

## RESEARCH ARTICLE

# Identification and expression analysis of primary auxin-responsive *Aux/IAA* gene family in cucumber (*Cucumis sativus*)

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## Abstract

*Aux/IAA* is an important gene family involved in many aspects of growth and development. *Aux/IAA* proteins are short-lived nuclear proteins that are induced primarily by various phytohormones. In this study, 29 *Aux/IAA* family genes (*CsIAA01–CsIAA29*) were identified and characterized in cucumber, including gene structures, phylogenetic relationships, conserved protein motifs and chromosomal locations. These genes show distinct organizational patterns of their putative motifs. The distributions of the genes vary: except for five *CsIAA* genes in cucumber that were not located, seven *CsIAA* genes were found on scaffold, while the other 17 *CsIAA* genes were distributed on seven other chromosomes. Based on a phylogenetic analysis of the *Aux/IAA* protein sequences from cucumber, *Arabidopsis* and other plants, the *Aux/IAA* genes in cucumber were categorized into seven subfamilies. To investigate whether the expression of *CsIAA* genes is associated with auxin induction, their transcript levels were monitored in seedlings treated with IAA (indole-3-acetic acid), and their expression patterns were analysed by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR). The results showed that 11/29 *CsIAA* genes were expressed in leaves whether treated with IAA or not and the time course of processing and compared with the control, five *CsIAA* genes showed low expression only after 60 min treatment with IAA, while 11 genes showed no expression. These results provide useful information for further functional analysis of *Aux/IAA* gene family in cucumber.

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## Introduction

The phytohormone auxin/indole-3-acetic acid (*Aux/IAA*) genes are regarded as primary/early auxin-responsive genes (Abel and Theologis 1996; Wang *et al.* 2008) that play vital roles in regulating many aspects of plant growth, development and differentiation (Teale *et al.* 2006). At the cellular level, auxin regulates cell division, extension and differentiation (Friml 2003). At the level of the whole plant, auxin plays an indispensable role in processes such as embryogenesis, vascular elongation, lateral root initiation, axis formation and patterning, leaf expansion, inflorescence, fruit set and development, tropisms and apical dominance (Liu *et al.* 2011).

*Aux/IAA* genes are transcriptional repression factors, and the proteins encoded play important roles during the early

stages of the transduction of auxin signalling. Some gene families, such as *Aux/IAA*, Gretchen Hagen 3 (*GH3*) and small auxin-up RNA (*SAUR*) are responsive to auxin stimulation (Guilfoyle and Hagen 2007; Benjamins and Scheres 2008; Wang *et al.* 2008). Thus, these gene families are termed primary/early auxin-responsive genes (Abel and Theologis 1996). Some *Aux/IAA* genes have been isolated from *Arabidopsis* (Dharmasiri and Estelle 2004), rice (Jain *et al.* 2006), poplar (Kalluri *et al.* 2007) and maize (Wang *et al.* 2010a), but to date only three *Aux/IAA* genes have been isolated in cucumber (Fujii *et al.* 2000; Leyser 2006). Cucumber genomes are now available (Huang *et al.* 2009) and these are valuable references for studying the *Aux/IAA* genes contained in the whole cucumber genome.

According to the identification of *Aux/IAA* genes in *Arabidopsis*, rice and maize (Wang *et al.* 2010a), typical *Aux/IAA* proteins are short-lived nuclear proteins that contain four conserved domains (namely domains I, II, III and IV), though some predicted proteins lack one or more of these domains. *Aux/IAA* proteins have a nuclear-localization

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sequence and representative Aux/IAA protein fusions localize to the nucleus. Domain I at the N terminus has three repeat leucine residues, referred to as 'LxLxL' motif (where L, leucine; x, any amino acid [aa] residue), and this is the smallest and least strictly conserved of the conserved domains. Domain I has been ascribed with the function of repressing transcription (Tiwari *et al.* 2004; Song *et al.* 2009). Domain II is highly conserved and related to Aux/IAA protein stability, as mutations in this domain can increase the activities of corresponding proteins by increasing their stability (Worley *et al.* 2000; Tiwari *et al.* 2001). Domain III probably forms a true domain in the protein structural sense and it might be sufficient by itself for dimerization because the conserved sequence can fold into a  $\beta\alpha\alpha$  structure (one  $\beta$  sheet and two  $\alpha$  helices) and a synthetic peptide containing domain III can fold and dimerize *in vitro* (Abel *et al.* 1994). Domain IV has a functional NLS and might also contribute to dimerization (Song *et al.* 2009; Poutrain *et al.* 2011). Domains III and IV are responsible for dimerization (homodimerization and heterodimerization) of the Aux/IAA proteins and between the Aux/IAA proteins (Hardtke *et al.* 2004; Tiwari *et al.* 2004).

