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ORIGINAL PAPER

Genome-wide analysis of *Aux/IAA* gene family in Solanaceae species using tomato as a model

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Abstract Auxin plays key roles in a wide variety of plant activities, including embryo development, leaf formation, phototropism, fruit development and root initiation and development. Auxin/indoleacetic acid (*Aux/IAA*) genes, encoding short-lived nuclear proteins, are key regulators in the auxin transduction pathway. But how they work is still unknown. In order to conduct a systematic analysis of this gene family in Solanaceae species, a genome-wide search for the homologues of auxin response genes was carried out. Here, 26 and 27 non redundant *AUX/IAAs* were identified in tomato and potato, respectively. Using tomato as a model, a comprehensive overview of *SlIAA* gene family is presented, including the gene structures, phylogeny, chromosome locations, conserved motifs and cis-elements in promoter sequences. A phylogenetic tree generated from alignments of the predicted protein sequences of 31 OsIAAs, 29 AtIAAs, 31 ZmIAAs, and 26 SlIAAs revealed that these IAAs were clustered into three major groups and ten subgroups. Among them, seven subgroups were present in both monocot and dicot species, which indicated that the major functional diversification within the IAA family predated the monocot/dicot divergence. In contrast, group C and some other subgroups seemed to be species-specific. Quantitative real-time PCR (qRT-PCR) analysis showed that 19 of the 26 *SlIAA* genes could be detected in all tomato organs/tissues, however, seven of them were specifically expressed in some of tomato tissues. The transcript abundance of 17 *SlIAA* genes were increased within a few hours when the seedlings were treated with exogenous IAA. However, those of other six *SlIAAs* were decreased. The results of stress treatments showed that most *SIIAA* family genes responded to at least one of the three stress treatments, however, they exhibited diverse expression levels under different abiotic stress conditions in tomato seedlings. *SlIAA20, SlIAA21* and *SlIAA22* were not significantly influenced by stress treatments even though at least one stress-related cis-element was identified in their promoter regions. In conclusion, our comparative analysis provides an insight into the evolution and expression patterns in various tissues and in response to auxin or stresses of the *Aux/IAA* family members in tomato, which will provide a very useful reference for cloning and functional analysis of each member of *AUX/IAA* gene family in Solanaceae crops.

Keywords *Solanum lycopersicum* · Solanaceae species · SlIAA · Expression analysis · qRT-PCR · Phylogenetic analysis

Abbreviations

ARF	Auxin response factor
Aux/IAA	Auxin/indoleacetic acid
AuxRE	Auxin responsive elements
NLS	Nuclear localization signal
qRT-PCR	Quantitative real-time PCR

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Introduction

Auxin, as one of the most important hormones, plays a key role in many processes of plant development, including embryo, root, flower and fruit development. *AUX/IAA* family genes are early auxin response genes that encode short-lived nuclear proteins (Abel and Theologis 1995; Quint and Gray 2006). Increasing evidences emerge that *AUX/IAA* genes act as transcription repressors in auxin signal transduction pathway by dimerizing with auxin response factor (ARF) (Leyser 2002). Most Aux/IAA proteins contain four highly conserved domains, called domain I, II, III and IV (Abel and Theologis 1995; Tiwari et al. 2001). Domain I, represented by an "LxLxL" motif (Tiwari et al. 2004), is a repression domain that can interact with the TOPLESS (TPL) co-repressor (Szemenyei et al. 2008). Domain II is required for auxin-regulated signaling by interacting with a component of the ubiquitin–proteasome protein (TIR1) degradation pathway (Dharmasiri et al. 2005), and this interaction is abolished by mutations within motif II (Gray et al. 2001). Auxin and Aux/IAA bind to the same TIR1 pocket. Auxin might act as a "molecular glue", increasing the affinity of the two kinds of proteins (AUX/IAAs and TIR1) by simultaneously interacting in a cavity at the protein interface (Tan et al. 2007; Hayashi et al. 2008). Domains III and IV are responsible for the homo- and hetero-dimerization among Aux/IAA family members and between the Aux/IAA proteins, and auxin response factors (ARFs) (Kim et al. 1997; Ulmasov et al. 1997a; Ouellet et al. 2001; Hardtke et al. 2004). Domain III in Aux/IAAs might be sufficient for dimerization by itself, but domain IV which contains a dimerization region and a functional nuclear localization signal (NLS) sequence (Reed 2001) is also thought to be responsible for the dimerization. Under low auxin concentration, ARFs are thought to be inhibited by dimerize with the Aux/IAAs via domains III and IV that are conserved between the two protein families (Ulmasov et al. 1997b; Hagen and Guilfoyle 2002). Elevated auxin concentration releases ARFs from repressor heterodimer by promoting the degradation of Aux/IAA proteins through the ubiquitin–proteasome protein (TIR1) pathway (Tiwari et al. 2003; Berleth et al. 2004; Dharmasiri et al. 2005).

AUX/IAA family genes play important roles in many aspects of plant development. In *Arabidopsis*, an auxin-resistant mutant, iaa28-1, is insensitive to auxin, cytokinin, and ethylene. Rogg et al. (2001) proved that *iaa28-1* might act as transcription repressor in promoting lateral root initiation in response to auxin signals. SLR/IAA14 was regarded as a fundamental regulator in lateral root formation because its mutant completely lacked lateral roots (Fukaki et al. 2002). In tomato, the down-regulation of *Sl-IAA3* resulted in the alteration of several auxin-related vegetative growth phenotypes, such as apical dominance, apical hook curvature and petiole epinasty (Chaabouni et al. 2009). When tomato *SlIAA9* was down-regulated, the compound leaves were replaced by the simple leaves, and the order of fruit development was also reversed and the ovary began to develop before fertilization and produce the parthenocarpic fruit (Wang et al. 2005, 2009). In potato, down-regulation of *StIAA2* resulted in an increased plant height petiole hyponasty and extreme curvature of growing leaf primordia in the shoot apex (Kloosterman et al. 2006).

Aux/IAA gene was first isolated in soybean (Walker and Key 1982). Subsequently, many *Aux/IAAs* genes have been characterized based on the analysis of gain-of-function mutants in *Arabidopsis* (Park et al. 2002; Yang et al. 2004), mung bean (Yamamoto 1994), rice (Thakur et al. 2001; Nakamura et al. 2006) and *Populus* (Kalluri et al. 2007). In *Arabidopsis*, 29 *Aux/IAA* genes were distributed in 5 different chromosomes (Liscum and Reed 2002). In rice, a total of 31 *AUX/IAAs* distributed on 10 of the 12 rice chromosomes have been reported (Jain et al. 2006). In maize, a total of 31 *AUX/IAAs* genes were also identified, which are distributed in all the 10 chromosomes except chromosome 2 (Wang et al. 2010a, b). In tomato, only three complete *SlIAAs* sequences (*SlIAA3*, *SlIAA4* and *SlIAA9*) and eight partial sequences (*SlIAA1-2*, *SlIAA5-8*, *SlIAA10*, and *SlIAA11)* shown to be homologous to *AtIAAs* were identified (Nebenführ et al. 2000; Wang et al. 2005; Chaabouni et al. 2009). Until now, 17 *IAA* genes in potato genome and several *IAA* genes in other Solanaceae species have been reported (Cle'ment et al. 2006; Kloosterman et al. 2006; Terrile et al. 2010; Zanetti et al. 2003). However, to our knowledge, no systematic investigations of *IAA* gene family have been conducted in Solanaceae species. Tomato, as the representation of Solanaceae plants, is not only one of the most important vegetables but also is considered one of the model dicot plants for fruit development. The Genome Sequencing Project for tomato genome has been completed lately (http://solgenomics.net/organism/Solanum_lycopersicum/genome). Recently, the potato genome was also released (Xu et al. 2011). In the present study, taking advantage of the available SGN database, we carry out a genome-wide search for the homologues of *AUX/IAA* family genes in Solanaceae crops. As a result, we identified 26 and 27 putative genes with IAA domains in tomato and potato genome, respectively. The detailed information on the genomic structures, chromosomal locations, sequence homologies and expression patterns of tomato *IAA* genes was presented. In addition, the phylogenetic relationships among *IAA* genes in *Arabidopsis*, tomato, rice and maize were also compared. Furthermore, the different expression patterns during flower and fruit development and in response to various abiotic stress conditions in tomato plants were determined

for each *SlIAA* gene using quantitative real-time PCR (qRT-PCR) analysis. The data would facilitate future studies on elucidating the biological functions of AUX/IAA in Solanaceae crops.

Materials and methods

Searching for Aux/IAA family genes

To find all *IAA* genes in *Arabidopsis* (*AtIAAs*), rice (*OsIAAs*) and maize (*ZmIAAs*),"early auxin-responsive Aux/IAA" was used as a query to search the protein and nucleotide databases of NCBI (The National Center for Biotechnology Information) and the matching genes were confirmed by previous reports (Nebenführ et al. 2000; Wang et al. 2010a, b).

To find previously identified and potential *IAA* family genes in Solanaceae species, multiple database searches were performed. First, "early auxin-responsive Aux/IAA" was used as a query to search the SGN database (http://solgenomics.net). In tomato, three formerly known *SlIAA* family genes (*SlIAA3, SlIAA4* and *SlIAA9*) with full-length cDNA sequences and eight other *SlIAA* genes with partial sequences (*SlIAA1-2, SlIAA5-8, SlIAA10,* and *SlIAA11*) were identified. Similarly, four formerly known *IAA* genes (*StIAA1-4*) in potato were found. To find other potential *IAAs* in tomato and potato, we initially surveyed the tomato and potato genome database of SGN by TBLASTN (Search translated nucleotide database using a protein query) using the whole amino acid sequences of the conserved IAA domains from all the known IAA families (including AtIAAs, OsIAAs, ZmIAAs, and PoptrIAA) as queries. Based on the combined results from all above searches, we finally identified all members of tomato and potato IAA family from the currently available genomic databases. After searching for *IAA* genes, bioinformatics tools, such as DNASTAR and FGENESH (http://linux1.softberry.com/berry) were used to analyze and predict those unknown *IAAs*.

