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CLONING AND CHARACTERIZATION OF AN *AUX/IAA* GENE IN *POPULUS DAVIDIANA* × *P. ALBA* VAR. *PYRAMIDALIS* AND THE CORRELATION BETWEEN ITS TIME-COURSE EXPRESSION AND THE LEVELS OF INDOLE-3-ACETIC IN SAPLINGS INOCULATED WITH *TRICHODERMA*

ZHIHONG YAO[1], ABDUL MAJEED BALOCH[1], ZHIHUA LIU[2], TONGTONG ZHAI[1], CHUANYING JIANG[1], ZHAOYING LIU[1] AND RONGSHU ZHANG[1*]

[1]College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China
[2]School of Forestry, Northeast Forestry University, Harbin 150040, China
[*]Corresponding author's email: zrs_nefu@163.com

Abstract

PodaAUX/IAA gene, encoding an early-stage responsive protein to auxin in *Populus davidiana* × *P. alba* var. *pyramidalis* (Shanxin poplar), was cloned. The length of mRNA transcript of *Poda AUX/IAA* was 741bp, encoding a 248-amino-acid protein product, Poda AUX/IAA ORF analysis suggested that Poda AUX/IAA contained one conserved domain (pfam02309). Predicted molecular weight of Poda AUX/IAA was found to be 27kDa and its theoretical isoelectric point was determined as 8.21. Poda AUX/IAA was predicted to be a hydrophilic nucleoprotein and its multi-sequence alignment analysis showed that it shares high identity in four conserved domains with eight AUX/IAA proteins in other *Populus* species and these sequences of Poda AUX/IAA shared highest similarity with Pt-IAA14.1 in *P. trichocarpa*. In this study, we found that *Poda AUX/IAA* was expressed in both leaves and roots of Shanxin poplar. Three strains of *Trichoderma asperellum* were used to inoculate Shanxin poplar saplings. Inoculated saplings were cultured for 72 h. It was then found that IAA levels in both leaves and roots of inoculated saplings gradually increased and time-course expression patterns of *PodaAUX/IAA* was changed along with IAA levels. Results of Pearson correlation analysis demonstrated a negative correlation between expression levels of *Poda AUX/IAA* and IAA levels in both leaves and roots of Shanxin poplar saplings when compared with control. Negative correlation in inoculated saplings were less significant, probably as a result of *Trichoderma* inducing.

Key words: *Populus davidiana* × *P. alba* var. *pyramidalis*, *AUX/IAA*, *Trichoderma*, LC-MS/MS, fluorescence quantitative real-time PCR.

Introduction

Populus davidiana× *P. alba* var. *pyramidalis* (Shanxin poplar) is selected bred cultivar that originally comes from the manual hybridization using *P. davidiana* as maternal plant (Lee *et al*., 2011) and *P. alba* var. *pyramidlis* as paternal plant. Shanxin poplar is a female-sterile cultivar whose infructescence merely falls off in spring and does not produce airborne catkin pollutant. In addition, Shanxin poplar grows fast and has narrow crown that makes it not only an excellent tree choice for urban/rural greening but also for windbreak afforestation and timber production in China, which ultimately brings significant economical and ecological value. Collectively, Shanxin poplar is a proper species for studying positive influences of *Trichoderma* on trees and the underlying mechanisms.

Trichoderma spp., are widely-known as beneficial fungi and biocontrol agents. *Trichoderma* can penetrate plant roots, colonize in epidermis and outer cortex and exert beneficial effects on plant metabolism, promoting growth, increasing nutrient uptake and improving crop productivity (Martínez-Medina *et al*., 2011; Mclean *et al*., 2012; Tripathi *et al*., 2013). *Trichoderma* spp., can synthesize and secrete antagonistic compounds including enzymes, proteins and antibiotics and micro-nutrients such as hormones, vitamins and minerals during symbiosis with plants. These secretions promote growth of plant and also improve resistance against diseases (Al-Taweil *et al*., 2009; Valenzuela *et al*., 2015). *Trichoderma* are eco-friendly immunity-inducing bio-fertilizer for plants and they are adaptable to a wide range of growth conditions (Contreras-Cornejo *et al*., 2009).

Phytohormoneauxin plays an important role during plant growth. Indole-3-acetic acid (IAA) is an important natural auxin that regulates plants' responses in certain biological events such as cell elongation, cell division and differentiation, apical dominance, tropism, caducity as well as blossom formation (Jain *et al*., 2006; Ljung, 2013; Gao *et al*., 2016). The effects of auxin on plant are largely related to its concentration. In order to ensure healthy growth and development, plants need a mechanism of auxin regulation to keep IAA level stabilization in various environmental conditions (Zhang *et al*., 2011).