Previous research has demonstrated that auxin induces and regulates the expression of numerous genes, which serves multiple regulatory functions (Zhang *et al.* 2007). Auxin induces a rapid and specific expression of many Aux/IAA genes without the requirement for *de novo* protein synthesis. While recent researches showed that some Aux/IAA genes can also be induced by cytokinin, kinetin, jasmonic acid, light or abiotic stress etc. *IAA3*/*SHY2*, an Aux/IAA gene from *Arabidopsis* can be induced by cytokinin (Nemhauser *et al.* 2006). The *OsIAA1* gene in rice is expressed in all the tissues and organs that have been examined and it is induced by various phytohormones including IAA, 2,4-D, kinetin, 24-epibrassinolide and jasmonic acid (Song *et al.* 2009). In maize, a total of 31 Aux/IAA genes have been identified and putative *cis*-acting regulatory DNA elements involved in auxin response, light signal transduction and abiotic stress adaption have been observed, and expression data mining suggests that maize Aux/IAA genes show differential temporal and spatial expression patterns (Wang *et al.* 2010a). *SbIAA1*, an Aux/IAA gene in sorghum, is highly induced by IAA, brassinosteroid, salt and drought conditions and expressed in leaf and root tissues differentially (Wang *et al.* 2010b). The regulatory mechanisms of Aux/IAA-ARF action in *Arabidopsis* have also been studied, and the transcriptome that is regulated downstream of *IAA1* has been identified during the auxin response using dexamethasone-controlled nuclear translocation (Lee *et al.* 2009).

Using cucumber genome sequences, we aimed to investigate the Aux/IAA genes in the whole genome (*CsIAAs*). Various characteristics of the cucumber Aux/IAA genes were analysed in detail, including total number, genomic organization, motif distributions and phylogenetic relatedness. In addition, the effect of IAA application on the expression of Aux/IAA genes in five different tissues was investigated. The

results of this paper provide new data for further study on the auxin signalling pathway.

## Materials and methods

### Identification of Aux/IAA genes in cucumber

The hidden Markov model (HMM) profile of the Aux/IAA gene family (PF02309) was downloaded from the Pfam database (<http://pfam.sanger.ac.uk>) (Finn *et al.* 2008). Based on the HMM profile, a 261 aa conserved sequence of the Aux/IAA proteins was obtained (Eddy 2008). First, for the *CsIAAs*, all the predicted *CsIAA* protein sequences were used as query sequences to search against the Cucumber Genome Database (<http://cucumber.genomics.org.cn/page/cucumber/index.jsp>) using the BLASTP program (with a  $P = 0.001$  to avoid false positives). In addition, the National Centre for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/>) database was mined using IAA as a keyword to avoid missing any other cucumber Aux/IAA genes. For Aux/IAA genes in *Arabidopsis*, the sequences obtained were used as queries to search against the DATF (Database of Arabidopsis Transcription Factor, <http://datf.cbi.pku.edu.cn/browsefamily.php?fn=Aux/IAA>). The Pfam and SMART (Simple Model Architecture Research Tool, <http://smart.embl-heidelberg.de>) (Letunic *et al.* 2004) databases were used to determine whether any candidate Aux/IAA protein sequences were members of the Aux/IAA family. To exclude any overlapping genes, all of the candidate Aux/IAA genes were aligned using Clustal W (Larkin *et al.* 2007) and the sequences were checked manually. All nonoverlapping Aux/IAA genes were used for further analyses.

### Structural analysis of *CsIAAs*

Information for the *CsIAA* genes was retrieved from the Cucumber Genome Database, including the sequence ID, its chromosomal location and deduced polypeptide sequence. The position of each *CsIAA* gene on the cucumber chromosomes was determined by BLAST searching against the genomic sequences of each cucumber chromosome. Molecular weights (MW) and isoelectric points (PI) were determined using the ProtParam program on the ExPASy website (<http://au.expasy.org/tools/protparam.html>).

### Analysis of conserved motifs

To identify the conserved motifs within the Aux/IAA proteins in cucumber, *Arabidopsis* and other dicotyledons, the online multiple expectation maximization for motif elicitation (MEME) utility was employed to display the motifs in Aux/IAA proteins ([http://meme.nbcr.net/meme4\\_1/cgi-bin/meme.cgi](http://meme.nbcr.net/meme4_1/cgi-bin/meme.cgi)) (Bailey *et al.* 2009). Parameters were set as follows: the occurrences of a single motif: zero or one per sequence, optimum motif width:  $\geq 6$  and  $\leq 50$ , maximum

number of motifs for identification: nine, all other parameters were set as the default values. The SMART (<http://smart.embl-heidelberg.de>) program and Pfam database were used to annotate the MEME motifs (<http://meme.sdsc.edu>) (Bailey *et al.* 2009). Multiple alignments of Aux/IAA proteins were conducted using Clustal X (ver. 1.83) software with all parameters defaulted (Larkin *et al.* 2007).