Isolation of the open reading frame (ORF) cDNA sequences

To identify the homologue unigenes or ESTs for the *AUX/IAA* family genes in tomato, the available SGN database was searched, and then 16 *SlIAAs* ORF sequences were found in the unigenes. Since only partial cDNAs sequences of *SlIAA2, 11, 18, 20* and *26* were found existing in the unigene or EST database of SGN database and the full-length of those five genes and other two *SlIAA* genes (*SlIAA15* and *SlIAA17*) without homologous EST or unigene in the SGN database were identified using BLASTN against ITAG Release 2 predicted CDS (SL2.31). Since *SlIAA12*, *SlIAA13*, and *SlIAA16* showed no significant homology to any known sequences using the BLAST approaches, they were amplified through RT-PCR using the primers designed by FGENESH (Table S1). Total RNA was extracted from tomato variety "Micro-Tom" young ovaries using TRIZOL reagent (Invitrogen, Germany) according to the manufacturer's instructions. After DNAse (Qiagen, Germany) treatment, RNA was reverse transcribed to cDNA using the Improm-TM Reverse Transcription system (Promega, Madison, USA) following the manufacture's protocol. RT-PCR was performed as described in our previous study (Wu et al. 2011). In potato, homologue unigenes or ESTs were found from potato unigene database by BLASTN.

Mapping *SlIAA* genes on chromosomes

To determine the location of *SlIAA* genes on tomato chromosomes, each above *SlIAA* cDNA sequence was further used as query sequence for the BLASTN search against SGN tomato whole genome scaffolds data (2.30) (http://www.sgn.cornell.edu/tools/blast/).

Multiple-sequence alignments and phylogenetic analysis

All the identified *SlIAA* DNA sequences were analyzed by DNASTAR software and the net service ExPASy Proteomics Server (http://ca.expasy.org). All the conserved domains were investigated by multiple alignment analyses using ClustalX v1.81 (Hompson et al. 1997). Phylogenetic analysis was performed using MEGA 4.1 program by the neighbor-joining (NJ) method (Saitou and Nei 1987). MEME utility was used to display motifs of Aux/IAA proteins from tomato, maize, rice and *Arabidopsis* (http://meme.nbcr.net) (Bailey et al. 2009). Parameters were set as the following: (i) the occurrence of a single motif distributed among the sequences was zero or one per sequence; (ii) the motif width ranged from 10 to 300 amino acids; (iii) the maximum number of motifs to find was five. Other parameters were defaulted.

Promoter regions analysis of *SlIAA* genes

To investigate cis-elements in promoter sequences of tomato *Aux/IAA* genes, 2,000 bp of genomic sequences upstream of the initiation codon from the SGN database were analyzed for cis regulatory elements (Suppl Fig. 1). Only 1,098 bp and 896 bp for *SlIAA18* and *SlIAA19*, respectively were used for analysis because of the unavailability of their 5′-upstream full DNA sequences in SGN database. The PLACE website (http://www.dna.affrc.go.jp/PLACE/) was applied to identify putative cis-

regulatory elements along the promoter sequences of each *SlIAA* family gene (Higo et al. 1999).

Plant growth and treatments

Tomato (*S. lycopersicum* L. cv. Micro-Tom) seeds were obtained from Tomato Genetics Resource Center (University of California, Davis, USA). All the plants were grown in a temperature-controlled chamber until flowering at the experimental farm in Zhejiang University. To analyze tissue or organ-specific expression, leaves, stems, roots, and flower buds were collected from flowering plants, meanwhile the various floral organs (sepal, petal, stamen, and ovary) were collected from the flower buds (about 3 days before opening). To analyze the expression pattern during early flower developmental stages, flower buds were collected at three stages of early floral development (before flowering), which was roughly defined by the length of flower buds as follows: stage I: 3–4 mm, stage II: 5–6 mm, and stage III at 7–8 mm (Brukhin et al. 2003). In addition, the ovaries were sampled at 0, 3, 6, and 9 days after the flower fully opened.

For stress treatments, 'Micro-Tom' tomato plants were grown in a growth chamber at 28 ± 1°C with a photoperiod of 14 h light and 10 h dark. The 3-week-old seedlings with three fully opened leaves were selected for different stress treatments. For heat stress (HS) treatment, the seedlings were treated with 42 ± 1°C for 1 h. Then the leaves were sampled immediately. Drought stress was initiated by withholding water supply to 3-week-old seedlings after seedlings were fully watered. On the 6th day after stress, the leaves were harvested when some of them started to curl due to the drought stress. Salt stress was performed by the addition of 200 mM sodium chloride to the planter box and the seedlings were sampled after 6 h. For IAA treatment, 3-week-old seedlings were sprayed with 100 mM IAA, and then the leaves were sampled at 0, 6, and 12 h after spraying. The seedlings without treatment grown at 28 ± 1°C with normal irrigation were used as control (CK). Each above experiment was repeated two times. Fifteen seedlings were used in each treatment in each replication. All the samples were stored at −75°C.

QRT-PCR analysis

Total RNA and the first cDNA strand were prepared as described above. QRT-PCR was carried out using the primer pairs listed in Table S2. Specificity of each primer to its corresponding gene was checked using the BLASTN program of the NCBI. One milligram aliquots of cDNA was subjected to each qRT-PCR reaction in a final volume of 20 µl containing 12.5 µl SYBR Green Master Mix Reagent (Takara, Japan) and specific primers (3 pmol). QRT-PCR reactions were carried out in a StepOne real-time PCR machine (Applied Biosystems, USA) as described by Wu et al. (2011). Two biological replicas were performed with three technical replicates for each sample. To normalize the total amount of cDNA present in each reaction, the *Ubi3* gene (accession number X58253) was co-amplified as an endogenous control for calibration of relative expression. The comparative Ct method (ΔΔCT method) of relative gene quantification recommended by Applied Biosystems (CA, USA) was used to calculate the expression levels of different treatments.

Results

Identification and isolation of IAA family genes in Solanaceae species

To identify the IAA family genes in Solanaceae species, BLAST searches of the SGN database were performed using the whole amino acid sequences of all four conserved IAA domains of the *Arabidopsis,* rice, *Populus* and maize protein as a query sequence. A total of 40 genomic DNA sequences and 63 unigenes in tomato were obtained from tomato genome database and unigene database using the TBLASTN program with an *e* value cutoff of $1e^{-1}$. Meanwhile, 45 candidates in potato were also found by TBLASTN against potato genome sequence with an *e* value cut-off $1e^{-1}$ and 35 homologous DNA sequences were indentified in tobacco.

Different sequences presenting on the same or overlapping contigs in different databases were identified and removed to obtain a set of nonredundant IAA sequences. All predicted sequences were confirmed by FGENESH (http://www.softberry.com/berry.phtml?topic=fgenesh). These predicted amino acid sequences were analyzed by ExPASy Proteomics server to find their conserved domains, followed by homologous alignment with known *IAA* genes in Solanaceae species. The overall revealed that the tomato genomes appeared to have 26 members which contained the IAA domains. In addition to the 11 previously known *SlIAA* genes (Nebenführ et al. 2000; Wang et al. 2005), 15 other putative novel *SlIAA* genes were found (Table 1). Meanwhile, a total of 27 *StIAA* family genes in potato and 18 *NtIAA* family genes in tobacco were identified (Table 2; Table S3).

The open reading frames (ORFs) of 16 *SlIAAs* were identified from the 23 unigenes. Meanwhile, the full-length cDNA sequences of *SlIAA12, SlIAA13,* and *SlIAA16* were isolated by PCR-based methods. The primers for PCR reaction are listed in Table S1. The ORF sequences of the other seven *SlIAA* genes were obtained based on ITAG Release 2 predicted CDS (SL2.31) of SGN database. The

Table 1 IAA gene in tomato

Gene	Predicted protein (aa)	Molecular weight (kDa)	PI	Domain	Unigene or EST	Intron	Chromosome number	Location
SlIAA1	190	21.30	6.22	I II III IV	SGN-U215090[a]	4	6	33204953–33205715
SlIAA2	156	17.50	8.61	I II III IV	SGN-U599474[b]	3	6	45618648–45619226
SlIAA3	185	20.80	6.61	I II III IV	SGN-U577993[a]	2	9	59714102–59714846
SlIAA4	349	37.40	6.84	I II III IV	SGN-U214220[a]	2	4	59353677–59357365
SlIAA5	233	26.16	6.83	I II III IV	SGN-U577813[a]	4	12	64013323–64015668
SlIAA6	278	30.50	6.90	I II III IV	SGN-U218763[a]	3	3	62931225–62932952
SlIAA7	218	24.70	8.86	I II III IV	SGN-U579168 SGN-U590099[a]	4	6	33179424–33181343
SlIAA8	252	28.10	9.17	I II III IV	SGN-U579568[a]	2	3	62863659–62864743
SlIAA9	251	27.60	8.22	I II III IV	SGN-U239038[a]	4	1	79957819–79960332
SlIAA10	208	23.50	8.86	I II III IV	SGN-U593495[a]	2	6	2498399–2501180
SlIAA11	192	21.80	6.01	I II III IV	SGN-E714899 SGN-E747636[b]	2	3	62856237–62857626
SlIAA12	189	21.60	6.22	I II III IV	No	2	3	210557–211725
SlIAA13	75	8.60	4.39	III IV	No	0	3	57617631–57617858
SlIAA14	287	31.90	9.05	I II III IV	SGN-U573372[a]	4	3	63351160–63353184
SlIAA15	166	19.30	8.84	I II III IV	No	3	4	51421118–51422604
SlIAA16	132	15.20	9.30	III IV	No	5	5	3078259–3080096
SlIAA17	225	25.20	6.84	I II III IV	No	3	6	2489840–2490930
SlIAA18	242	28.20	5.21	I II III IV	SGN-U241455[b]	2	6	37766839–37768956
SlIAA19	195	22.20	7.80	I II III IV	SGN-U589819[a]	3	7	2730927–2732468
SlIAA20	147	16.40	6.72	III IV	SGN-U603679[b]	3	7	11422820–11424037
SlIAA21	227	26.70	8.66	I II III IV	SGN-U568970[a]	1	8	9927234–9928187
SlIAA22	320	34.00	9.12	I II III IV	SGN-U579795[a]	3	9	57406259–57407670
SlIAA23	196	22.02	5.85	I II III IV	SGN-U579410[a]	4	9	64331489–64332734
SlIAA24	236	26.24	7.51	I II III IV	SGN-U216526[a]	2	9	64364767–64367498
SlIAA25	282	30.55	8.62	I II III IV	SGN-U579354[a]	2	9	65663277–65665939
SlIAA26	295	32.04	8.49	I II III IV	SGN-U581702[b]	4	12	1650063–1653493