Auxin signal transduction is mainly conducted by proteins encoded by *Aux/IAA*, *SAUR* and *GH3* gene families (Ramos *et al*., 2001); amongst them Aux/IAAs, which are present as multigene families in plants, are key regulators (Han *et al*., 2012). A total of 35 *AUX/IAA* genes have been discovered in *P. trichocarpa*; *PoptrIAA16.1* are expressed in seed, root, cortex, phloem and xylem, whereas *PoptrIAA16.3* and *16.4* are expressed in tender leaves (Kalluri *et al*., 2007). In *Arabidopsis thaliana*, a study on the presence of 29 Aux/IAA proteins showed that auxin signal transduction relied on interactions between Aux/IAA and auxin response factors (ARF) (Liscum & Reed, 2002; Woodward & Bartel 2005). When auxin level is low, Aux/IAA binds with *ARFs* thus suppresses the transcription of auxin-responsive genes. However, with increasing auxin level the *Aux/IAA* degrades and dissociated *ARFs* then bind with the auxin responsive element *AuxRE* (TGTCNC) of auxin responsive genes to facilitate transcription (Reed, 2001; Liacum & Reed., 2002; Yang *et al*., 2004; Tan *et al*., 2007).

Researches have already been conducted to determine the effects of growth promotion induced by *Trichoderma* in plants, which are mainly focused on herbaceous (Wijesinghe *et al*., 2010; Sant *et al*., 2010; Valenzuela *et al*., 2015). The present study was conducted on the interacting mechanisms between three separate *T. asperellum* strains and Shanxin poplar, a model woody plant with economic value. Here, we cloned the *PodaAux/IAA* gene in Shanxin poplar and subsequently bioinformatics analysis was done for its protein product. We also analyzed the correlations between the time-course expression of *PodaAux/IAA* gene and levels of indole-3-acetic acid in Shanxin poplar saplings inoculated with *Trichoderma*.

Materials and Methods

Preparation of Shanxin poplar saplings and inoculation: Shanxin poplar seedlings were provided by Dr. Zhihua Liu (School of Forestry, Northeast Forestry University) and reproduced through tissue-culturing. Seedlings were subcultured on woody-plant medium (WPM) (Lloyd &McCown, 1981) supplemented with 6-BA (0.5 mg/L) and NAA (0.1 mg/L). Rooting of subcultured seedlings was carried out on solidified WPM medium supplemented with IBA (0.4mg/L). Seedlings having 8-12 leaves were transplanted individually into pots containing 4 L of surface soil collected in suburban farmland in Harbin, China (45°44′ N, 126°36′ E) and moved into greenhouse for acclimation for 30 days. Acclimatized seedlings of Shanxin poplar were then moved directly into the open and were grown under natural conditions in nursery of College of Landscape Architecture, Northeast Forestry University, Harbin from June 10th, 2015. In total 120 saplings were prepared and divided into four groups for inoculation experiments using each of *T. asperellum*ACCC32492 (the T1 group), *T. asperellum*ACCC30536 (T2) and *T. asperellum* 4 (T3) and one control group (Con) inoculated with tap water (for each group n=10 and three biological replicates were performed).

Preparation of the *Trichoderma* strains and conidiuminocula: *T. asperellum* ACCC30536 (Ta536) and *T. asperellum* ACCC32492 (Ta492) strains were purchased from the Center of Agricultural Culture Collection of China. *T. Asperellum*4 (Ta4) strain was provided by Dr. Zhihua Liu, Northeast Forestry University of China. Each *T. asperellum* strain was cultured on potato dextrose agar (PDA) medium in petri dishes at 28°C for 6 days for harvesting adequate conidia. Conidia were washed off gently with tap water. Concentration of conidia in water suspension was then measured using microscope and hemocytometer. Final concentrations of all conidia suspensions were adjusted to 1×10^5cfu/mL (colony forming units, cfu) with tap water and adjusted suspensions were then used as inocula.

Inoculation and sample collection: For each pot, 200 ml of inoculum was poured into the soil, equally around stem base of the sapling. For Con, saplings were treated with the same volume of tap water. At 0, 0.5, 1, 2, 4, 24 and 72 hours post-inoculation, shoot tips (in which leaves were less than 1cm long) of the poplar saplings of each of the four groups were collected and frozen in liquid nitrogen at once and stored at -80ºC for RNA extraction and determination of endogenous IAA concentration.

Cloning of genes: Previously transcriptome libraries of roots and leaves of Shanxin poplar seedlings treated with *Trichoderma* for 24 hours were constructed using Solexa technology. In total 50051 non-redundant unigenes of >200bp in length were generated using Trinity *de novo* software. Based on principle that orthologous genes have similar functions, electronic cloning technology was used to find cDNA fragments of *Poda AUX/IAA*, *Podaactin1*, *PodaEF1-α* and *Podaubiquitin* genes, respectively. Then, primers for these four genes were designed according to the fragments. RT-PCR and resequencing were used in order to confirm *PodaAUX/IAA*, *Podaactin1*, *PodaEF1-α* and *Podaubiquitin* sequences, respectively.