### Phylogenetic analysis

To further understand the evolutionary relationships of the Aux/IAA proteins, phylogenetic trees of cucumber, *Arabidopsis* and other dicotyledon Aux/IAA genes were constructed, and the sequences of the Aux/IAA domain-containing proteins were aligned using ClustalX 1.83 (Larkin *et al.* 2007). Phylogenetic analysis was performed using the MEGA 4.0 program (Tamura *et al.* 2007) according to the neighbour-joining (NJ) method. Moreover, the maximum parsimony method was used with a bootstrap value of 1000 replicates to create a phylogenetic tree and to validate the results from the NJ method. According to their phylogenetic relatedness with *Arabidopsis* Aux/IAA genes, the cucumber and other dicotyledon Aux/IAA genes were named accordingly.

### Plant materials and treatments

Seeds of cucumber (*Cucumis sativus*, Jinlü No. 5; Horticultural Lüfeng Ltd., Tianjin, China) were germinated in flow-erpots. Plants were grown in a greenhouse at 24–28°C, and three main-stem nodes stage seedlings were prepared for the IAA treatment. The seedlings were incubated in 40 mol.L<sup>-1</sup> IAA liquid media and leaves were collected at 0, 15, 30 and 60 min after treatment.

### RNA extraction and semiquantitative RT-PCR

Total RNA of the collected samples was extracted using the RNeasy Plant Mini Kit (Qiagen, Hefei, China). For semiquantitative RT-PCR analysis, first-strand cDNA was synthesized using the PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa, Hefei, China) according to the manufacturer's instructions. The gene-specific primers for the RT-PCR analysis were designed using Primer 5.0 (table 1). The expression level of the cucumber *ACTIN* gene (DQ115882.1) was used as the internal control and was amplified with the primers: 5'-ggaaggagcagcttgatgg-3' and 5'-ggagaagatctggcatcacac-3'.

## Results

### Identification of CsIAAs

To identify all the Aux/IAA genes in the cucumber genome, a conserved sequence of 261 aa in Aux/IAA proteins generated from the HMM profile in the Pfam program was employed to search the annotated cucumber database. Additionally, the

NCBI database was queried using 'IAA' as the search term in case some cucumber Aux/IAA genes were missed. Using this approach, 29 putative Aux/IAA genes designated CsIAA01–CsIAA29 were identified. Information on these Aux/IAA family genes, including the sequence ID, aa length, MW, PI and the number of Aux/IAA motifs and their physical locations on the chromosomes are listed in table 1. The PIs of 13 Aux/IAA gene products are below 7.0, while nine gene products have PIs above 8.0. The lengths of the Aux/IAA proteins vary between 160 aa and 427 aa (table 1).

### Chromosomal locations of CsIAAs

Except for five CsIAA genes in cucumber that remains to be unlocated, seven CsIAA genes were found to be distributed on scaffold, while the other 17 CsIAA genes in cucumber are distributed on a total of seven chromosomes. The distribution of the CsIAA genes varies: there are seven genes on chromosome 3, four genes on chromosome 2, three genes on chromosome 7 and just a single gene on each of the chromosomes 1, 5 and 6 (table 1).

### Sequence analysis of the CsIAA proteins

Alignment of the cucumber Aux/IAA protein sequences revealed four highly conserved characterized regions designated domains I, II, III and IV. Except for three CsIAA proteins (CsIAA07, CsIAA22 and CsIAA24), the conserved domains I to IV are present in all of the other CsIAA protein sequences. Domain I is missing in CsIAA07, domain IV is absent in CsIAA22 and CsIAA24. Two NLSs of Aux/IAA proteins were identified separately in domains II and IV, the CsIAA proteins that contain a domain II degron partly implying that the CsIAA proteins are short-lived proteins, and the  $\beta\alpha\alpha$  motif that plays an important role in the dimerization of the Aux/IAA proteins was found in domain III. Moreover, multiple phosphorylation sites of kinases were found in the CsIAA proteins (figure 1).

The online MEME server was used to identify the distribution of conserved motifs in Aux/IAA proteins of cucumber, *Arabidopsis* and other dicotyledons. Five major motifs (motifs 1, 2, 3, 4 and 6) were detected in most of the Aux/IAA proteins. Meanwhile, motif 6 is absent in CsIAA14 and CsIAA28; motif 1 is missing in CsIAA17 and CsIAA22; motif 3 is shortage in CsIAA5 and CsIAA27; motif 4 is absent in CsIAA7 and CsIAA20; motif 2 is absent in CsIAA19; while two motifs (motifs 1, 2 or 4) are absent in CsIAA4, CsIAA25 and CsIAA24 (figure 2).

