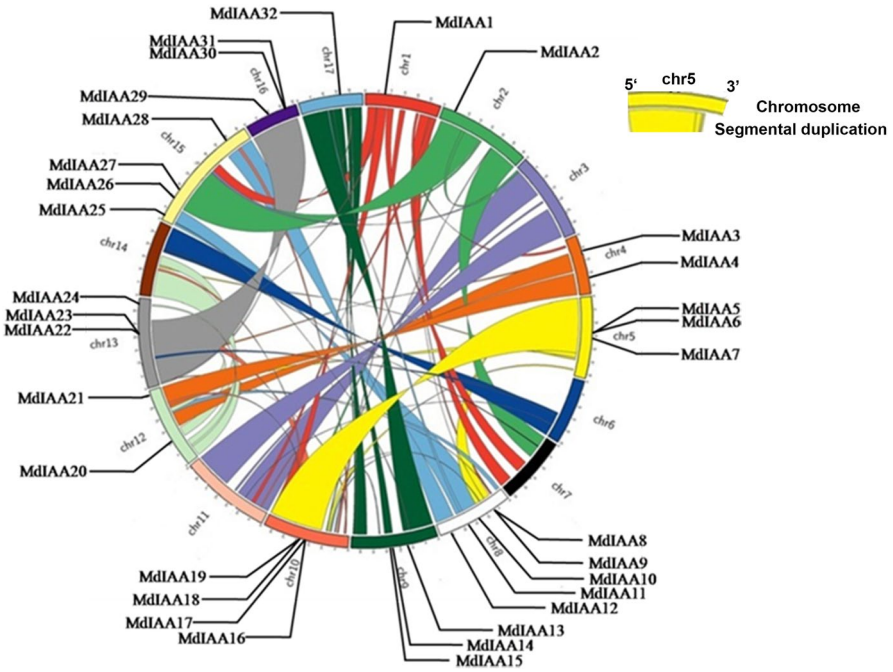

Fig. 1 Mapping of MdIAAs and segmental duplication regions on apple chromosomes based on BLAST-retrieved results. The outside of the ring stood for chromosomes (Chr). A map connecting segmental duplication regions of the apple genome is shown inside the figure

most introns while *MdIAA4/16/21/24* had just one intron. Consensus motifs were found in the *MdIAA* family such as the LXLXLX motif in Domain I, VVGWPP in Domain II, $VKVXMX_6RK$ in Domain III, and GDVPW and $KRXRX_2K$ in Domain IV. There were two types of putative NLS, a bipartite structure comprising a conserved KR basic doublet (Part 1) between domains I and II and RX_2RK (Part 2) in Domain II, and an SV40-like NLS motif $KRXRX_2K$ in domain IV. These putative NLSs might guide MdIAA family proteins to the nucleus. In total, 81.81% MdIAA proteins harbored four conserved domains; MdIAA11, 16, and 26 lacked domain I; MdIAA3 and 24 lacked domain I and II; and MdIAA17 lacked domain IV. Phylogenetic trees indicated that these genes with incomplete domains clustered closely. Domain III is clearly included in all members, suggesting the important function of this family. Thirty-three MdIAAs and IAA proteins from other species were divided into 14 clades (Fig. 3). Fourteen sister pairs of *MdIAA*s were found in apple (Table S2) with each pair showing greater than 80% amino acid sequence similarity.

Analysis of the Promoters of MdIAA Family Genes

To predict the *cis*-functioning elements in *MdIAA* family genes, 2 kb of sequence upstream of the initiation codon was obtained for each of the 33 genes except

Table 1 Identification of the *MdIAA* genes in apple

Name	Genomic position (bp)	Gene structure		Polypeptide			
		ORF length (bp)	Introns	Size (aa)	Mass	pI	Domains
MdIAA1	chr1: 7,408,512–7,411,458	1122	4	373	40,236.94	6.91	I II III IV
MdIAA2	chr2: 4,852,465–4,854,655	915	4	304	31,554.69	8.57	I II III IV
MdIAA3	chr4: 6,703,020–6,704,143	603	3	200	23,111.12	9.05	III IV
MdIAA4	chr4: 21,376,016–21,376,825	591	1	196	22,134.21	5.70	I II III IV
MdIAA5	chr5: 14,261,485–14,262,232	528	2	175	19,588.14	5.51	I II III IV
MdIAA6	chr5: 14,275,465–14,278,161	774	4	257	28,011.78	8.73	I II III IV
MdIAA7	chr5: 15,666,759–15,668,840	894	4	297	30,983.35	8.36	I II III IV
MdIAA8	chr8: 6,520,236–6,524,060	573	2	190	21,423.56	5.84	I II III IV
MdIAA9	chr8: 6,615,803–6,617,245	618	3	205	22,399.56	7.50	I II III IV
MdIAA10	chr8: 14,108,289–14,110,031	825	4	274	30,294.16	8.83	I II III IV
MdIAA11	chr8: 18,938,281–18,939,653	732	3	243	27,541.90	7.16	II III IV
MdIAA12	chr8: 25,116,270–25,119,707	1092	5	363	39,557.36	6.22	I II III IV
MdIAA13	chr9: 11,583,350–11,584,808	615	3	204	22,662.80	6.73	I II III IV
MdIAA14	chr9: 18,989,588–18,991,860	978	4	325	35,956.72	9.08	I II III IV
MdIAA15	chr9: 19,755,375–19,758,142	966	4	321	34,611.22	8.56	I II III IV
MdIAA16	chr10 :17,551,191–17,551,978	477	1	158	17,183.25	5.93	II III IV
MdIAA17	chr10: 17,566,197–17,568,055	804	3	267	28,087.19	7.69	I II III
MdIAA18	chr10: 19,773,424–19,774,161	522	2	173	19,292.97	7.62	I II III IV
MdIAA19	chr10: 19,786,568–19,789,335	741	3	246	26,970.44	6.45	I II III IV
MdIAA20	chr12: 10,412,318–10,430,879	975	4	324	35,938.80	9.06	I II III IV
MdIAA21	chr12: 30,230,865–30,231,571	588	1	195	22,068.13	6.04	I II III IV
MdIAA22	chr13: 21,548,682–21,549,461	573	2	190	21,342.48	8.95	I II III IV
MdIAA23	chr13: 21,624,958–21,627,301	735	4	244	26,846.90	8.87	I II III IV

Table 1 (continued)

Name	Genomic position (bp)	Gene structure		Polypeptide			
		ORF length (bp)	Introns	Size (aa)	Mass	pI	Domains
MdIAA24	chr13: 33,903,039–33,903,790	498	1	165	18,206.59	9.06	III IV
MdIAA25	chr15: 3,174,787–3,176,719	891	4	296	32,621.64	8.75	I II III IV
MdIAA26	chr15: 10,242,835–10,244,103	753	3	250	28,113.21	7.04	I II III IV
MdIAA27	chr15: 13,021,773–13,024,008	957	4	318	33,249.62	8.41	I II III IV
MdIAA28	chr15: 39,374,032–39,376,816	1017	5	338	36,858.27	8.45	I II III IV
MdIAA29	chr16: 9,903,364–9,905,701	927	4	308	32,488.63	7.74	I II III IV
MdIAA30	chr16: 16,289,039–16,291,841	735	4	244	26,749.71	8.06	I II III IV
MdIAA31	chr16: 16,430,457–16,432,404	576	2	191	21,339.53	7.61	I II III IV
MdIAA32	chr17: 15,807,534–15,810,215	996	4	331	35,954.68	8.36	I II III IV
MdIAA33	Chr13: 21,514,499–21,516,421	921	4	306	32,239.29	7.72	I II III IV

MdIAA8 and *MdIAA20*. All of these promoters contained both hormone response elements and stress response elements (Table 2). The presence of multiple hormone and stress response elements in their promoters indicated that *MdIAAs* play a vital role in plant development and stress response.