[a] The unigenes which contain the whole ORF of relevant SlIAAs

[b] The unigenes which only contain the partial ORF of relevant SlIAAs

NA not available

peptide sequences of all 26 SlIAAs were listed in Suppl Fig. 2. All the results were verified by BLASTN against the ITAG Release 2 predicted CDS (SL2.31) of SGN database. The ORF length of 26 *SlIAAs* genes varied from 228 bp (*SlIAA13*) to 1,050 bp (*SlIAA4*), encoding polypeptides of 75–349 aa, with a predicted molecular mass range of 8.6–37.4 kDa. The theoretical pI ranged from 4.39 to 9.30 (Table 1), similar with the IAAs polypeptides previously determined in other plants (Nebenführ et al. 2000; Wang et al. 2010a, b).The whole-length ORFs of 11 *StIAAs* were identified from the 11 unigenes in potato unigene database, the CDS of other *StIAAs* were predicted from PGSC DM v3.4 CDS sequences (Table 2).

Chromosomal locations of SlIAAs

All *SlIAA* clones from tomato genome have been anchored to tomato chromosomes. The chromosomal locations and transcription directions of 26 *SlIAA* genes were demonstrated using BLASTN analysis on Tomato WGS Chromosomes (Fig. 1). Like in maize and rice, tomato *SlIAA* family genes were distributed over 9 of the 12 tomato genomes, except chromosomes 2, 10 and 11. The number of *SlIAAs* genes per chromosome ranged from one to six. Six *SlIAA* genes were anchored on chromosomes 3 and 6. Five *SlIAA* genes were anchored to chromosome 9, while three of them were clustered on the same region with the different transcriptional orientation. Two *SlIAA* genes were found on chromosomes 4, 7 and 12 each with the same transcriptional orientation. Chromosomes 1, 5, and 8 only carried one *SlIAA* gene each (Table 1; Fig. 1).

It is worth mentioning that the nomenclature system for *SlIAAs* is used in the present study. Since sequence analysis indicated that the similarity between the SlIAA in tomato with AtIAAs in *Arabidopsis* was quite low, it is difficult to assign all SlIAA names based on their homolog proteins in

Table 2 IAA gene in potato

Gene	Predicted protein (aa)	Molecular weight (kDa)	PI	Domain	Unigene or est	Intron	Sequence ID
StIAA1	190	21.6	6.03	I II III IV	SGN-U273575[a]	2	PGSC0003DMC400028515
StIAA2	153	17.1	8.37	I II III IV	NA	2	PGSC0003DMC400034949
StIAA3	183	20.5	6.61	I II III IV	SGN-U284587[a]	3	PGSC0003DMC400033533
StIAA4	349	37.3	6.84	I II III IV	SGN-U269067[a]	5	PGSC0003DMC400011339
StIAA5	232	25.9	8.15	I II III IV	NA	4	PGSC0003DMC400051083
StIAA6	261	28.7	8.62	I II III IV	SGN-U269107[a]	4	PGSC0003DMC400004537
StIAA7	213	24.0	9.12	I II III IV	SGN-U269817[a]	4	PGSC0003DMC400028445
StIAA8	212	23.5	6.63	I II III IV	SGN-U272708[a]	4	PGSC0003DMC400004724
StIAA9	249	27.3	8.22	I II III IV	SGN-U269815[a]	3	PGSC0003DMC400033829
StIAA10	214	24.3	7.66	I II III IV	NA	4	PGSC0003DMC400009432
StIAA11	195	21.9	6.63	I II III IV	NA	2	PGSC0003DMC400004726
StIAA12	164	18.8	5.22	I II III IV	NA	2	PGSC0003DMC400023793
StIAA13	382	42.3	4.90	III IV	NA	5	PGSC0003DMC400010270
StIAA14	283	31.4	9.12	I II III IV	SGN-U272966[b]	3	PGSC0003DMC400004659
StIAA15	361	39.8	5.32	III IV	NA	3	PGSC0003DMC400000263
StIAA16	108	12.6	6.09	III IV	SGN-U282222[a]	2	PGSC0003DMC400028876
StIAA17	190	21.3	7.66	I II III IV	NA	2	PGSC0003DMC400009456
StIAA18	91	10.7	6.26	III IV	NA	3	PGSC0003DMC400035574
StIAA19	202	22.7	5.28	I II III IV	NA	4	PGSC0003DMC400053819
StIAA20	116	13.0	5.43	III IV	NA	0	PGSC0003DMC400065246
StIAA21	219	25.8	9.02	I II III IV	SGN-U276575[a]	3	PGSC0003DMC400024324
StIAA22	315	33.5	8.81	I II III IV	SGN-U283259[b]	4	PGSC0003DMC400015088
StIAA23	195	21.9	5.85	I II III IV	SGN-U271094[a]	2	PGSC0003DMC400010843
StIAA24	238	26.4	7.51	I II III IV	SGN-U269816[a]	4	PGSC0003DMC400010816
StIAA25	271	29.3	8.65	I II III IV	SGN-U281093[b]	4	PGSC0003DMC400002698
StIAA26	279	30.4	8.74	I II III IV	SGN-U278053[b]	4	PGSC0003DMC400000747
StIAA27	299	32.4	6.05	III IV	SGN-U277793[b]	1	PGSC0003DMC400037380

[a] The unigenes which contain the whole ORF of relevant StIAAs

[b] The unigenes which only contain the partial ORF of relevant StIAAs

NA not available

Arabidopsis. So the established names were used for 11 previously known genes or sequences in Genbank (SlIAA1-11). Other SlIAA genes (SlIAA12-26) were named according to their position from the top to the bottom on the tomato chromosomes 1–12. All the genes in other Solanaceae crops were named according to their homology with SlIAAs (Suppl Fig. 11).

Sequence analysis of IAAs proteins

All the tomato SlIAAs protein sequences were found to contain domain III and IV which act as C-terminal dimerization domains, mediating homodimerization and heterodimerization among Aux/IAA family members and between the Aux/IAA proteins and auxin response factors (ARFs). Among 26 SlIAAs, 23 proteins contain domain I and II, only three including SlIAA13, SlIAA16 and SlIAA20, lack these two domains (Table 1; Fig. 2). In potato, all the IAA proteins, except StIAA13, StIAA15, StIAA16, StIAA18, StIAA20 and StIAA27, contain all four conserved domains (Table 2; Suppl Fig. 3). Putative domain of NtIAA family genes in tobacco were also analyzed (Table S3; Suppl Fig. 4).

Gene structure and phylogenetic analysis

A comparison of the full-length cDNA sequences with the corresponding genomic DNA sequences revealed the numbers and positions of exons and introns for each individual *SlIAA* and *StIAA* gene. The coding sequences of all the *SlIAAs* and *StIAAs* except *SlIAA13* and *StIAA20* were disrupted by introns. The number of introns varied from 1 to 5 both in tomato and potato (Fig. 3). The IAA genes possessed a complex distribution of exons and introns both

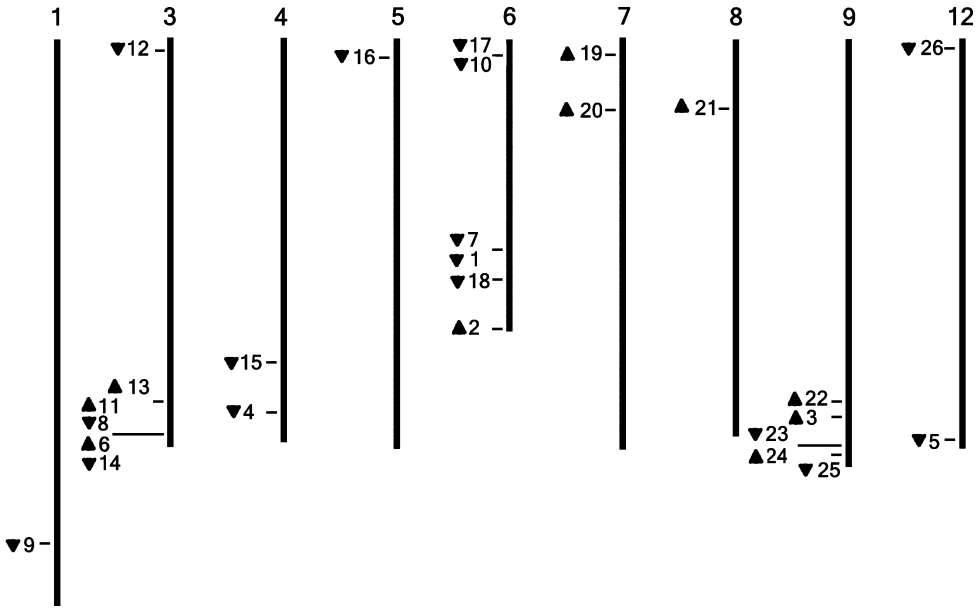

Fig. 1 Genomic distribution of *SlIAAs* genes on tomato chromosomes. The *arrows* next to gene names show the direction of transcription

in tomato and potato. The IAA members displayed different structural pattern of exon–intron junctions even within the same phylogenetic subgroup.