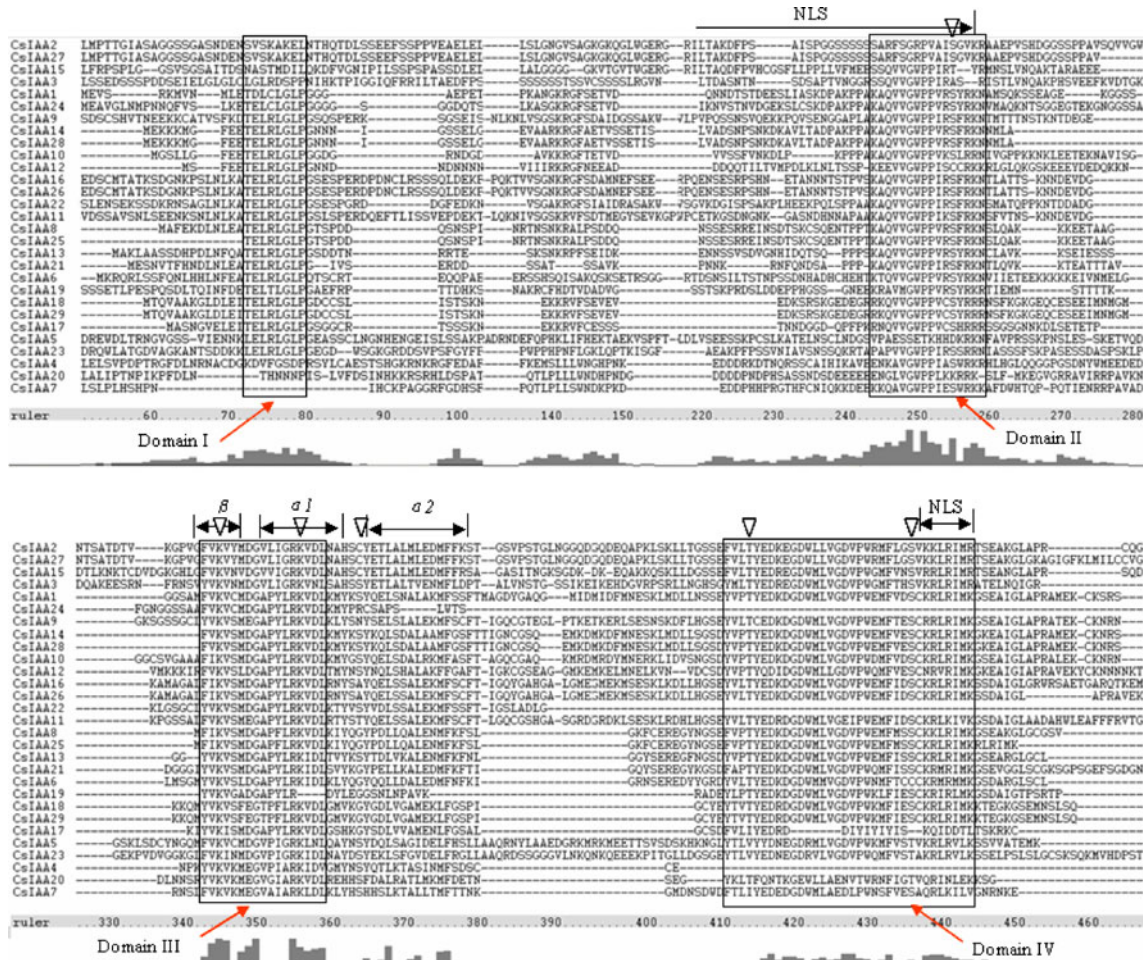
The four conserved domains of the Aux/IAA proteins shown in figure 1 were divided into five conserved motifs according to analysis with the MEME tool. Motifs 1 and 2 correspond to domain IV, while domains I, II and III correspond to motifs 4, 3 and 6, respectively (figure 2; table 2).

The multiple alignment results clearly show the high conservation of Aux/IAA proteins in cucumber. Therefore, the

**Table 1.** Basic information of the Aux/IAA proteins and primer sequences of semiquantitative PCR analysis of *Aux/IAA* family genes in cucumber.