Expression Pattern Analysis of *MdIAAs* in Apple

To obtain a preliminary understanding of the potential function of the *MdIAA* genes in apple, qRT–PCR analysis was performed to investigate their expression patterns in different organs (Fig. 4 and Table S1). The results showed that *MdIAA3*, *MdIAA15*, *MdIAA16*, *MdIAA19*, *MdIAA21*, *MdIAA22*, *MdIAA28*, *MdIAA31*, and *MdIAA32* could be detected in both vegetative and reproductive organs, while *MdIAA1*, *MdIAA2*, *MdIAA4*, *MdIAA5*, *MdIAA6*, *MdIAA7*, *MdIAA8*, *MdIAA9*, *MdIAA10*, *MdIAA12*, *MdIAA13*, *MdIAA17*, *MdIAA18*, *MdIAA20*, *MdIAA24*, *MdIAA25*, *MdIAA26,* and *MdIAA33* were mainly expressed in reproductive organs, *MdIAA11*, *MdIAA14*, *MdIAA23*, *MdIAA27*, *MdIAA29,* and *MdIAA30* were mostly expressed in vegetative organs. The expression levels of different genes varied in apple organs from high (more than 500 the relative levels of (*MdIAA5/6/23*) to low (less than 10 the relative levels of *MdIAA15/21/31*), suggesting that the *MdIAAs* function diversely.

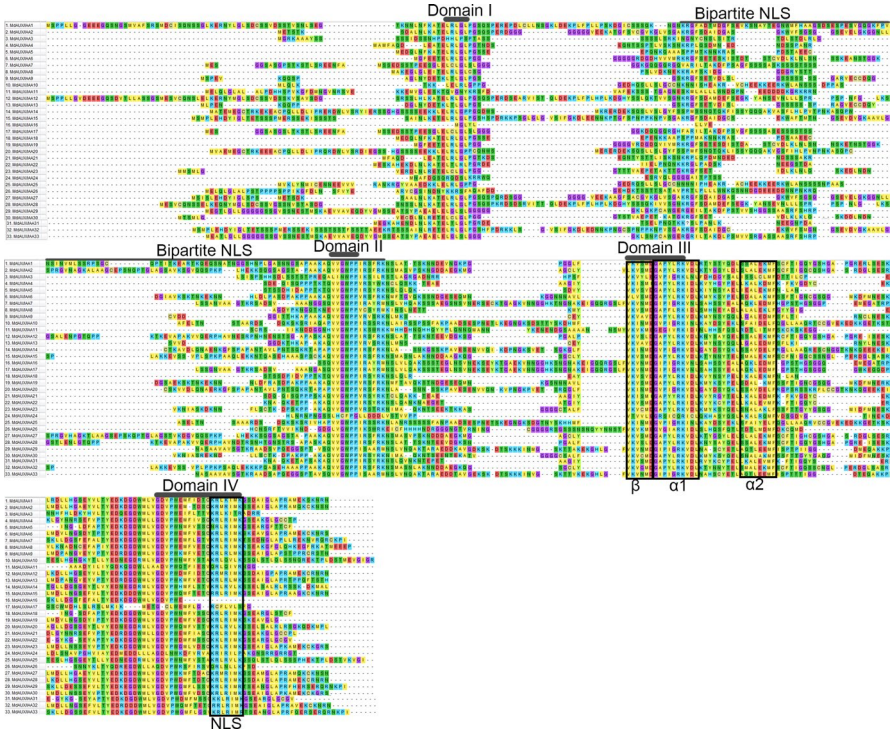

Fig. 2 Alignment of amino acid sequences of MdIAAs. All of the four conserved domains from Domain I to Domain IV were remarked by thick black lines. Bipartite NLS were remarked by thin black line. And NSL in domain IV as well as α helixes and β corner were remarked by black box

Interestingly, genes clustered into the same clade by the phylogenetic analysis showed similar expression patterns. *MdIAA4*/*21*/*22*/*31* in clade E had high expression levels in apples' reproductive organs while *MdIAA19*/*23*/*30* in clade B was mainly expressed in leaf buds. *MdIAA1*/*2*/*12*/*15*/*28*/*32* in clade A were mainly expressed in productive organs; *MdIAA9* and *13* in clade F were mainly expressed in flower buds and flowers; and *MdIAA10*, *20*, and *25* in clade I showed high expression levels in flowers and young fruit.

Expression Analysis of *MdIAA18* in Columnar and Non-columnar Apple Trees

To explore the expression of *MdIAA18* (identified closest to the *Co* region) in a columnar apple cultivar, tissue-specific expression analysis in columnar and non-columnar apple cultivars was performed by qRT-PCR. *MdIAA18* was highly expressed in young leaves and flower buds in both non-columnar apple 'McIntosh' and columnar apple 'Wijcik' (Fig. 5), similar to its expression pattern in 'Fuji.' Except of ceased shoot apex, the expression levels of *MdIAA18* in bark tissues, young shoot apex, leaves, and floral buds were significantly higher in the non-columnar cultivar than that of columnar apple.

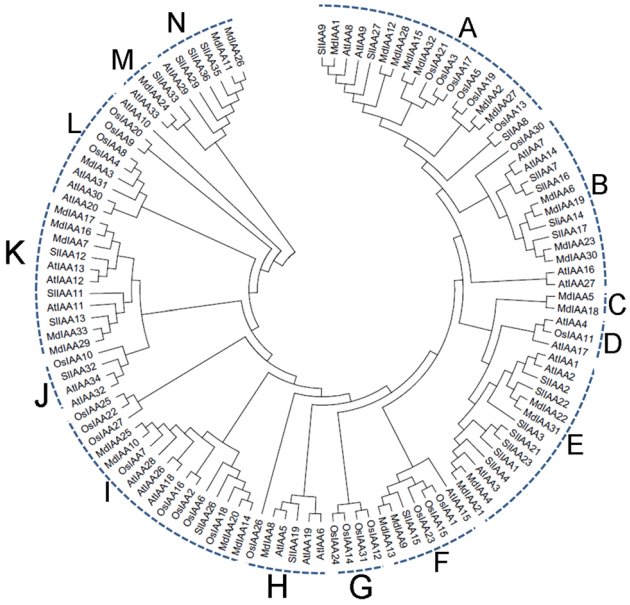

Fig. 3 Phylogenetic tree analysis among apple IAA proteins (MdIAAs), *Arabidopsis* IAA proteins (AtIAAs), *Solanum lycopersicum* IAA proteins (SlIAAs), and *Oryza sativa* IAA proteins (OsIAAs). A-N stood for IAA proteins clustered into the different clades

Analysis of the Biological Function of *MdIAA18*

To explain the biological function of *MdIAA18*, ectopic overexpression of *MdIAA18* in *Arabidopsis* was conducted. The phenotypic changes were characterized in transgenic T_3*Arabidopsis* seedlings (Fig. 6). Compared with wild-type plants, the root growth of transgenic plants was significantly restricted at 7 d after germination (Fig. 6a, b). *MdIAA18*-ox plants showed a compact dwarf phenotype (Fig. 6c) with small flat rosette leaves (Figs. 6e, f and S2) and short siliques (Figs. 6g, i); the total number of siliques was also lower (Fig. 6c).