There are two types of putative nuclear localization signals (NLS) detected in most of the identified Aux/IAA proteins. A bipartite NLS containing two stretches of K/R residues was found between a conserved basic doublet KR and basic amino acids in domain II, whereas a SV40-like NLS was located in domain III, consisting of one cluster of positively charged amino acid residues such as lysine (K) or/and arginine (R) (Fig. 2; Suppl Fig. 3; Suppl Fig. 4) (Raikhel 1992). These putative NLSs may direct Aux/IAA proteins to the nucleus.

An unrooted phylogenetic tree was generated from the alignment of full-length protein sequences of all SlIAAs. The 26 SlIAA protein sequences could be divided into two major groups (group A and B) with well-supported bootstrap value, which was similar to rice (Jain et al. 2006) and *Arabidopsis* (Remington et al. 2004). Group A was further divided into five subgroups (Fig. 3a). Among them, subgroup A1 and A5 each contained seven members, but subgroup A2 and A6 only contained one IAA member each. Group B was further divided into three subgroups: B1, B2 and B3, which contained 3, 1 and 6 SlIAA proteins, respectively. Unlike in rice, where many sister pairs were found in 31 OsIAA proteins (Jain et al. 2006), the 26 SlIAA proteins only formed four sister pairs (Fig. 4a) with strong bootstrap support (>90%). All the 27 StIAAs formed two major groups (group A and B), containing 16 and 11 members, respectively. The phylogenetic relationships between the predicated StIAAs protein sequences were also analyzed. Similarly, group A and group B could be further divided into five and three subgroups, respectively (Fig. 3b).

Evolution relationships analysis that 117 IAA protein sequences including 29 AtIAAs, 31 OsIAAs, 31 ZmIAAs, and 26 SlIAAs (Fig. 4) fell into three broad groups, namely group A, B and C including 65, 46, and 6 IAA proteins, respectively. Group A was further divided into six subgroups A1, A2, A3, A4, A5, and A6, containing 12, 11, 6, 11, 14, and 11 members, respectively. Group B was further divided into three subgroups, namely B1, B2 and B3 (Fig. 4). This classification is consistent with previous analyses (Remington et al. 2004; Jain et al. 2006). All the IAAs proteins contained conserved motifs except SlIAA13, ZmIAA3, ZmIAA13, ZmIAA25 and ZmIAA30 (Fig. 4).

In this joint phylogenetic tree, a total of 21 sister pairs were found, including 2 SlIAA–SlIAA pairs, 4 ZmIAA–ZmIAA pairs, 7 AtIAA–AtIAA pairs, 2 OsIAA–OsIAA pairs, 4 OsIAA-ZmIAA pairs and 2 SlIAA-AtIAA pairs. Interestingly, subgroups A2, A5, A6, B1, B2 and B3 contained IAA genes from all the four species, but subgroup A3 only contained the IAAs from dicotyledon (*Arabidopsis* and tomato), meanwhile, all the IAA proteins except ZmIAA26 presented in subgroup A1 came from dicotyledon plants. By contraries, all IAA proteins in subgroup A4 and B2 except SlIAA14 were from monocot (rice or maize), while the group C, the most divergent part, only contained IAAs from maize (Fig. 4).

The motif distributions in maize, rice, tomato, and *Arabidopsis* Aux/IAA proteins were analyzed using Multiple Expectation Maximization for Motif Elicitation (MEME) tool. Four conserved domains of Aux/IAA proteins were divided into five motifs by MEME tool. Domain I, II and IV were represented by motif 4, 3 and 1, while domain III was constituted by motif 2 and 5 (Fig. 4; Suppl Fig. 5). The number of sites and *e* value for each motif were also presented in Suppl Fig. 5.

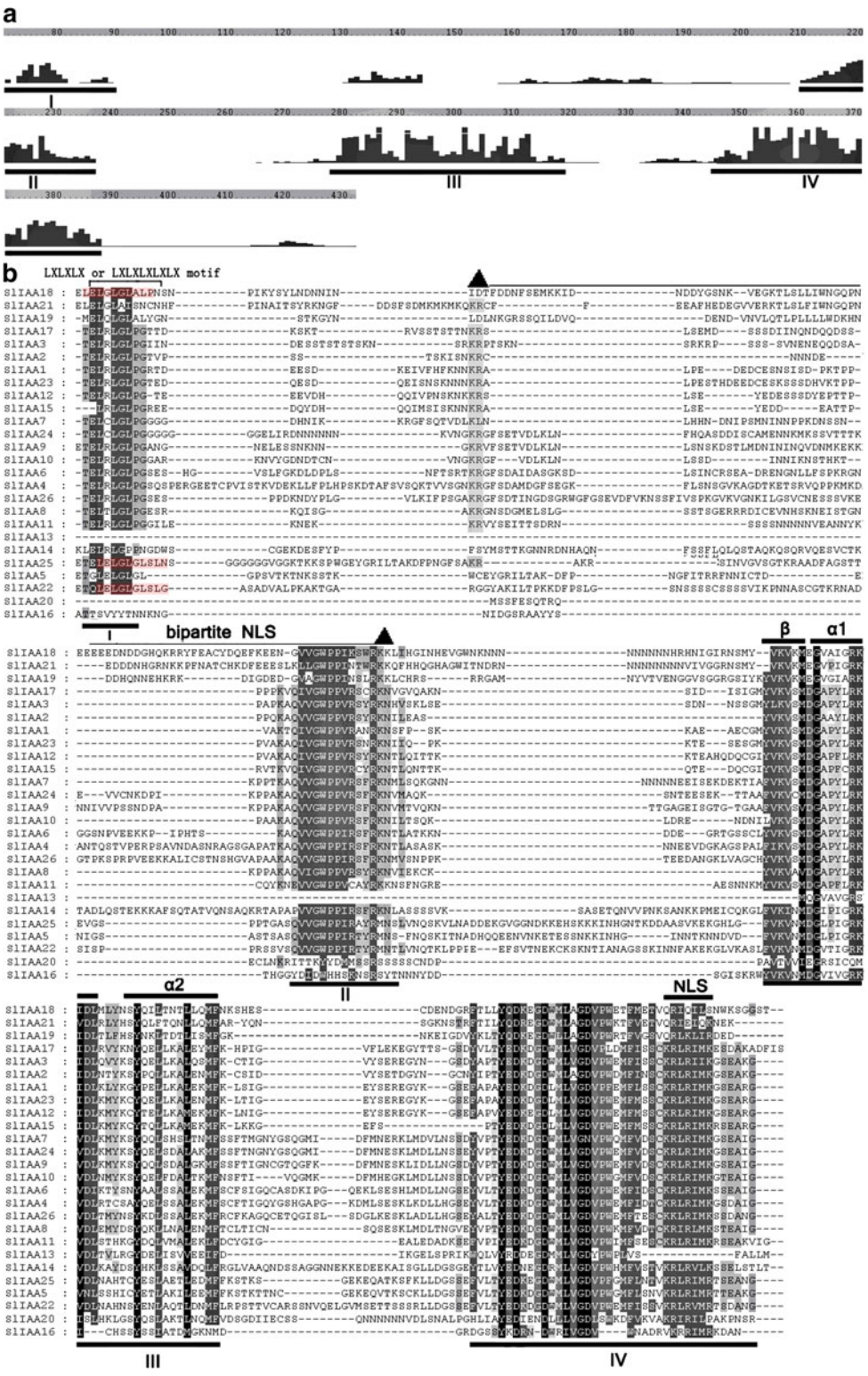

Fig. 2 **a** Alignment of tomato Aux/IAA proteins obtained with the ClustalX program. The height of the *bars* indicates the number of identical residues per position. **b** Multiple alignments of the domains I–IV of the tomato Aux/IAA proteins obtained with ClustalX and manual correction. *Black and light gray* shading indicates identical and conversed amino acid residues, respectively. Conserved domains are also *underlined* and correspond to part (**a**). The LxLxLx and LxLxLxLxLx motif were also marked

Cis-elements in promoter sequences of *SlIAA* genes

The investigation of 5′-upstream sequences of *SlIAAs* representing their promoter regions by PLACE and manual search revealed the presence of four types of cis-elements including seven auxin signaling transduction-related cis-element, 13 drought stress-related cis-element, one salt stress-related cis-element and one heat shock element (Table S4). All the locations of three AuxREs and other auxin signaling transduction-related cis-element were