Gene name	Sequence ID	Deduced polypeptide			Number of motifs	Chromosome	Primers for semiquantitative RT-PCR	
		Length (aa)	MW (kDa)	PI			Forward primer	Reverse primer
<i>CsIAA01</i>	Csa000125	237	25897.63	5.76	7	3	GGTCAACATGCTGGAAACTG	CTGTGGAGTGAGATCGAGC
<i>CsIAA02</i>	Csa000184	321	33861.19	8.73	7	3	CTAAGGAGTTGAACACCCACC	ACGACCCACAGGAGAGAT
<i>CsIAA03</i>	Csa001390	281	30512.19	7.07	5	3	TCCCTGAGGACTCTTCATC	CACGAAGAGAAAGAGAGGA
<i>CsIAA04</i>	Csa002030	187	21226.77	5.78	3	3	CAGGAATGCTTGATGG	TGCACAAGAGCTTCTCTGG
<i>CsIAA05</i>	Csa002198	342	38028.78	6.76	4	3	TGTCCTGAGCTTCTTGACC	TCCATCTCCAGCTATTCC
<i>CsIAA06</i>	Csa003118	222	25660.05	8.46	5	5	TGAGCAACAACCCAGCAGAG	CATCCAACCACTTGCGTC
<i>CsIAA07</i>	Csa006091	207	23864.91	6.26	4	2	CAACTTGGCCTTCTCTTCC	CCATCCTACTGCTTGGTTC
<i>CsIAA08</i>	Csa006680	197	22020.91	6.41	5	2	GATCTCAACTTGGAAAGCCAC	CAACTTGTGCCTTGGTAGGA
<i>CsIAA09</i>	Csa009839	356	38385.99	7.46	7	7	CAGAGGCTTCTTCGATGGA	GACTGAGAAACAGGTAAACCG
<i>CsIAA10</i>	Csa010933	236	25885.65	9.03	7	1	TGAAGAGACAGAGCTCCGT	TGACGAAGCTCGAAAGGAC
<i>CsIAA11</i>	Csa012115	404	44285.62	6.39	8	6	TGTAAGTGCTGCTTCACT	GGTAGACCAAGTCTCAACTCG
<i>CsIAA12</i>	Csa014661	222	25258.88	6.63	6	2	TCGTTGAGGAGACAGAGC	CCAACCCACCACTTCTTC
<i>CsIAA13</i>	Csa016714	196	21830.53	6.74	5	7	CCAGATCTCAACTTCCAAGC	CTTGGATGGAGGAGGTTG
<i>CsIAA14</i>	Csa016715	230	25381.18	8.28	6	7	ATTGGTGAGGTTGCTGC	GTCTTTGTGCTAGGTTGG
<i>CsIAA15</i>	Csa016993	285	30832.04	6.01	6	Scaffold0000084	CTCGATGGAGAGTGTCAAGTC	TTCAGATCCAGGAGACCA
<i>CsIAA16</i>	Csa018571	427	47510.39	5.86	7	2	CTCGATGGAGAGTGTCAAGTC	CAAGCCTCAGTTCTGTAGCCT
<i>CsIAA17</i>	Csa020458	160	17695.89	7.59	4	3	CGAAGAAGAGTAGTGGTAGC	ATCAGCACAAAGTCGGAGC
<i>CsIAA18</i>	Csa020459	188	21341.61	8.45	5	3	ACCCAGTGGCAGCTAA	TCCCTCATCCTCACCTTTG
<i>CsIAA19</i>	Csa020481	184	20415.65	5.15	4	Scaffold0000163	TCCTCCGAGACACTTCC	GCATCAACAGTGTCAATGG
<i>CsIAA20</i>	Csa020973	218	24986.20	7.08	4	Scaffold0000187	AGCGTTCTCGTCACTCG	CCATTCTCCTTCTCTCCT
<i>CsIAA21</i>	Csa021321	203	22287.11	7.68	5	Scaffold0000210	TGAAGGAGACTGAGCTCTGC	ACACCAACACCCAGACCTC
<i>CsIAA22</i>	Csa021532	233	24583.56	7.78	4	Scaffold0000225	GAGCTTCGCTTGGACTTC	GCCTCTGTTTCTTCACTG
<i>CsIAA23</i>	Csa021916	326	35118.65	6.85	5	Scaffold0000259	AGGCTACTGAACTGAGGTTG	CCATTGTGGAGGAGTC
<i>CsIAA24</i>	Csa021312	179	26805.27	8.49	3	Scaffold0000210	CCAACACCTCAGATGACAAG	CCATGGATTCTCAGAGGGA
<i>CsIAA25</i>	BAA85822.1	180	20538.37	9.11	4	unlocated	ACGGAAGCTGAGATTGGGA	CCACAACCTGTGCTTGG
<i>CsIAA26</i>	BAA85821.1	355	39030.65	5.38	8	unlocated	GTGACACAACATGGAGAACC	GACCAAGCCTCAGTCTGTAG
<i>CsIAA27</i>	ACA35267.1	328	34617.49	7.60	6	unlocated	GTGACACAACATGGAGAACC	TGAGCTCAGCTCAACAG
<i>CsIAA28</i>	BAA81687.1	230	25381.18	8.28	6	unlocated	CAACATCGGAAGCAGTGA	GTCTTTGTGCTAGGTTGG
<i>CsIAA29</i>	BAA85820.1	188	21341.61	8.45	5	unlocated	CTCTGAGGTGGAAAGTAGAAGAC	TCTTCACTCTCACACTGCTCTC





**Figure 1.** Sequence alignment of *Aux/IAA* family proteins. The protein sequences were aligned using ClustalX (1.83). Regions I, II, III and IV represent four conserved domains (black boxes). Two NLSs and one  $\beta\alpha\alpha$  motif are shown by bi-directional arrows. Phosphorylation sites are emphasized by triangles. The positions of *Aux/IAA* proteins are indicated by figures at the bottom of the sequences.

configuration of the motifs identified by MEME reflects the conservation and diversity within *Aux/IAA* subfamilies.