Discussion

The Expansion of *MdIAA* Family Genes

Auxin is an important plant hormone that plays a role in virtually all aspects of plant development by regulating auxin response genes. *Aux/IAA* are some of the key early auxin response genes (Liscum and Reed 2002). A previous study had predicted 40 members of the *MdIAA* family in apple (Devoghalaere et al. 2012). However, five members that were instead predicted to be ARF genes with DBD domains and two members with unknown function were excluded in this paper. Thirty-three *Aux/IAA* members were identified that were not evenly distributed in the apple genome

Table 2 *cis*-element analysis in the promoters of *MdIAAs* in apple

Gene	Hormone-related *cis*-elements						Stress-related *cis*-element				
	ABA	GA	IAA	SA	Ethylene	MeJA	Low temp	Drought	Heat	Wound	TC-Rich
MdIAA1	ABRE 1	GARE-motif 2	TGA ELEMENT 1	TCA-element 1			LTR 1	MBS 4	HSE 1	WUN-motif 1	
		P-Box 1									
		TATC-BOX 1									
MdIAA2	ABRE 1	GARE-motif 1	TGA-ELEMENT 1	TCA-element 3		CGTCA-motif 1		MBS 1		WUN-motif 1	TC-Rich 2
MdIAA3	ABRE 1		AuxRE 1					MBS 3		WUN-motif 1	TC-Rich 1
MdIAA4	ABRE 5	GARE-motif 1	TGA-ELEMENT 2			CGTCA-motif 1	LTR 1	MBS 3			
		P-Box 2									
		TATC-BOX 1									
MdIAA5	ABRE 1	TATC-BOX 1		TCA-element 1	ERE 3	CGTCA-motif 1		MBS 3			TC-Rich 2
MdIAA6	ABRE 3		AuxRE 1	TCA-element 1		CGTCA-motif 2	LTR 3		HSE 1		TC-Rich 5
MdIAA7	ABRE 4		AuxRE 1	TCA-element 1	ERE 1	CGTCA-motif 1	LTR 2	MBS 3	HSE 1		TC-Rich 1
			TGA-element 2								

Table 2 (continued)

Gene	Hormone-related *cis*-elements						Stress-related *cis*-element				
	ABA	GA	IAA	SA	Ethylene	MeJA	Low temp	Drought	Heat	Wound	TC-Rich
MdIAA9	ABRE 3		TGA-element 1	TCA-element 2		CGTCA-motif 4		MBS 1	HSE 1	WUN-motif 1	TC-Rich 1
MdIAA10	ABRE 1	P-box 1	AuxRE 1	TCA-element 4				MBS 3	HSE 1		TC-Rich 2
			AuxRR-core 1								
MdIAA11	ABRE 2	P-box 2	TGA-element 1		ERE 1	CGTCA-motif 2		MBS 4	HSE 2		
		GARE-motif 1									
MdIAA12	ABRE 1	GARE-motif 1	TGA-element 1	TCA-element 2	ERE 1			MBS 1	HSE 4		TC-Rich 2
MdIAA13	ABRE 3			TCA-element 2	ERE 2	CGTCA-motif 2		MBS 2	HSE 1	WUN-motif 1	TC-Rich 1
MdIAA14	ABRE 1	GARE-motif 3		TCA-element 2	ERE 1			MBS 2	HSE 5		
		P-box 1									
MdIAA15	ABRE 1	GARE-motif 1	TGA-box 1	TCA-element 1		CGTCA-motif 6			HSE 1		TC-Rich 1

Table 2 (continued)

Gene	Hormone-related *cis*-elements						Stress-related *cis*-element				
	ABA	GA	IAA	SA	Ethylene	MeJA	Low temp	Drought	Heat	Wound	TC-Rich
			TGA-element 1								
MdIAA16	ABRE 1	GARE-motif 3	TGA-element 2		ERE 1	CGTCA-motif 1	LTR 1	MBS 1			
MdIAA17	ABRE 2		TGA-element 1	TCA-element 1	ERE 1	CGTCA-motif 3	LTR 1	MBS 1			
MdIAA18	ABRE 4	GARE-motif 1	TGA-element 2	TCA-element 1	ERE 1			MBS 2	HSE 1		TC-Rich 2
MdIAA19	ABRE 5		TGA-element 2			CGTCA-motif 2		MBS 2	HSE 2		TC-Rich 4
MdIAA21	ABRE 2	GARE-motif 1	TGA-element 2	TCA-element 4		CGTCA-motif 3	LTR 1	MBS 2	HSE 1		TC-Rich 2
		P-box 1									
MdIAA22	ABRE 1					CGTCA-motif 1		MBS 3			
MdIAA23	ABRE 2			TCA-element 1		CGTCA-motif 1		MBS 2	HSE 2		TC-Rich 2
MdIAA24	ABRE 1	TATC-box 1	AuxRR-core 1			CGTCA-motif 2		MBS 3		WUN-motif 1	TC-Rich 2
MdIAA25	ABRE 2	P-box 1		TCA-element 4					HSE 1		TC-Rich 1

Table 2 (continued)

Gene	Hormone-related *cis*-elements						Stress-related *cis*-element				
	ABA	GA	IAA	SA	Ethylene	MeJA	Low temp	Drought	Heat	Wound	TC-Rich
MdIAA26	ABRE 3		TGA-element 1			CGTCA-motif 1	LTR 1	MBS 2			
MdIAA27		P-box 1	TGA-element 1	TCA-element 1	ERE 2			MBS 4	HSE 2		TC-Rich 2
MdIAA28	ABRE 1	GARE-motif 1	TGA-element 1		ERE 2	CGTCA-motif 1	LTR 1	MBS 2	HSE 2		TC-Rich 2
		P-box 1									
MdIAA29		TATC-box 2		TCA-element 5	ERE 1	CGTCA-motif 1		MBS 4		WUN-motif 1	TC-Rich 2
MdIAA30		GARE-motif 2		TCA-element 1	ERE 1	CGTCA-motif 1		MBS 1	HSE 2		TC-Rich 2
MdIAA31	ABRE 1	GARE-motif 1				CGTCA-motif 1		MBS 2	HSE 1		TC-Rich 3
		TATC-box 1									
MdIAA32	ABRE 1			TCA-element 1				MBS 1	HSE 3		TC-Rich 4
MdIAA33	ABRE 1	GARE-motif 1	TGA-element 1	TCA-element 2		CGTCA-motif 1	LTR 1	MBS 1	HSE 2		TC-Rich 2
		P-box 1									

2-kb upstream sequences of *MdIAAs* were obtained from GDR database based on the *MdIAA* genomic sequences. The *cis*-elements were analyzed by PlantCARE database