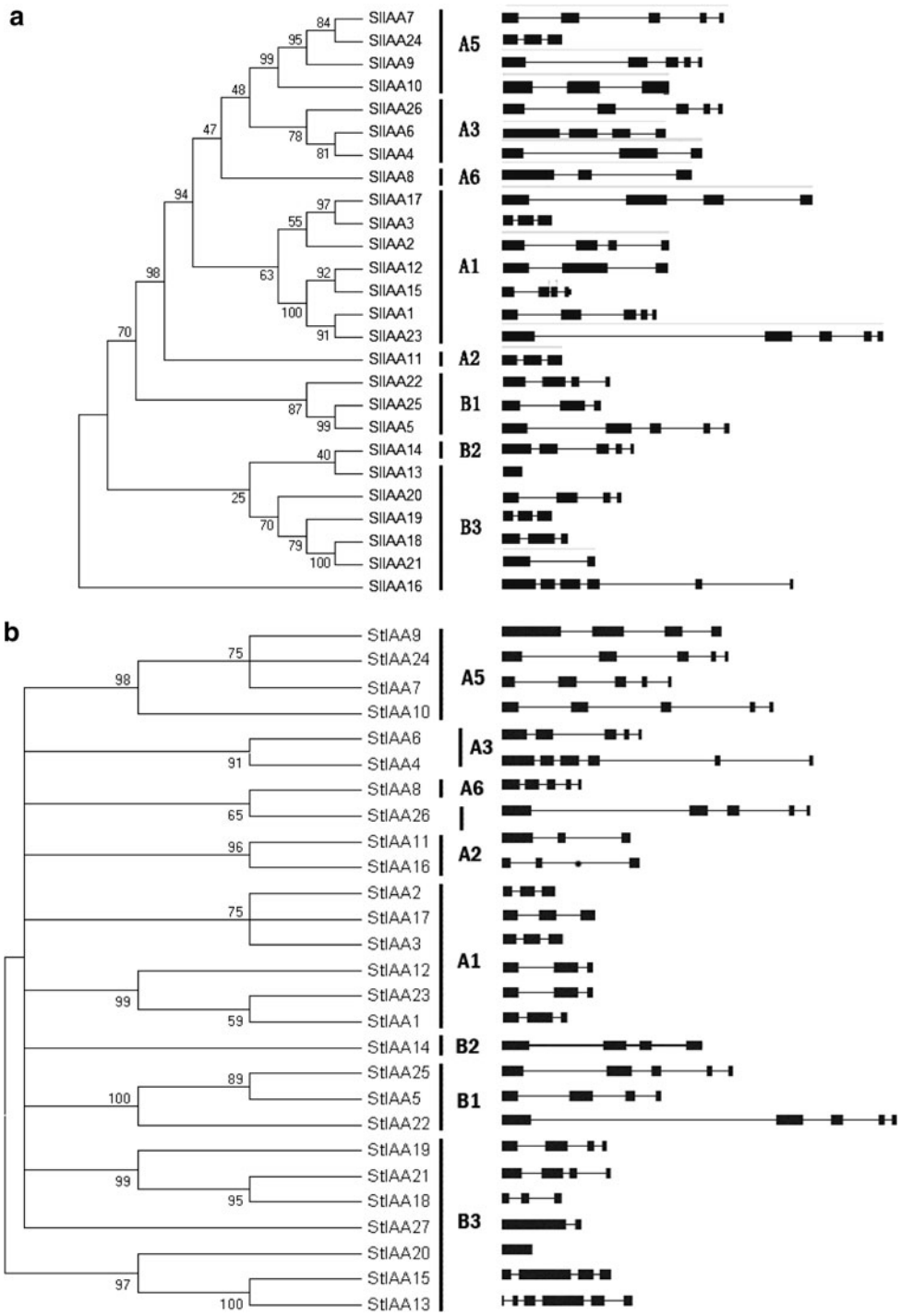

Fig. 3 **a** *Left part* illustrates the phylogenetic relationships among the tomato Aux/IAA proteins. **b** *Left part* illustrates the phylogenetic relationships among the patoto Aux/IAA proteins. The unrooted tree was generated using MEGA4.1 program by the neighbor-joining method. Bootstrap supports from 1,000 replicates are indicated at each branch. *Right part* illustrates the exon–intron organization of corresponding IAA genes. The exons and introns are represented by *black boxes and lines*, respectively

presented in Suppl Fig. 6. Three auxin-responsive elements (AuxREs)-S000026, S000234, and S000270, have been defined within upstream promoter regions of *AUX/IAA* genes. S000270 could be found in the promoter regions of 11 *SlIAAs*, while S000026 only in *SlIAA5* and *SlIAA25*, S000234 in *SlIAA14* and *SlIAA21*. However, no auxin signaling transduction-related cis-element was found in the 1,098 bp upstream region of *SlIAA18*.

Expression characterization of *SlIAA* genes

Most of the *SlIAAs* could be detected in root, stem, leaf, flower bud and ovary using qRT-PCR (Fig. 5). Some *SlIAAs* showed organ/tissue-specific expression pattern in tomato. *SlIAA2, SlIAA12*, *SlIAA13*, *SlIAA15*, *SlIAA16*, *SlIAA20* and *SlIAA25* were highly expressed in tomato roots, especially *SlIAA16* and *SlIAA20* exhibited root-

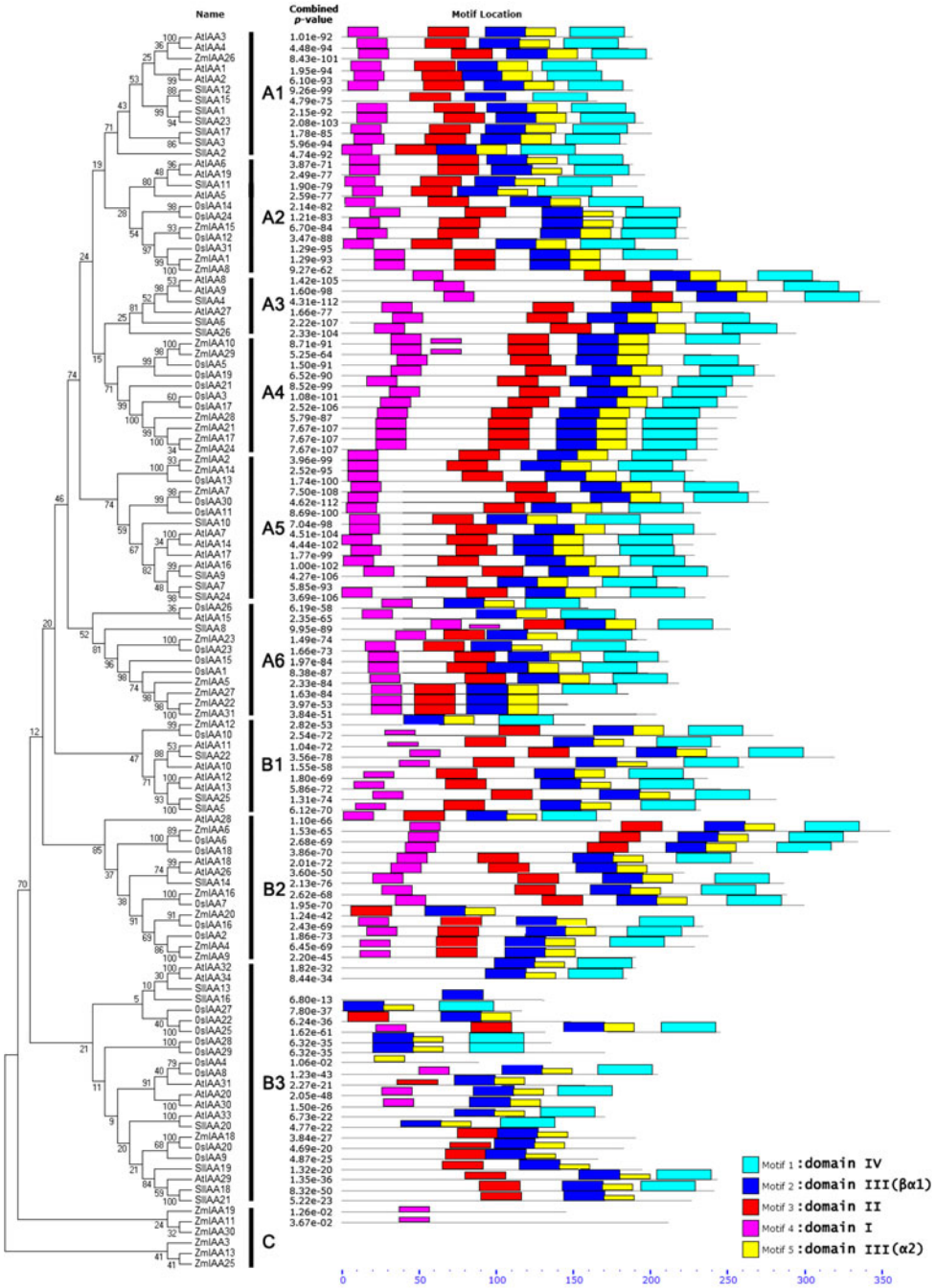

Fig. 4 *Left part* illustrates the phylogenetic relationships among tomato, rice, maize and *Arabidopsis* IAA proteins. The unrooted tree was generated using MEGA4.1 program by the neighbor-joining method. Bootstrap supports from 1,000 replicates are indicated at each branch. *Right part* shows motif distribution in tomato (Sl), maize (Zm), rice (Os) and *Arabidopsis* (At) Aux/IAA proteins. Motifs of Aux/IAA proteins were investigated by MEME web server. Five motifs representing four domains I, II, III and IV were not observed in all Aux/IAA proteins. The heights of each *box* represent the conservation of each motif

specific expression in tomato. *SlIAA4*, *SlIAA5, SlIAA6*, *SlIAA17*, *SlIAA21*, and *SlIAA26* exhibited a higher expression level in stem than in the other organs. *SlIAA1*, *SlIAA7*, *SlIAA19* and *SlIAA24* mRNA were highly expressed in leaves. Interestingly, *SlIAA19* only expressed in leaf and root and *SlIAA24* only in leaf and stem. *SlIAA1* and *SlIAA15* especially expressed in vegetative organs. In general, most of the *SlIAAs* exhibited relatively low expression level in reproductive organs, except that *SlIAA8* and *SlIAA18* highly expressed in ovary.