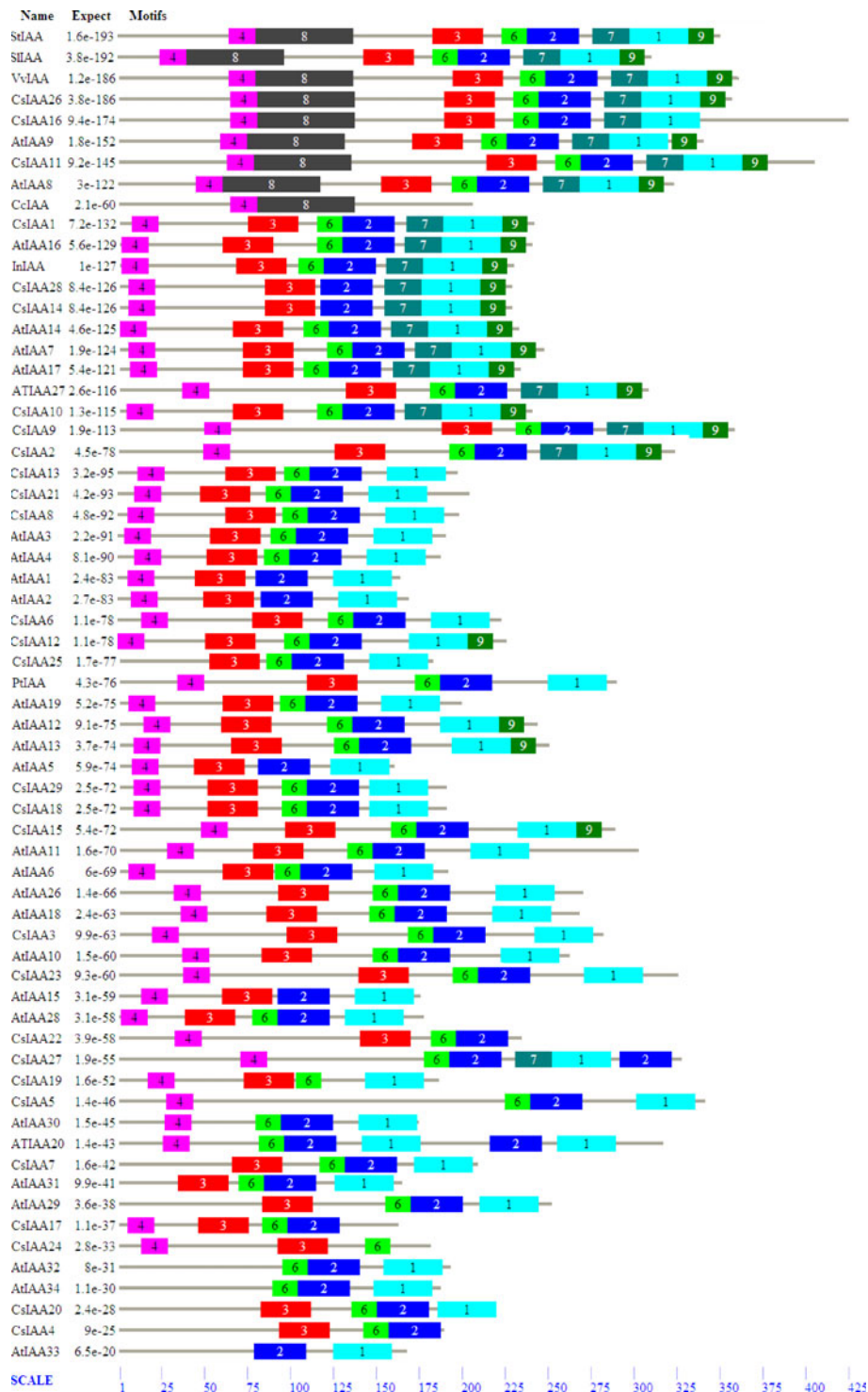
### Phylogenetic analysis of *Aux/IAA*s

To investigate the phylogenetic relationships of the *Aux/IAA* proteins and infer the evolutionary history of this gene family, a phylogenetic tree was constructed from 29 cucumber, 29 *Arabidopsis* and six other dicotyledon *Aux/IAA* proteins. Based on the domain compositions and phylogenetic relationships of the protein sequences, these 64 *Aux/IAA* proteins were divided into seven groups: four groups (classes I, II, IV and VII) contain only *CsIAA*s and *AtIAA*s, while three groups (classes III, V and VI) contain sequences from other dicotyledons, such as *Vitis vinifera* (*VvIAA*), *Solanum lycopersicum* (*SlIAA*), *Solanum tuberosum* (*StIAA*), *Cap-sicum chinense* (*CcIAA*), *Ipomoea nil* (*InIAA*) and *Popu-lus tremula* (*PtIAA*). There are only two *Aux/IAA* proteins (*CsIAA19* and *AtIAA15*) in class VII, while six or more

*Aux/IAA* proteins are included in the other cluster of the phylogenetic tree (figure 3).