(Table 1 and Fig. 1). The number of *MdIAAs* in apple is comparable to that of Arabidopsis (29) (Liscum and Reed 2002), rice (31) (Jain et al. 2006a), *Zea mays* (31/34) (Wang et al. 2010), and *P. trichocarpa* (35) (Kalluri et al. 2007), although their genome size is quite different. Additionally, many sister pairs were found in apple that were highly similar with the same number of exons and introns, similar gene structure, and were located on homologous chromosomes at overlapping regions of the apple genome. The results of Table 1 and Fig. 1 show that *MdIAA* family genes were distributed on all chromosomes except Chr.17, suggesting the expansion occurred during apple genome-wide duplication, and their functional redundancy or specificity in auxin-regulated developmental processes. On average, 64.5% of plant genes are paralogous; in apple, this number is 84.4% (Panchy et al. 2016). This is consistent with the relatively recent (> 50 million years ago) GWD in the Pyreae which led to the evolution from a 9-ancestral chromosome to the 17-chromosome karyotype of extant Malus (Velasco et al. 2010; Wu et al. 2017). A similar phenomenon is also observed in other species such as *G. Max* (Singh and Jain 2015), *P. trichocarpa* (Kalluri et al. 2007), *Z. Mays* (Wang et al. 2010), and *Arabidopsis* (Liscum and Reed 2002); there were no sister pairs in rice (Jain et al. 2006a). Pairwise homologs of *MdIAAs* might share the same or overlapping functions. Based on their gene structure and sequence similarity (Fig. 3), *MdIAA16* and *MdIAA17*, which are located at the region of 16.46 kb on chromosome 10, may arise from tandem duplication. Tandem duplications in the apple genome have been identified for isopentenyl transferases and CK oxidase/dehydrogenases family genes that respond to the auxin pathway (Tan et al. 2018). A similar phenomenon has been detected in eucalyptus for genes such as *EgrIAA29A* and *EgrIAA29B* (Yu et al. 2015). *Aux/IAA* genes widely exist among plants and their expansion is likely to have assisted in the development of their specialized functions (Nemhauser 2018). Similarly, the qRT-PCR results of Fig. 4 show that each *MdIAA* gene had its specific expression pattern and likely role in development, and indicated that *MdIAA* family genes play important roles during apple growth and underwent functional specialization during plant evolution.

The Importance of the Different Domains of *MdIAA* Family Genes

Some members of the *Aux/IAA* family are rapidly induced in response to auxin (Abel and Theologis 1996) and display qualitative and quantitative differences in their regulation by auxin (Abel and Theologis 1995; Rogg et al. 2001). Thirty-three *MdIAA* members were identified in the apple genome that possess different domains (Fig. 2). Based on previous studies, there are four key domains with varied functions. The dimerization domains (domains III and IV) can form homodimers and heterodimers with either a second *Aux/IAA* or an ARF to activate or suppress the expression of downstream genes (Kim et al. 1997; Ulmasov et al. 1997, 1999). Domain II is related to the homeostasis of Aux/IAA proteins by regulating their instability and recognition by the ubiquitin–proteasome protein (TIR1) degradation pathway (Tan et al. 2007). Domain I contains a leucine

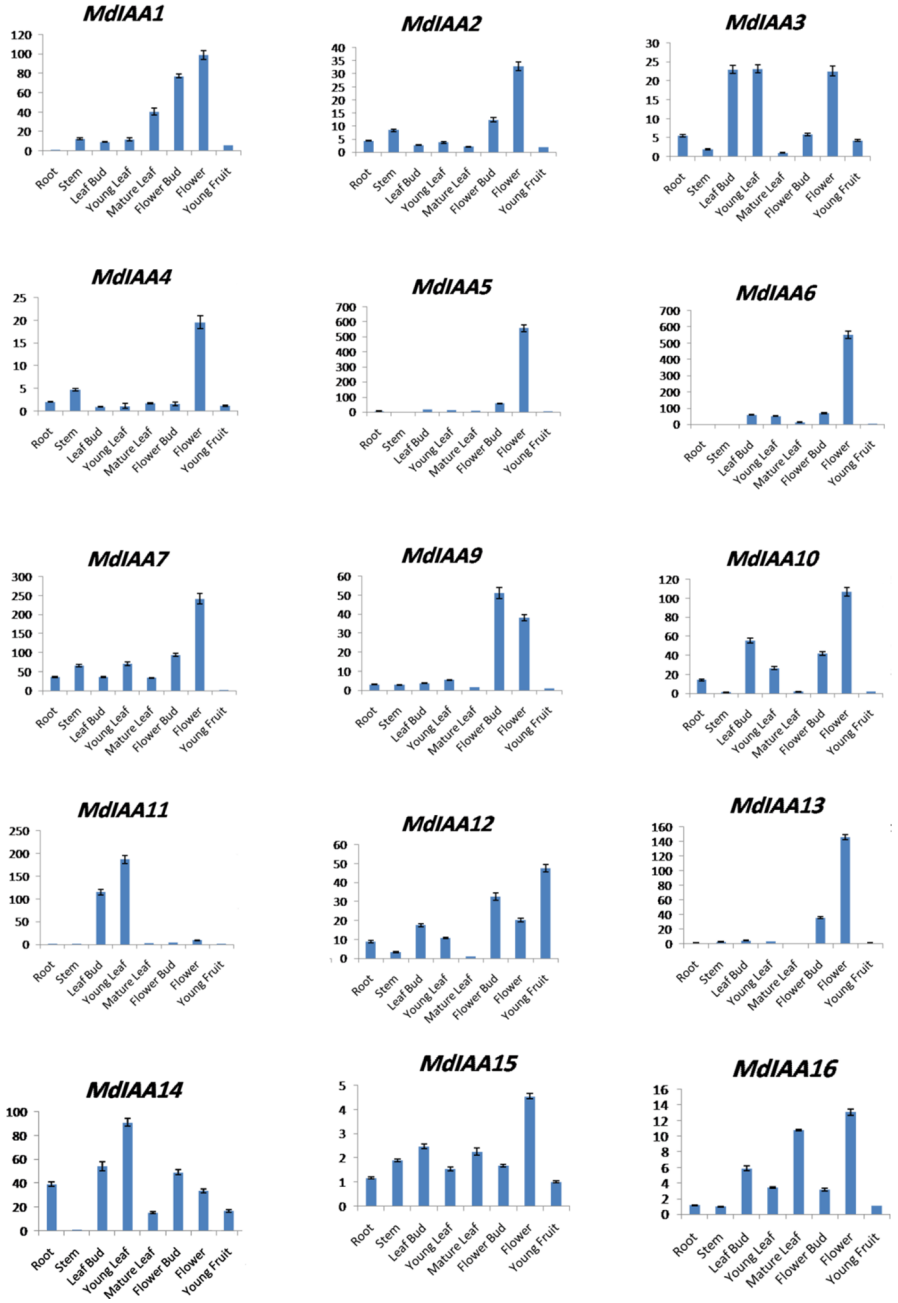

Fig. 4 Expression patterns of *MdIAAs* in different tissues of 'Fuji' by quantitative real-time PCR. *MdActin* acted as reference genes