The mRNA expression of most *SlIAA* genes could be detected in different tissues of the tomato flower (Suppl Fig. 7). In general, the transcript levels of most *SlIAA* genes were higher in petal than in other parts. However, higher mRNA levels of *SlIAA3, SlIAA13, SlIAA14, SlIAA16, SlIAA18,* and *SlIAA19* were detected in stamen, while *SlIAA20, SlIAA21* and *SlIAA25* mRNA exhibited a higher transcript level in ovary than in other tissues. In contrast, only *SlIAA24* and *SlIAA26* exhibited relatively strong expression in sepal.

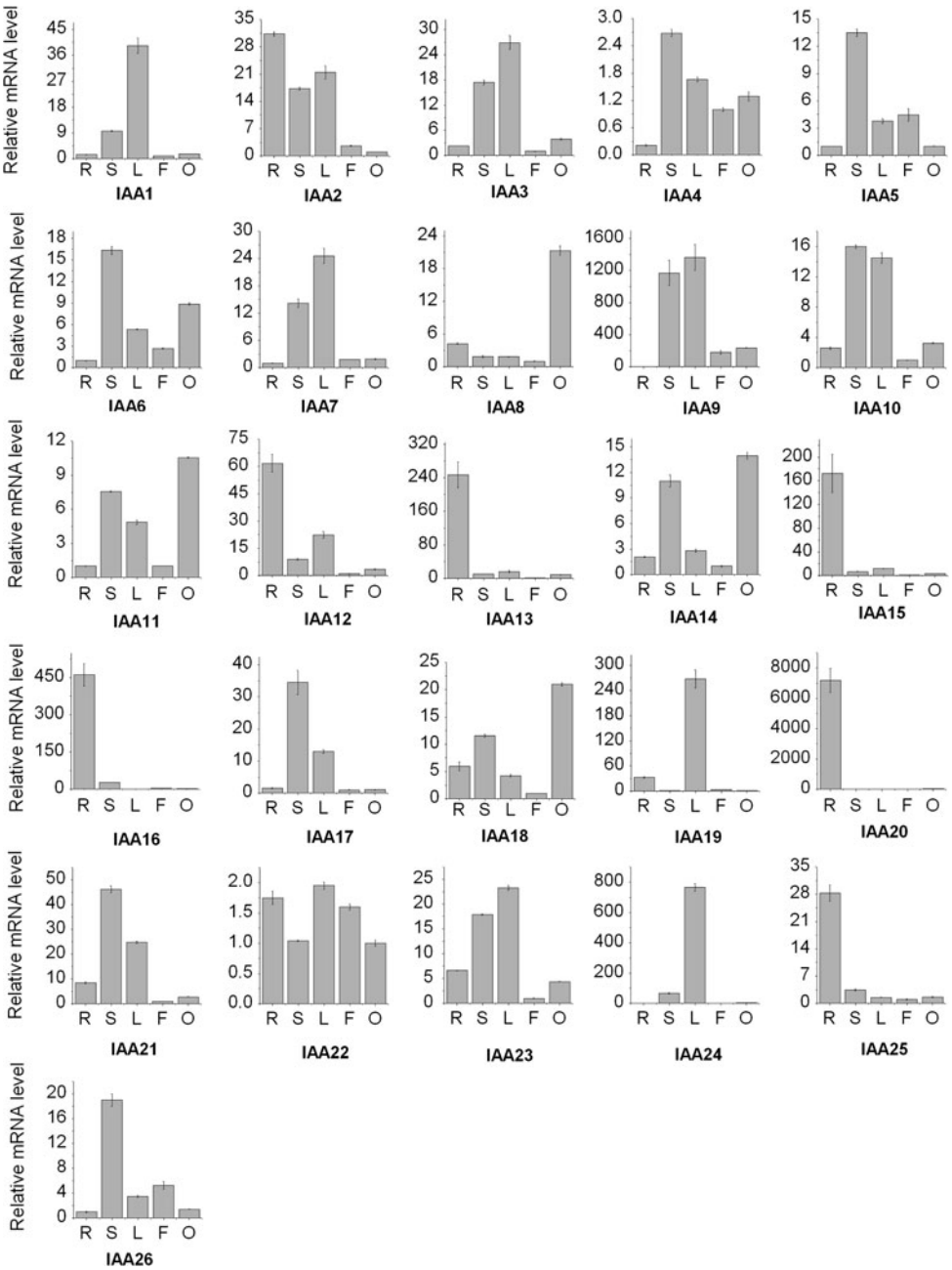

Fig. 5 Expression profiles of all the 26 *SlIAA* genes in different tomato organs. QRT-PCR analyses of total RNA isolated from root (R), stem (S), leaf (L), buds (B), and ovary (O) were used to assess SlIAA transcript levels in flowering tomato plants. The data on represented mean ± SD normalized relative to the Ubi3 (accession number X58253) related protein transcript levels. All samples were run in triplicate and the entire assay was performed twice for each biological pool

Most of the *SlIAA* family genes exhibited a similar expression pattern of mRNA accumulation during flower development (Suppl Fig. 8). However, *SlIAA2, SlIAA22, SlIAA24* and *SlIAA25* mRNA were markedly down-regulated at stage II and then up-regulated at stage III. Different expression patterns of *SlIAA* genes during the early development of tomato fruit were found using qRT-PCR analysis (Fig. 6). The relative mRNA levels of most *SlIAA* genes increased during flowering and reached the maximum value on the 3rd or 6th day after the flower opened (DAF), and finally drastically decreased again till the end of observation. However, the relative mRNA levels of *SlIAA6, SlIAA11, SlIAA13, SlIAA15, SlIAA16, SlIAA18, SlIAA19, SlIAA21* and *SlIAA25* continuously decreased during the early fruit development. Higher expression levels were observed in *SlIAA5*, *SlIAA10* and *SlIAA20* at the time of flower opening, then their mRNA levels were markedly decreased at 3 DAF, but increased at 6 DAF and drastically decreased again at 9 DAF. The expression level of *SlIAA17* also decreased at 3 DAF, but it continuously increased during the early fruit development.

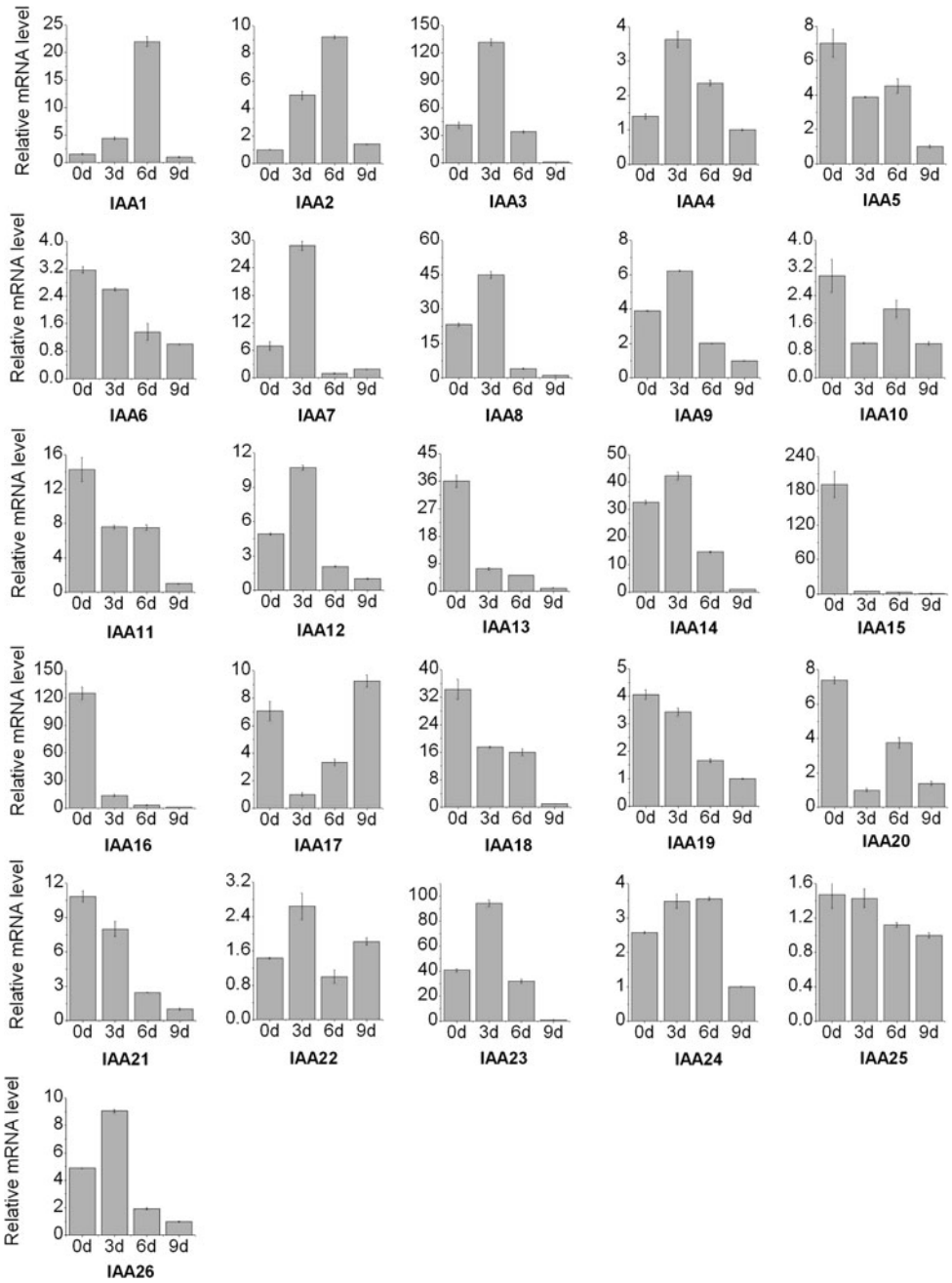

Fig. 6 Expression profiles of all the 26 *SlIAA* genes during tomato early fruit developmental stages. QRT-PCR analyses were performed using RNA generated from tomato ovaries at different number of days (0, 3, 6, and 9 days) after the flower opening. For other details see Fig. 5

Expression of *SlIAAs* genes in response to IAA and stress treatments

QRT-PCR was performed with total RNA isolated from the tomato leaves treated with IAA. As expected, most of *SlIAA* genes were activated by IAA treatment (Fig. 7). The mRNA levels of these *SlIAAs* were increased from less than onefold (*SlIAA14*) to more than tenfolds (*SlIAA16)* at 6 h after the IAA treatment. Among them, most *SlIAA* genes were down-regulated at 12 h except *SlIAA4, SlIAA8, SlIAA9*, and *SlIAA14.* It is worth mentioning that *SlIAA20, SlIAA21* and *SlIAA22* were markedly down-regulated just after IAA treatment (Fig. 7; Table S5). *SlIAA7* and *SlIAA26* mRNA abundance was also slightly decreased in IAA-treated seedlings.