### Expression of *CsIAA* genes

To investigate whether the expression of *CsIAA* genes in cucumber is associated with auxin induction, the transcript levels of each *CsIAA* gene was monitored in seedlings after IAA treatment, and their expression patterns were analysed using semiquantitative RT-PCR. The results showed that 11/29 *CsIAA* genes (*CsIAA02*, *CsIAA05*, *CsIAA07*, *CsIAA12*, *CsIAA15*, *CsIAA16*, *CsIAA18*, *CsIAA20*, *CsIAA21*, *CsIAA22* and *CsIAA27*) were normally expressed in the leaves regard-less of whether the treatment processing and the time course of processing time, one gene (*CsIAA23*) showed weak expression, while 11 genes (*CsIAA01*, *CsIAA03*, *CsIAA06*, *CsIAA08*, *CsIAA10*, *CsIAA11*, *CsIAA24*, *CsIAA25*, *CsIAA26*, *CsIAA28* and *CsIAA29*) showed no expression at all. While compared with the control, five *CsIAA* genes (*CsIAA04*,



**Figure 2.** Multiple alignment of Aux/IAA proteins. Ten motifs were identified using the MEME software. Different motifs are indicated by different colours. Five conserved motifs (motifs 1, 2, 3, 4 and 5) were located within most members of the Aux/IAA family. Order of the motifs corresponds to the position of the motifs in the individual protein sequences. Names of all the members from different subfamilies and combined *P* values are shown on the left side of the figure, and a scale is located at the bottom to indicate the relative size of each motif.

**Table 2.** Motif distribution of cucumber Aux/IAA proteins.

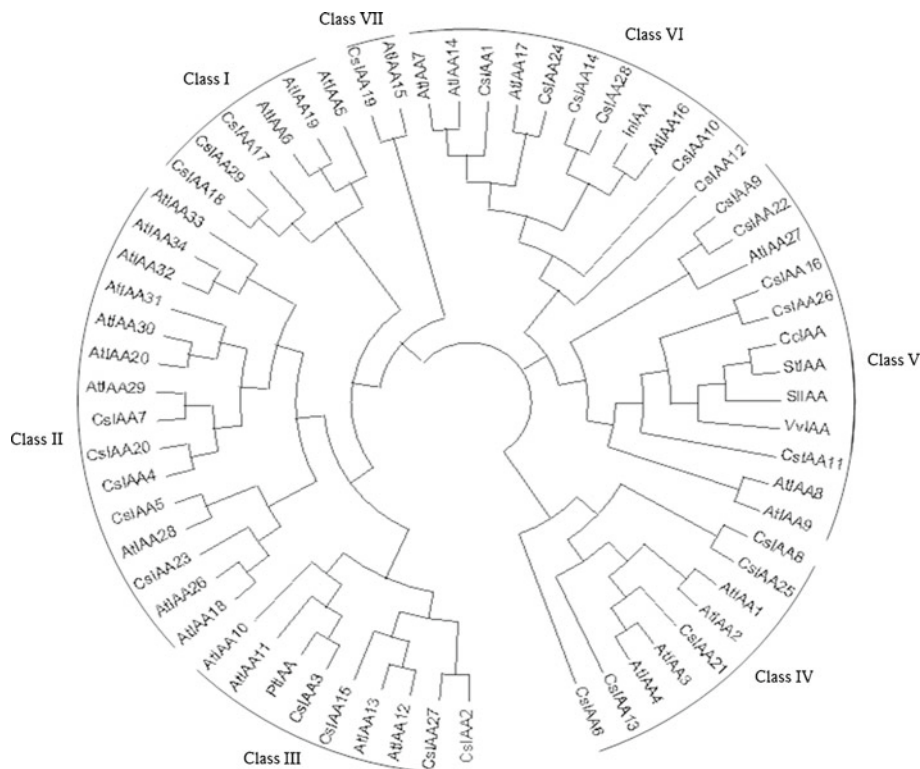
Motif name	Length (aa)	Database sequences	Corresponding domain
Motif1	35	LTYEDKDGDWMLVGDVPWEMFIESCKRLRIMKGSE	Domain IV
Motif 2	31	GAPYLRKIDLKMYKCYQELSHALEKMFGCFT	Domain IV
Motif 3	30	HAKPPAKAQVVGWPPIRSRYKNTMATQKKN	Domain II
Motif 4	16	LNFKATELRLGLPGGQ	Domain I
Motif 5	80	YFCKTLTASDTSTHGGFSVPRRAAEKIFPPLDFSMQPPAQE LIARDLHDNEWKFRHIYRGQPKRHLLTTGWSVFVSAKRL	
Motif 6	9	AMYVKVSMD	
Motif 7	22	QGMKDFMNESKLMDLLHGSEYV	Domain III
Motif 8	58	SPERDPETCLISSTKLDEKPLFPLHPSKDTAYSVS QKTVVSGNKRGFSDAMDGFSEGG	
Motif 9	12	AIGLAPRAMEKC	

*CsIAA13*, *CsIAA14*, *CsIAA17* and *CsIAA19*) showed low expression only after 60 min treatment with IAA (figure 4).