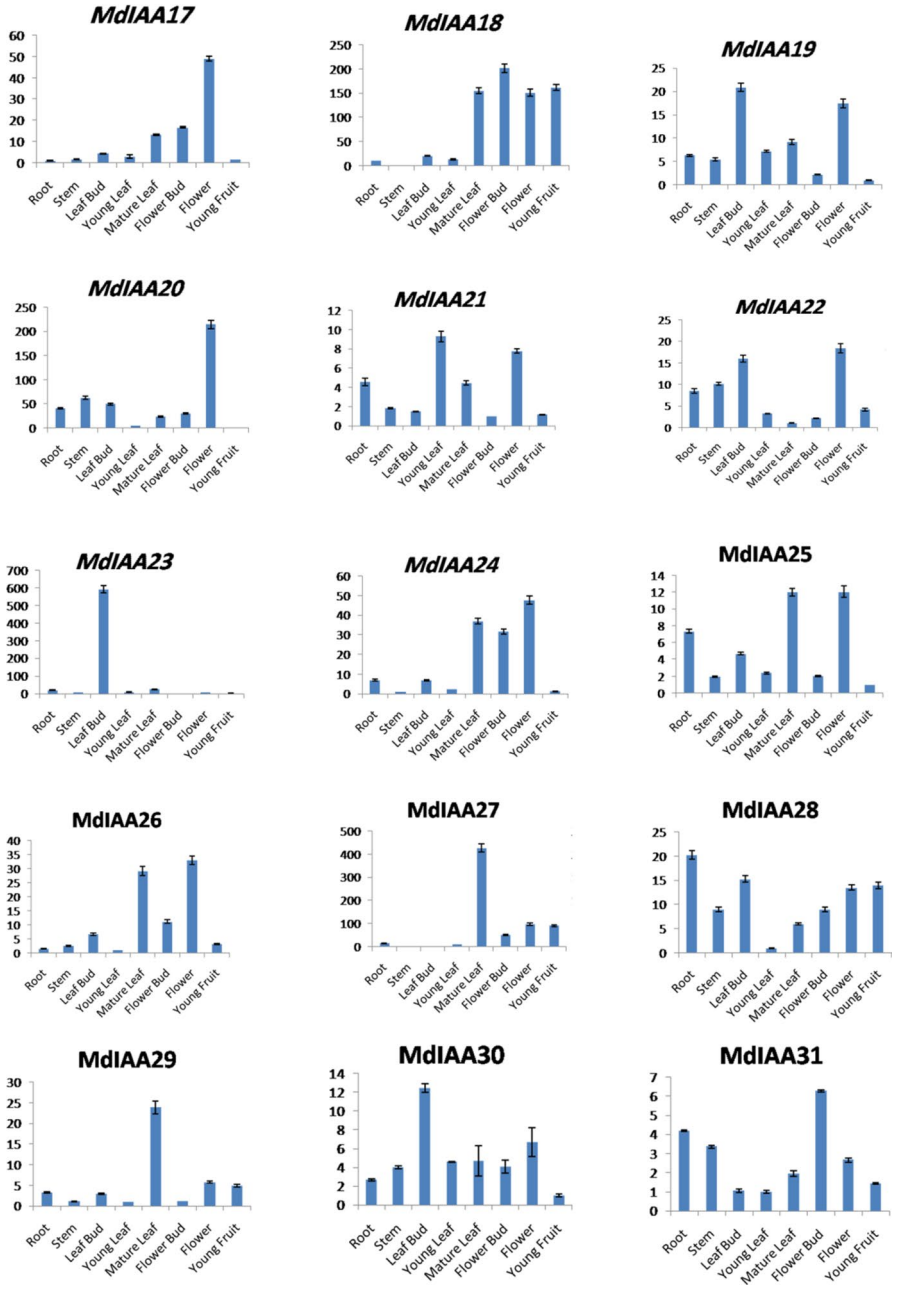

Fig. 4 (continued)

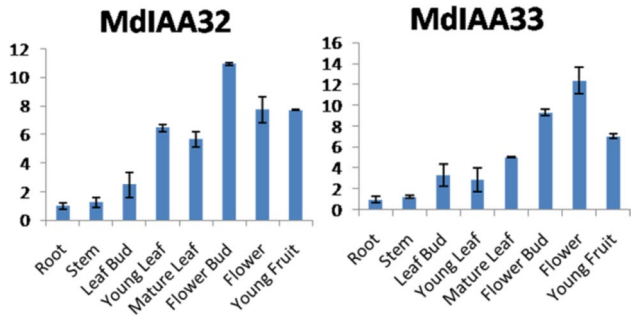

Fig. 4 (continued)

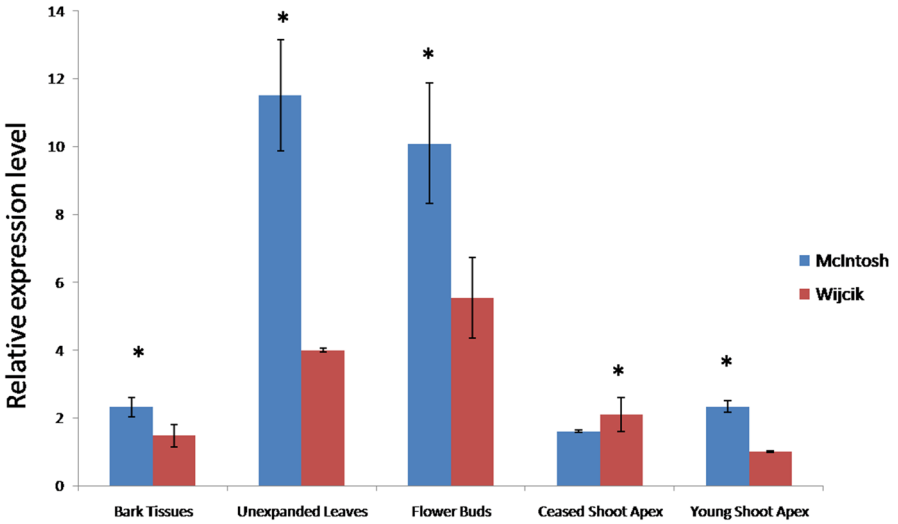

Fig. 5 Different expression levels of *MdIAA18* in columnar apple 'Wijcik' and non-columnar apple 'McIntosh.' The results of quantitative real-time PCR were normalized by *MdActin*, *Represented significant differences ($P < 0.05$)

repeat motif (L × L × L) and functions as a repressor when homodimers occur between domains III and IV (Tiwari et al. 2004). The auxin response depends on the auxin-dependent degradation of Aux/IAA proteins (Yang et al. 2004). Figure 3 indicates that 33 non-redundant *MdIAA* genes in apple, including 27 canonical members and six members lacking domains I and/or II. *MdIAA* genes lacking domains I and/or II have been shown to be involved in the auxin signaling pathway but no information is available regarding genes lacking domains III and/or IV (Sato and Yamamoto 2008; Song et al. 2009; Wu et al. 2012). Therefore, all *MdIAA*s identified in the current result contain either four domains or domains III and IV, indicating that they have physiological functions during plant growth and development. Amino acids located in Aux/IAA domain regions