The *SlIAA* genes exhibited altered responses to salt, drought and heat stress. *SlIAA20, SlIAA21* and *SlIAA22* showed no significant changes after all stress treatment (Suppl Fig 9; Table S5). Most *SlIAA* mRNA transcripts were enhanced under the drought treatment, whereas *SlIAA3, SlIAA11*, and *SlIAA14* were down-regulated after drought treatment. Salt stress treatment increased the transcript levels of 19 out of 26 *SlIAA* genes in tomato seedlings, especially *SlIAA15* mRNA was increased even

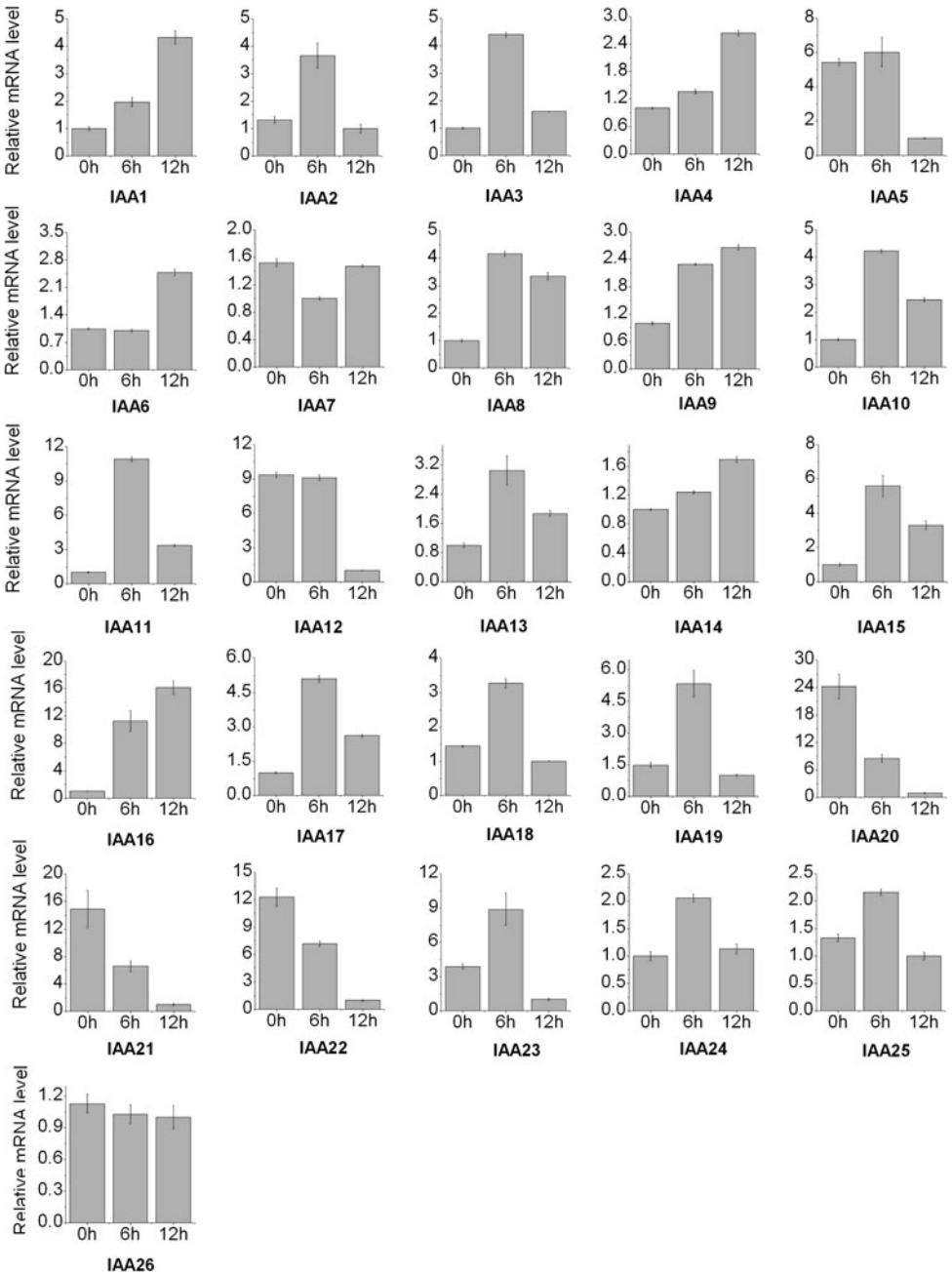

Fig. 7 Expression profiles of all the 26 *SlIAA* genes in response to IAA treatment. QRT-PCR analyses were used to assess *SlIAA* transcript levels in the leaves sampled at 0, 6, and 12 h after spraying 100 mM IAA in 3-week tomato seedlings. For other details see Fig. 5

more than sixfolds. In contrast, *SlIAA3, SlIAA11*, and *SlIAA14* were down-regulated, especially the transcript level of *SlIAA11* was decreased over 46-fold subjected to salt treatment. Heat treatment significantly enhanced the accumulation of *SlIAA6, SlIAA8, SlIAA15* and *SlIAA16* mRNA over twofolds, but caused a decrease in the transcript level of *SlIAA3, SlIAA11* and *SlIAA14* by 12-, 4- and 2.4-fold, respectively. Other 14 *SlIAA* genes, including *SlIAA2, SlIAA5, SlIAA9*, *SlIAA10*, *SlIAA12*, and *SlIAA18-26* seemed to be not significantly regulated by heat stress treatment (Suppl Fig. 9; Table S5).

Discussion

In this study, a comprehensive set of 26 and 27 non-redundant *AUX/IAA* genes were identified and characterized from the current version of the SGN database for tomato and potato genome, respectively. The number of *SlIAAs* and *StIAAs* members from tomato and potato is comparable to that of *Arabidopsis* (29), rice (31) and maize (31), although the genome size of tomato, potato, maize, *Arabidopsis* and rice is quite different. These partially accounts for the *AUX/IAA* conservation in these five

species during the evolutionary process (Liscum and Reed 2002; Jain et al. 2006; Wang et al. 2010a, b). The phylogenetic analysis showed that subgroups A2, A5, A6, B1, B2 and B3 contained IAA genes from all four species, which implies that those genes originated prior to the divergence of monocots and dicots. IAAs in subgroup A1 and A3 were from dicot crops, while subgroup A4 only contained the IAAs from monocotyledon, which indicated that those proteins were either lost or evolved after the divergence of monocots and dicots, consequently, they might play an important role in the development of dicotyledonous or monocotyledonous plants. Interestingly, the SlIAA proteins in main group C all came from maize, indicating that they may play an important role in determining species-specific traits and functions. Furthermore, the phylogenetic analysis indicated that there were four sister pairs between OsIAAs and ZmIAAs and two sister pairs between SlIAAs and AtIAAs, but no sister pairs between dicotyledon and monocotyledon were found in the phylogenetic tree (Fig. 4), which is also consistent with the evolutionary relationships of IAA family genes among these four species.

Twenty of the 28 Aux/IAA loci formed 10 sister pairs in the neighbor-joining reconstructions, nine of which had strong bootstrap support (≥96% in all three trees) (Remington et al. 2004). In this study, six sister pairs of AtIAAs, five sister pairs of ZmIAAs, seven sister pairs and two triplets of OsIAAs were found (Suppl Fig. 12–14). However, only two sister pairs of SlIAAs and one sister pair of StIAAs were found (Fig. 3), indicating that *AUX/IAA* genes in tomato and potato may play non-redundant roles during plant development. As expectedly, *SlIAA3, 23* and *24* distributed in chromosome 9 formed clusters, were comparative with their closely related genes *SlIAA7, 10* and *17* with highly similar sequences, the clusters of in chromosome 6, which indicated that they might originate from local duplication events (Fig. 2). This pattern probably reflects the series of chromosomal and large segmental duplication events existing in tomato genome. Some ancient tandem duplications which preceded the divergence of some chromosomal segments were reported by Ku et al. (2000). However, although there are some other *SlIAA* paralogs displaying high levels of sequence similarity, they are distributed all across the genome. Conversely, some closely linked SlIAAs, such as *SlIAA6, 8,11,14* on chromosome 3, *SlIAA10* and *SlIAA17* on chromosome 6, and *SlIAA23* and *SlIAA24* on chromosome 9 were not grouped in sister pairs. Consequently, we may conclude that the contribution of whole genome and chromosomal segment duplications in tomato was not as obvious as in other three species (Liscum and Reed 2002; Jain et al. 2006; Wang et al. 2010a, b).