## Discussion

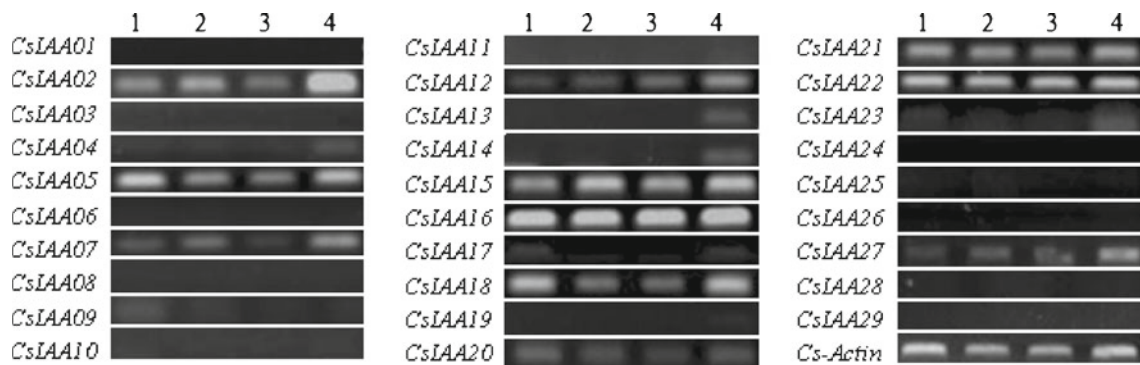
Auxin plays an important role in plant growth and development, *Aux/IAA* genes which respond primarily to auxin induction (Kalluri *et al.* 2007) are important regulatory genes in the auxin signalling pathway. The expression of *Aux/IAA* genes is increased transiently by auxin treatment, and thus

regulates the expression of other genes in the auxin signalling network (Reed 2001; Rogg *et al.* 2001). Recently, *Aux/IAA* genes have been studied in *Arabidopsis*, rice and potato (Dreher *et al.* 2006; Terrile *et al.* 2010; Uchiumi and Okamoto 2010), and the expression and regulation of the early auxin-responsive *Aux/IAA* genes in plants and organs have also been studied. Transcription of the *VvIAA19* gene in grapes is upregulated and maximum expression is maintained until the end of ripening, while in grape leaves expression could not be induced by exogenous IAA, which suggests that *VvIAA19* might be auxin nonresponsive (Kohno



**Figure 3.** Phylogenetic relationships of cucumber, *Arabidopsis* and some other dicotyledon *Aux/IAA* genes. At, *Arabidopsis thaliana*; Vv, *Vitis vinifera*; Sl, *Solanum lycopersicon*; St, *Solanum tuberosum*; Cc, *Capsicum chinense*; In, *Ipomoea nil*; Pt, *Populus tremula*; Cs, *Cucumis sativus*.





**Figure 4.** Expression profiles of *CsIAA* genes. 1, expression with no treatment (as a control); 2, 15 min; 3, 30 min; 4, 60 min. IAA treatment and the time course are described in the ‘Materials and methods’ section.

et al. 2012). The *CrIAA1* gene is an early auxin-related gene in *Catharanthus roseus* cells, and its expression can be induced dramatically by treatment with auxin via a feedback mechanism (Poutrain et al. 2011).

According to the locations of 29 *CsIAA* genes on chromosomes, 5/29 genes remained unlocated up to now, 17 *CsIAA* genes were well positioned in the genome and seven genes were located on chromosome 3, so it may be possible that several genes are organized in genomic clusters.

Based on the phylogenetic analysis in this study, *CsIAA* and *AtIAA* are distributed uniformly, about 50% in each branch (except for class VI). Among seven subfamilies, only one subfamily (class VII) is composed of two proteins (*AtIAA15* and *CsIAA19*), and some *Aux/IAA* proteins from other dicotyledons are only distributed into three subfamilies (classes III, V and VI).

Recent researches showed that some *Aux/IAA* genes can be induced by auxin, cytokinin, kinetin, jasmonic acid, light or abiotic stress etc. *FaAux/IAA1* and *FaAux/IAA2*, the two auxin-responsive *Aux/IAA* genes, play important roles in regulating fruit growth and ripening in strawberry, which can be induced by NAA treatment (Liu et al. 2011). Nemhauser et al. (2006) found that only one *Arabidopsis Aux/IAA* gene could be responsive to cytokinin. The expression of *VvAux/IAA4* in *Vitis vinifera* was rapidly induced in response to NAA treatment, but was decreased by salt, drought and salicylic acid (SA) treatments which provide evidence of crosstalk between phytohormone and abiotic stresses, and support a role for auxin in stress responses (Cakir et al. 2013). In our study, most *CsIAA* genes showed high expression in almost all of the leaves whether treated with auxin (IAA) induction or not.

Cucumber is one of the most important vegetable with abnormal stems–tendrils, and its growth may be related to auxin, cytokinins and other factors. Characterization and expression of cucumber *Aux/IAA* genes is the aim of our research. Research shows auxin induces the rapid and specific expression of many *Aux/IAA* genes, without the requirement for *de novo* protein synthesis. But in the present study, 11 genes were expressed properly regardless of whether

induced by auxin or not, or the length of the induction time. It is strange that only five *CsIAA* genes are expressed after 60 min induction, while other 11 *CsIAA* genes showed no expression which may be related to the limited induction time. Experiments to examine their induction time and spatial expression patterns are in progress. The results presented here provide significant clues for exploring the expressions and functions of the cucumber *Aux/IAA* genes in further studies.

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