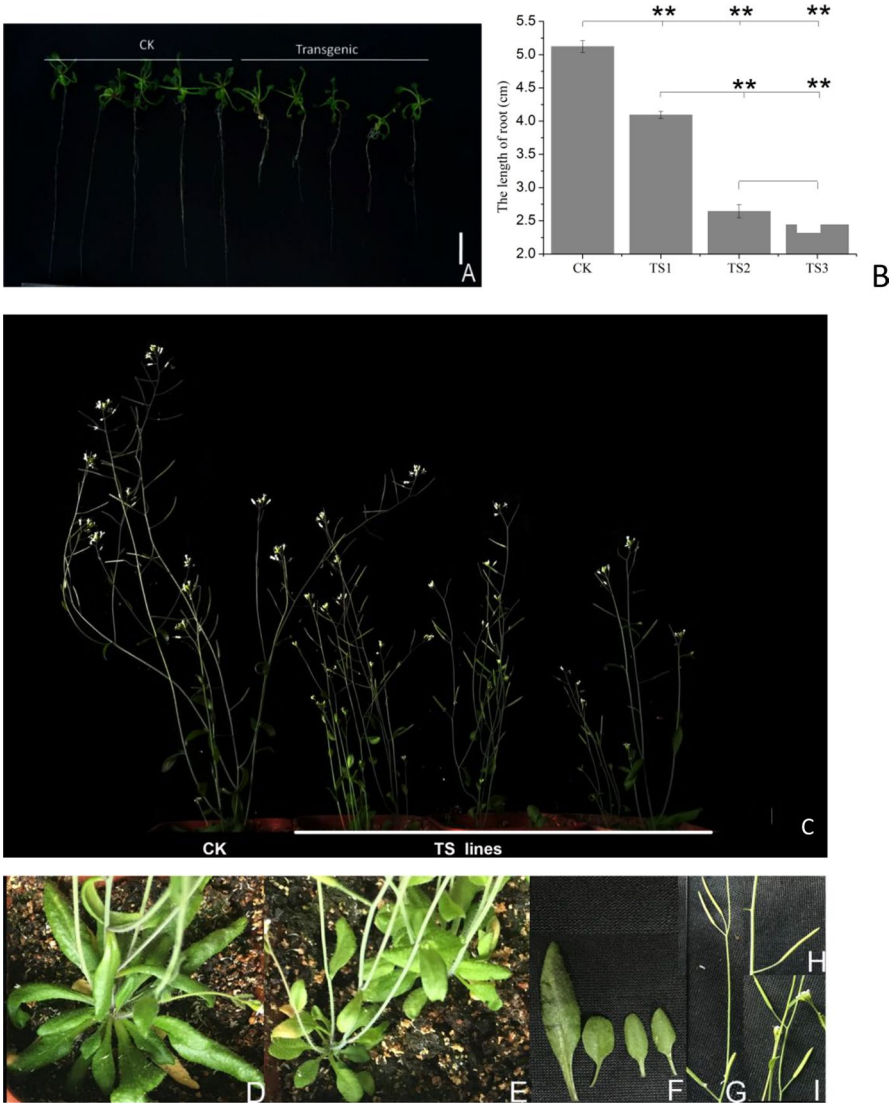

Fig. 6 Phenotypes of *MdIAA18*-ox *Arabidopsis* plants. **a** The comparison of root lengths in *MdIAA18*-ox *Arabidopsis* (Transgenic) and wild-type plants (CK) at 7 days after germination (Scale bar represents 1 cm); **b** histogram of the length of roots from *MdIAA18*-ox and CK plants (*$P < 0.05$; **$P < 0.01$); **c** the compact plant form in TS lines; comparison of rosette leaves in CK (**d**) and *MdIAA18*-ox plants (**e**, **f**); comparison of siliques in CK (**h**) and *MdIAA18*-ox plants (**g**, **i**); the same scale bar representing 1 cm for (**c**–**i**) and the phenotype were detected after one month growth

showed high similarities in apple (Devoghalaere et al. 2012) and Chinese hickory (Yuan et al. 2018), while they were highly variable in other regions, indicating that the function of these genes is likely to be highly conserved across species even as it differs among members.

Functional Analysis of *MdIAA* Family Genes

The stability of Aux/IAA proteins is crucial for their functions in regulating plant growth and development; several plant *Aux/IAA* genes have, therefore, been characterized using gain-of-function (Abel and Theologis 1995; Uehara et al. 2008) or loss-of-function transgenic lines (Chaabouni et al. 2009). The resulting transgenic plants have pleiotropic phenotypes, some of which are consistent with increased auxin sensitivity and others with decreased auxin sensitivity. Overexpression of *MdIAA18* in Arabidopsis decreases auxin sensitivity in terms of plant height and root length (Fig. 6), similar to other canonical *Aux/IAA* genes such as *OsIAA1* (Song et al. 2009), *OsIAA9* (Luo et al. 2015), and *PtrIAA14* (Liu et al. 2015). An earlier report indicated that an E3 ubiquitin-ligase SOR1 interacting with OsIAA26 acts downstream of OsTIR1/AFB2-auxin-OsIAA9 signaling to modulate ethylene inhibition of root growth in rice seedlings (Chen et al. 2018). In Arabidopsis, reduced inhibition of root elongation by IAA has been identified in gain-of-function mutations of several Aux/IAA genes such as *iaa28-1* (Rogg et al. 2001), *iaa3/shy2* (Tian and Reed 1999), *iaa7/axr2* (Wilson et al. 1990), *iaa17/axr3* (Leyser et al. 1996), and *slr1/iaa14* (Fukaki et al. 2002). Downregulation of *SlIAA9* enhances auxin sensitivity and *SlIAA9* antisense plants have increased hypocotyl/stem elongation, increased leaf vascularization, reduced apical dominance, and parthenocarpy (Wang et al. 2005). In *Carica papaya*, different *CpIAA* expression levels were reported in response to fruit development and during ripening (Liu et al. 2017). Overexpression of *MdIAA18* in transgenic Arabidopsis plants results in auxin-related phenotypes, including inhibition of primary root length and rosette leaf growth as well as reduced apical dominance, indicating the functional specificity of different members of the *Aux/IAA* family. Ectopic expression *MdIAA18* in Arabidopsis suppressed root elongation. Root growth is regulated by the interaction between ethylene and auxin. Such ethylene-regulated root growth is dependent on the transport of auxin from the root apex via the lateral root cap and auxin responses occurring in multiple elongation zone tissues (Swarup et al. 2007). The presence of auxin facilitates the degradation of the Aux/IAA proteins while the increased concentration of free auxin interacts with ethylene to suppress root elongation (Chen et al. 2018). Plant root elongation was suppressed with IAA concentrations of more 10^{-3} μM (Paul and Bari 1979).

Analysis of *MdIAA18* in Relation to Different Apple Architecture

The apple columnar phenotype is controlled by a single dominant gene, *Co*, that is located in the region of 18.5–19.1 Mb on chromosome 10 (Bai et al. 2012; Baldi et al. 2013; Petersen and Krost 2013). Among the 33 *MdIAA* genes examined here, four members were located on Chr.10 with *MdIAA18* being the closest to the *Co* region (Fig. 1 and Table 1). According to Fig. 3, MdIAA5 and MdIAA18 are grouped in sub-clade C that contains no homologous *AtIAA*s. The expression level of *MdIAA18* was significantly lower in tested tissues and organs (except ceased

shoot apex) in the 'Wijcik' cultivar than in 'McIntosh' (Fig. 5) suggesting that the expression of *MdIAA18* facilitated the vigorous growth of the non-columnar trees. This result was consistent with the high IAA and cytokinin level in shoots of columnar apple trees compared with non-columnar trees (Petersen and Krost 2013). The relatively higher auxin promotes the degradation of *Aux/IAA* genes that then release ARFs to activate the expression of downstream auxin-responsive genes (Choi et al. 2018). The current result speculate that *MdIAA18* may be involved in the columnar growth phenotype. Additionally, *MdIAA18* is involved in fruit ripening and fruit size, which indicates that the *MdIAAs* function in pleiotropic phenotype, consistent with their ubiquitous expression pattern (Devoghalaere et al. 2012). Further experiments are required to clarify the mechanism underlying the effect of *MdIAA18* on columnar growth in apple.