Transcript abundance in particular organs at a given time is an important prerequisite to subsequent elucidation of the corresponding protein required for proper execution of developmental, metabolic and signaling processes. Virtually all 26 *SlIAA* genes were expressed in all organs/tissues analyzed, but their expression levels varied considerably. The mRNA levels of *SlIAA12, SlIAA13, SlIAA15, SlIAA16, SlIAA20* and *SlIAA25* in root were significantly higher than other organs (Fig. 5), implying that they might play an important role in the development of root. *SlIAA4, SlIAA5, SlIAA6, SlIAA17, SlIAA21,* and *SlIAA26* might play a crucial role in stem due to the higher expression levels than the other organs (Fig. 5). Similarly, *SlIAA1*, *SlIAA7*, *SlIAA19* and *SlIAA24* mRNA might have important functions in leaf, and *SlIAA11, SlIAA8* and *SlIAA18* might affect the development of fruit (Fig. 5). Chaabouni et al. (2009) proved that the relative mRNA level of *SlIAA13* in tomato stamen, leaf, and flower was much higher than root. Similar expression pattern was also found in the present study, although much lower in flower buds due to the different flower stage. When compared to the gene expression data of ARF family genes which are proved to interact with AUX/IAA (Kumar et al. 2011; Wang et al. 2007; Wu et al. 2011), Several *SlIAA* genes showed additional tissue-specific expression patterns suggesting the complexity of the Aux/IAA and ARF interactions (Jain et al. 2006; Wang et al. 2010a, b).

Aux/IAA genes encoding short-lived nuclear proteins are responsive primarily to auxin induction. The *Aux/IAA* genes in *Arabidopsis*, rice, maize and sorghum were induced by exogenous auxin, but displayed differential expression pattern (Yamamoto et al. 1992; Thakur et al. 2001). According to previous microarray data, *AtIAA6* (At1g52830) and *AtIAA3* (At1g04240) were up-regulated by IAA treatments (Nemhauser et al. 2004). IAA treatment up-regulated ten *AUX/IAA* genes in *Arabidopsis* (*IAA1, 2, 3, 5, 6, 7, 11, 13, 19* and *26*) (Goda et al. 2004). In sorghum, most *SbIAA* genes were slightly up-regulated under IAA treatment, especially in roots. But *SbIAA3, SbIAA4, SbIAA5, SbIAA6, SbIAA14, SbIAA15,* and *SbIAA21* in leaves and *SbIAA21* in roots were down-regulated under IAA treatment (Shibasaki et al. 2009). In tomato, *SlIAA11, SlIAA15, SlIAA16, SlIAA17, SlIAA19* and *SlIAA23* were up-regulated (over fourfolds), whereas *SlIAA20, SlIAA21,* and *SlIAA22* were drastically down-regulated after IAA treatment in leaves (Fig. 6). Our promotor analysis identified seven auxin signaling transduction-related cis-elements presenting in the promoter regions of all the SlIAA genes except *SlIAA18*. The diversity of numbers and locations of their auxin signaling transduction-related cis-elements may partially account for the different expression patterns of *SlIAAs* under IAA treatment. However, although none of the auxin signaling transductions-related cis-elements were

found in the promoter of *SlIAA18* (Table S4), the relatively mRNA level of *SlIAA18* increased after the IAA treatment (Fig. 7).

Interestingly, the investigation of conserved AUX/IAA domains indicated that three *SlIAA* genes (*SlIAA13, SlIAA16* and *SlIAA20*) and six *StIAA* genes (*StIAA13, StIAA15, StIAA16, StIAA18, StIAA20* and *StIAA27*) lacked domain I and domain II (Tables 1, 2; Fig. 2; Suppl Fig. 3). Domain I is a repression domain (Szemenyei et al. 2008), and domain II physically interacts with TIR1 which leading to the degradation of AUX/IAA proteins under a high level of auxin (Gray et al. 2001). It has been shown that the half-lives of these proteins which lack domain II are much longer than those of the canonical Aux/IAA proteins (Sato and Yamamoto 2008). It seems that these nine *SlIAA* genes should be insensitive to IAA treatment since they have longer half-lives than other *SlIAAs* proteins. However, *SlIAA13* and *SlIAA16* mRNA were both drastically up-regulated when exposed to exogenous IAA, especially *SlIAA16* transcript level increased 9- to 15-fold at 6 and 12 h, respectively, after IAA treatment. On the other hand, *SlIAA20* mRNA was found to be markedly down-regulated (23-fold at 12 h) after the IAA treatment. Similar results have been reported in previous researches where proved that *OsIAA8*, as a domain II-lacking IAA gene (Jain et al. 2006), was highly sensitive to IAA treatment (Song et al. 2009). So a possible unknown auxin-related pathway may exist in the degradation of the proteins encoded by these genes and the negative (*SlIAA20*) or positive (*SlIAA13* and *SlIAA16*) regulations in the gene expression. These non-canonical *Aux/IAA* genes (*IAA20*, *IAA30*, and *IAA31*) could cause auxin-related aberrant phenotypes in *Arabidopsis*, which suggests that these noncanonial genes have potential functions in auxin signaling (Sato and Yamamoto 2008). Recently, Li et al. (2011) found that when the first Leu was replaced by Ala in the LxLxL motif of *AtIAA3, AtIAA6* and *AtIAA19*, the "low-auxin" phenotypes were repressed and "high-auxin" phenotypes were activated. However, in addition to a single Leu-to-Ala substitution of AtIAA12 in LxLxLxLxL motif, a second and third Leu residues in the LxLxLxLxL motif should be replaced by Ala to active the "high-auxin" phenotypes. In tomato, most of the IAA genes contain the LxLxL motif (Fig. 2). However, AtIAA12, SlIAA20, 22 and 25 were found to contain the LxLxLxLxL motif (Fig. 2), indicating that these two motifs might play similar function in auxin signaling.

Extensive researches have showed that various environmental signals are integrated into changes in auxin homeostasis, redistribution, and signaling (Park et al. 2007; Shibasaki et al. 2009). There are increasing evidences that the auxin-response genes, auxin/indole-3-acetic acid (Aux/IAA), auxin-response factor (ARF), Gretchen Hagen 3 (GH3) are involved in stress/defense responses in *Arabidopsis*, rice, maize and sorghum (Ghanashyam and Jain 2009; Jain and Khurana 2009; Wang et al. 2010a, b). In our study, many different *SlIAAs* showed similar expression patterns under diverse stress treatments, the expression levels of most of *SlIAAs* increased after drought, salt and heat treatments when compared with control treatment. However, *SlIAA3, SlIAA11,* and *SlIAA14* expression levels were down-regulated after abiotic stress treatments (Suppl Fig. 9). Similar results have been reported in other species. The transcript levels of most *OsIAA* genes in rice were up-regulated by both drought and salt stresses, but *OsIAA31* was down-regulated (Song et al. 2009). In the present study, 13 drought stress-related cis-element, 1 salt stress-related cis-element and 1 heat shock element were found and distributed differently in the promoter regions of most *SlIAAs*, those abiotic stress-related cis-elements combining with the cis-elements involved in the auxin signaling regulation pathway may lead to the specific expression and function of *SlIAA* genes. However, the results of qRT-PCR were not always consistent with those of the promoter region analysis on *SlIAA* genes. Although there were some stress-related cis-elements present in the *SlIAA20, SlIAA21* and *SlIAA22*, no significant changes of their expression levels were detected in response to abiotic stress treatment. On the other hand, there were no salt stress-related cis-elements detected in the promoter regions of *SlIAA11* (Table S4), but the real-time PCR data showed that it was significantly stress responsive (Suppl Fig. 9). Although the putative salt stress-related cis-element (S000453) and heat shock element (S000030) were found in promoters of *SlIAA14* and *SlIAA3* (Table S4), previously proved to enhance the expression levels of relevant genes (Park et al. 2004; Rieping and Sehfffl 1992), their expression levels were significantly down-regulated under the heat or salt stresses (Suppl Fig. 9). This might indicate that some unidentified cis-regulated elements may play an important role in regulating the expression of those *SlIAAs* in stress response in tomato.

In general, no significant expression similarity in terms of tissue distribution and in response to stresses were exhibited between *SlIAAs* and *AtIAAs* in the same group of the phylogenetic tree based on the comparison of the expression pattern of *IAA* genes in tomato and *Arabidopsis* (Winter et al. 2007) (Figs. 5, 6; Suppl Figs. 7–9; Tables S6, S7). However, the *SlIAAs* and *AtIAAs* from the same group, showed similar sensibility to auxin treatment. Consistent with analysis by Goda et al. 2004, the IAA genes in group A1 and A2 showed high sensibility to auxin, but the expression level of the genes from group A3, A5, B2 changed slightly after the auxin treatment (Figs. 5, 6; Suppl Figs. 7–9; Tables S6, S7). These findings suggest that although the diverse levels of *IAA* genes expressed in

different species, a similarity in response to auxin was inherited from their common ancestors through the long evolution.

Conclusion

Overall, 26 and 27 non-redundant *AUX/IAA* genes were identified and characterized in Solanaceae species, tomato and potato, respectively. A comprehensive genome-wide analysis of *SlIAA* gene family is presented, including the gene structures, chromosome locations, phylogeny, and conserved motifs. Our work demonstrates that different spatio-temporal transcript accumulation patterns exist for most members of the SlIAA gene family in tomato. The tomato *SlIAAs* could be transcriptionally induced by exogenous auxin, and most of them could also be induced by drought, salt and heat treatments in tomato leaves. However, it is needed to note that our qRT-PCR analysis only provides an estimate of the whole organ/tissue expression of AUX/IAA family genes, more detailed or exact expression pattern analysis of these IAA genes will be required using in situ hybridization or promoter–reporter fusion system. The final challenge is to define the specific functions of each individual *AUX/IAAs* gene during plant development and in response to environment stress.

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