Conclusion

In this study, a total of 33 *MdIAA* gene members were identified in apple, of which 27 gene members contained four conserved domains, whereas the others lost one or two conserved domains. Most *MdIAA* gene members were highly expressed in leaf buds and reproductive organs and their expression levels varied diversely in apple. Overexpression of *MdIAA18* in *Arabidopsis* lead to the auxin-related phenotype, dwarf plants with small restricted root growth, rosette leaves, short siliques, and decreased numbers of siliques. Additionally, the expression levels of *MdIAA18* were significantly higher in the non-columnar cultivar than that of columnar apple, suggesting its potential roles in the development of apple tree ideotype.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Abel S, Theologis A (1995) A polymorphic bipartite motif signals nuclear targeting of early auxin-inducible proteins related to PS-IAA4 from pea (*Pisum sativum*). Plant J 8:87–96

Abel S, Theologis A (1996) Early genes and auxin action. Plant Physiol 111:9–17

Asif M et al (2006) Isolation of high-quality RNA from apple (*Malus domestica*) fruit. J Agr Food Chem 54:5227–5229. https://doi.org/10.1021/jf053137n

Bai T et al (2012) Fine genetic mapping of the *Co* locus controlling columnar growth habit in apple. Mol Genet Genom 287:437–450. https://doi.org/10.1007/s00438-012-0689-5

Baldi P et al (2013) Genetic and physical characterisation of the locus controlling columnar habit in apple (*Malus* × *domestica* Borkh.). Mol Breed 31:429–440. https://doi.org/10.1007/s11032-012-9800-1

Benkova E et al (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell 115:591–602

Catala C, Rose JK, Bennett AB (2000) Auxin-regulated genes encoding cell wall-modifying proteins are expressed during early tomato fruit growth. Plant Physiol 122:527–534
Chaabouni S et al (2009) *Sl-IAA3*, a tomato *Aux/IAA* at the crossroads of auxin and ethylene signalling involved in differential growth. J Exp Bot 60:1349–1362. https://doi.org/10.1093/jxb/erp009
Chapman EJ, Estelle M (2009) Mechanism of auxin-regulated gene expression in plants. Annu Rev Genet 43:265–285. https://doi.org/10.1146/annurev-genet-102108-134148
Chen H et al (2018) E3 ubiquitin ligase SOR1 regulates ethylene response in rice root by modulating stability of Aux/IAA protein. Proc Natl Acad Sci USA 115:4513–4518. https://doi.org/10.1073/pnas.1719387115
Choi HS, Seo M, Cho HT (2018) Two TPL-binding motifs of *ARF2* are involved in repression of auxin responses. Front Plant Sci 9:372. https://doi.org/10.3389/fpls.2018.00372
Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J 16:735–743
Devoghalaere F et al (2012) A genomics approach to understanding the role of auxin in apple (*Malus x domestica*) fruit size control. BMC Plant Biol 12:7. https://doi.org/10.1186/1471-2229-12-7
Dharmasiri N, Dharmasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor. Nature 435:441–445. https://doi.org/10.1038/nature03543
Fukaki H et al (2002) Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14* gene of *Arabidopsis*. Plant J 29:153–168
Galli M et al (2015) Auxin signaling modules regulate maize inflorescence architecture. Proc Natl Acad Sci USA 112:13372–13377. https://doi.org/10.1073/pnas.1516473112
Gray WM et al (2001) Auxin regulates SCF(TIR1)-dependent degradation of AUX/IAA proteins. Nature 414:271
Guilfoyle TJ, Hagen G (2012) Getting a grasp on domain III/IV responsible for Auxin Response Factor-IAA protein interactions. Plant Sci 190:82–88. https://doi.org/10.1016/j.plantsci.2012.04.003
Hagen G, Guilfoyle T (2002) Auxin-responsive gene expression: genes, promoters and regulatory factors. Plant Mol Biol 49:373–385
Hofgen R, Willmitzer L (1988) Storage of competent cells for Agrobacterium transformation. Nucleic Acids Res 16:9877
Hollender CA, Dardick C (2015) Molecular basis of angiosperm tree architecture. New Phytol 206:541–556. https://doi.org/10.1111/nph.13204
Hu B et al (2015) GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics 31:1296–1297. https://doi.org/10.1093/bioinformatics/btu817
Jain M et al (2006) Structure and expression analysis of early auxin-responsive Aux/IAA gene family in rice (*Oryza sativa*). Funct Integr Genom 6:47–59. https://doi.org/10.1007/s10142-005-0005-0
Jain M et al (2006) The auxin-responsive *GH3* gene family in rice (*Oryza sativa*). Funct Integr Genom 6:36–46. https://doi.org/10.1007/s10142-005-0142-5
Kalluri UC et al (2007) Genome-wide analysis of *Aux/IAA* and *ARF* gene families in *Populus trichocarpa*. BMC Plant Biol 7:59. https://doi.org/10.1186/1471-2229-7-59
Kepinski S, Leyser O (2005) The *Arabidopsis* F-box protein TIR1 is an auxin receptor. Nature 435:446–451. https://doi.org/10.1038/nature03542
Kim J, Harter K, Theologis A (1997) Protein-protein interactions among the Aux/IAA proteins. Proc Natl Acad Sci USA 94:11786–11791
Knudsen S (1999) Promoter2.0: for the recognition of PolII promoter sequences. Bioinformatics 15:356–361
Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054
Lapins KO (1969) Segregation of compact growth types in certain apple seedling progenies. Can J Plant Sci 49:765–768
Lescot M et al (2002) PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Res 30:325–327
Leyser HM et al (1996) Mutations in the *AXR3* gene of *Arabidopsis* result in altered auxin response including ectopic expression from the *SAUR-AC1* promoter. Plant J 10:403–413
Liscum E, Reed JW (2002) Genetics of Aux/IAA and ARF action in plant growth and development. Plant Mol Biol 49:387–400
Liu S et al (2015) Expression of wild-type PtrIAA14.1, a poplar *Aux/IAA* gene causes morphological changes in *Arabidopsis*. Front Plant Sci 6:388. https://doi.org/10.3389/fpls.2015.00388

Liu K et al (2017) Genome-wide analysis and characterization of *Aux/IAA* family genes related to fruit ripening in papaya (*Carica papaya* L.). BMC Genom 18:351. https://doi.org/10.1186/s12864-017-3722-6

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408

Ljung K (2013) Auxin metabolism and homeostasis during plant development. Development 140:943–950. https://doi.org/10.1242/dev.086363

Looney NE, Lane WD (1984) Spur-type growth mutants of McIntosh apple: a review of their genetics, physiology and field performance. Acta Hortic 146:31–46

Lu G et al (2015) OsPIN5b modulates rice (*Oryza sativa*) plant architecture and yield by changing auxin homeostasis, transport and distribution. Plant J 83:913–925. https://doi.org/10.1111/tpj.12939

Ludwig Y et al (2014) Diversity of stability, localization, interaction and control of downstream gene activity in the Maize Aux/IAA protein family. PLoS ONE 9:e107346. https://doi.org/10.1371/journal.pone.0107346

Luo S et al (2015) Constitutive expression of *OsIAA9* affects starch granules accumulation and root gravitropic response in *Arabidopsis*. Front Plant Sci 6:1156. https://doi.org/10.3389/fpls.2015.01156

Mockaitis K, Estelle M (2008) Auxin receptors and plant development: a new signaling paradigm. Annu Rev Cell Dev Biol 24:55–80. https://doi.org/10.1146/annurev.cellbio.23.090506.123214

Nemhauser JL (2018) Back to basics: what is the function of an Aux/IAA in auxin response? New Phytol 218:1295–1297. https://doi.org/10.1111/nph.15172

Okushima Y et al (2005) AUXIN RESPONSE FACTOR 2 (ARF2): a pleiotropic developmental regulator. Plant J 43:29–46. https://doi.org/10.1111/j.1365-313X.2005.02426.x

Panchy N, Lehti-Shiu MD, Shiu SH (2016) Evolution of gene duplication in plants. Plant Physiol 171:2294–2316. https://doi.org/10.1104/pp.16.00523

Paul KK, Bari MA (1979) Protocol establishment for micro propagation and callus regeneration of *Maulavi Kachu* (L Schott.) From Cormel Axillary Bud Meristem

Peer WA (2013) From perception to attenuation: auxin signalling and responses. Curr Opin Plant Biol 16:561–568. https://doi.org/10.1016/j.pbi.2013.08.003

Petersen R, Krost C (2013) Tracing a key player in the regulation of plant architecture: the columnar growth habit of apple trees (*Malus x domestica*). Planta 238:1–22. https://doi.org/10.1007/s00425-013-1898-9

Robert X, Gouet P (2014) Deciphering key features in protein structures with the new ENDscript server. Nucleic Acids Res 42:W320–W324. https://doi.org/10.1093/nar/gku316

Rogg LE, Lasswell J, Bartel B (2001) A gain-of-function mutation in IAA28 suppresses lateral root development. Plant Cell 13:465–480

Roy S et al (2017) *MtLAX2*, a functional homologue of the auxin importer *AtAUX1*, is required for nodule organogenesis. Plant Physiol 174:326–338

Santner A, Estelle M (2009) Recent advances and emerging trends in plant hormone signalling. Nature 459:1071–1078. https://doi.org/10.1038/nature08122

Sato A, Yamamoto KT (2008) Overexpression of the non-canonical *Aux/IAA* genes causes auxin-related aberrant phenotypes in *Arabidopsis*. Physiol Plant 133:397–405. https://doi.org/10.1111/j.1399-3054.2008.01055.x

Shapiro BE et al (2015) Analysis of cell division patterns in the *Arabidopsis* shoot apical meristem. Proc Natl Acad Sci U S A 112:4815–4820

Singh VK, Jain M (2015) Genome-wide survey and comprehensive expression profiling of *Aux/IAA* gene family in chickpea and soybean. Front Plant Sci 6:918. https://doi.org/10.3389/fpls.2015.00918

Song Y, Wang L, Xiong L (2009) Comprehensive expression profiling analysis of *OsIAA* gene family in developmental processes and in response to phytohormone and stress treatments. Planta 229:577–591. https://doi.org/10.1007/s00425-008-0853-7

Swarup R et al (2007) Ethylene upregulates auxin biosynthesis in *Arabidopsis* seedlings to enhance inhibition of root cell elongation. Plant Cell 19:2186–2196. https://doi.org/10.1105/tpc.107.052100

Szemenyei H, Hannon M, Long JA (2008) TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis. Science 319:1384–1386. https://doi.org/10.1126/science.1151461

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci U S A 101:11030–11035. https://doi.org/10.1073/pnas.0404206101
Tan X et al (2007) Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature 446:640–645. https://doi.org/10.1038/nature05731
Tan M et al (2018) Identification and expression analysis of the *IPT* and *CKX* gene families during axillary bud outgrowth in apple (*Malus domestica* Borkh.). Gene 651:106–117. https://doi.org/10.1016/j.gene.2018.01.101
Tao S, Estelle M (2018) Mutational studies of the Aux/IAA proteins in Physcomitrella reveal novel insights into their function. New Phytol 218:1534–1542. https://doi.org/10.1111/nph.15039
Tian Q, Reed JW (1999) Control of auxin-regulated root development by the *Arabidopsis thaliana SHY2/IAA3* gene. Development 126:711–721
Tiwari SB et al (2001) AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin. Plant Cell 13:2809–2822
Tiwari SB, Hagen G, Guilfoyle T (2003) The roles of auxin response factor domains in auxin-responsive transcription. Plant Cell 15:533–543
Tiwari SB, Hagen G, Guilfoyle TJ (2004) Aux/IAA proteins contain a potent transcriptional repression domain. Plant Cell 16:533–543. https://doi.org/10.1105/tpc.017384
Uehara T et al (2008) Domain II mutations in CRANE/IAA18 suppress lateral root formation and affect shoot development in *Arabidopsis thaliana*. Plant Cell Physiol 49:1025–1038. https://doi.org/10.1093/pcp/pcn079
Ulmasov T et al (1995) Composite structure of auxin response elements. Plant Cell 7:1611–1623. https://doi.org/10.1105/tpc.7.10.1611
Ulmasov T, Hagen G, Guilfoyle TJ (1997) ARF1, a transcription factor that binds to auxin response elements. Science 276:1865–1868
Ulmasov T, Hagen G, Guilfoyle TJ (1999) Activation and repression of transcription by auxin-response factors. Proc Natl Acad Sci USA 96:5844–5849
Velasco R et al (2010) The genome of the domesticated apple (*Malus x domestica* Borkh.). Nat Genet 42:833–839. https://doi.org/10.1038/ng.654
Wan S et al (2014) Genome-wide identification, characterization and expression analysis of the auxin response factor gene family in *Vitis vinifera*. Plant Cell Rep 33:1365–1375. https://doi.org/10.1007/s00299-014-1622-7
Wang H et al (2005) The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. Plant Cell 17:2676–2692. https://doi.org/10.1105/tpc.105.033415
Wang Y et al (2010) Genome-wide analysis of primary auxin-responsive *Aux/IAA* gene family in maize (*Zea mays*. L.). Mol Biol Rep 37:3991–4001. https://doi.org/10.1007/s11033-010-0058-6
Wang Y et al (2014) Effect of root pruning and irrigation regimes on pear tree: growth, yield and yield components. Hortic Sci 41:34–43
Wang LM et al (2018) The isolation of the IGT family genes in *Malus* × *domestica* and their expressions in four idiotype apple cultivars. Tree Genet Genomes 14:46
Watanabe M et al (2008) Seasonal changes of IAA and cytokinin in shoots of columnar type apple trees. Acta Hortic 774:75–80
Wilson BF (2000) Apical control of branch growth and angle in woody plants. Am J Bot 87:601–607
Wilson AK et al (1990) A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene and abscisic acid. Mol Gen Genet 222:377–383
Winkler M et al (2017) Variation in auxin sensing guides AUX/IAA transcriptional repressor ubiquitylation and destruction. Nat Commun 8:15706. https://doi.org/10.1038/ncomms15706
Woodward AW, Bartel B (2005) Auxin: regulation, action, and interaction. Annals of Botany 95:707
Wu J et al (2012) Genome-wide analysis of *Aux/IAA* gene family in Solanaceae species using tomato as a model. Mol Genet Genom 287:295–311. https://doi.org/10.1007/s00438-012-0675-y
Wu J et al (2014) Genome-wide identification and transcriptional profiling analysis of auxin response-related gene families in cucumber. BMC Res Notes 7:218. https://doi.org/10.1186/1756-0500-7-218
Wu W et al (2017) Evolution analysis of the *Aux/IAA* gene family in plants shows dual origins and variable nuclear localization signals. Int J Mol Sci. https://doi.org/10.3390/ijms18102107
Xu K et al (2017) Characterization of a SUPERMAN-like Gene, *MdSUP11*, in apple (*Malus* × *domestica* Borkh.). Plant Physiol Biochem 125:136–142

Yang X et al (2004) The IAA1 protein is encoded by *AXR5* and is a substrate of SCF(TIR1). Plant J 40:772–782. https://doi.org/10.1111/j.1365-313X.2004.02254.x

Yu H et al (2015) Comprehensive genome-wide analysis of the *Aux/IAA* gene family in eucalyptus: evidence for the role of *EgrIAA4* in wood formation. Plant Cell Physiol 56:700–714

Yuan H et al (2018) Identification and expression profiling of the *Aux/IAA* gene family in Chinese hickory (*Carya cathayensis* Sarg.) during the grafting process. Plant Physiol Biochem 127:55–63. https://doi.org/10.1016/j.plaphy.2018.03.010

Zenser N et al (2001) Auxin modulates the degradation rate of Aux/IAA proteins. Proc Natl Acad Sci U S A 98:11795–11800. https://doi.org/10.1073/pnas.211312798

Zhang S et al (2018) Genome-wide identification of the *HKT* genes in five Rosaceae species and expression analysis of *HKT* genes in response to salt-stress in *Fragaria vesca*. Genes Genom. https://doi.org/10.1007/s13258-018-0767-0