Bioinformatics analysis: Prediction of open reading frames (ORF) and amino acid sequence were performed using the NCBI online tool, ORF finder (http://www.ncbi.nlm.nih. gov/gorf/gorf.html). Prediction of conserved sequence and search of homologous protein sequence were carried out using the BLAST X tool of NCBI (https://blast.ncbi.nlm.nih. gov/Blast.cgi?PROGRAM=blastx&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). Physical and chemical properties of protein were predicted employing the online tools Protaram (http://web.expasy.org/protparam/) and Signalp 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). Multiple sequence alignment and analysis were operated using the ClustalX (1.81) software with default parameters. Phylogenetic analysis was performed and phylogenetic tree was established in Neighbor-Joining (NJ) method using the MEGA 6.0 software. Sequences with close phylogenetic relationships of *PodaAux/IAA* gene were referred to while employing the tBLASTp tool again in the *P. trichocarpa* v3.0 database (https://phytozome. jgi.doe.gov/pz/portal.html#!search?show=BLAST) to acquire the reported *PrtroAux/IAA* gene.

Fluorescent Quantitative Real-Time RT-PCR (qRT-PCR): RNA was extracted from roots and leaves using CTAB method (Gehrig *et al*., 2000; Zhang *et al*., 2011). The residual DNA was eliminated from total RNA with DNaseI (Promega). About 1 µg of total RNA was used as template for synthesizing cDNA by reverse transcription in a 10 µL volume using Prime Script RT reagent Rit (TaKaRa) according to the manufacturer's protocol. 90 µL sterile ddH_2O was added into 10 µL synthesized cDNAs and used as templates for qRT-PCR.

A Light Cycler 96 real-time PCR detection system (Roche) and Fast Start Essential DNA Green Master Mix (Roche) were used to perform fluorescent quantitative real-time PCR experiments according to manufacturer's instructions. Three genes, *Podaactin1*, *PodaEF1-α* and *Podaubiquitin* were used as internal reference genes; sequences for primers are given in Table 1. A total of 20 µl was prepared as the reaction volume, containing 5 $\mu mol\cdot L^{-1}$ of the forward and reverse primers respectively and 100 ng of cDNA template. PCR reaction was set as 95°C 10 min followed by 45 cycles of 95°C 5 s, 59°C 15 s and 72°C 10 s. Procedures for melting curves were set as 95°C 10 s, 65°C

60 s and 97°C 1s. Three technical repeats were performed for each biological repeat of samples.

Extraction of IAA: Extraction of IAA was performed according to Zhang *et al*. (2011). Obtained extracts were diluted with methanol to 1 ml and filtered by a 0.45 µm PTFE filter (Waters, Milford, MA, USA) for detection. IAA and IBA were purchased from Sigma-Aldrich Co. Shanghai, China. HPLC-grade formic acid, methanol and acetonitrile were purchased from Merck (Merck Serono Co. Ltd).

LC-MS/MS analyses: Agilent 1100 series HPLC system (Agilent, San Jose, CA, USA) including a G1312A binary pump, a G1379A degasser and a 7725i manual injector were employed for chromatographic analyses. 10 µl of the filtered extracts of each sample was injected into the LC-MS system. For all analyzed extracts, a C18 guard cartridge and Agilent ODS C18 reversed-phase column (150 mm × 4.6 mm, I.D. 5 µm, Phenomenex, USA) were used for chromatographic separation. Mobile phase consisted of methanol (Me) and 0.1% formic acid aqueous solution. Gradient elution program was performed with 0-5 min, 45% (Me); 5-10 min, 45-65% (Me); 10-15 min, 65% (Me); 15-16 min, 45-65% (Me); and 16-20 min, 45% (Me) for separation. Flow rate was 1.0 ml per minute and column temperature was maintained as 25°C.

Detection was run on a triple quadrupole mass spectrometer (API 3000, Applied Biosystems, USA) coupled with electrospray ionization source operating in the negative ion mode, Multiple reaction monitoring (MRM) mode Mass spectrometer was acquired in order to enhance specificity and selectivity of low content IAA in poplar species. Conditions of nebulizing gas and curtain gas were 10 and 12 psi, respectively. The ion source temperature was 300°C. Ion spray voltage, focusing potential and entrance potential were -4500, -375, and -10 V, respectively. The other parameters are summarized in Table 2.

Data analysis: Expression levels of *PodaAUX/IAA* were calculated from threshold cycle by $2^{-\Delta\Delta Ct}$ method according to Livak*e t al.* (Livak *et al*., 2001). In this study Ct represented the fluorescence signal intensity detected by Thermal Cycler; $2^{-\Delta\Delta Ct}$ represented fold change of expression level of *PodaAUX/IAA* in each sample taken after inoculation compared to that of the sample taken at 0 h after inoculation within the same group. The data acquisition and processing of IAA levels detection were analyzed by Analyst software (Version 1.4).

Acquired data were processed using Excel 2007 (Microsoft company, USA), SigmaPlot 12.5 (SystatInc., USA) and SPSS 19.0 (SPSS Inc., IBM company, USA). The correlations between the expression of *PodaAUX/IAA* gene and the concentrations of endogenous IAA were analyzed through Pearson correlation analysis using SPSS 19.0.

Results

Characterization of PodaAux/IAA: The length of the protein coding region of Poda AUX/IAA cDNA is 747 bp and the cDNA sequence was deposited in GenBank under the accession number KP893247. Initiation codon atg was located at 1 bp of gene and termination codon was at 745 bp (Fig. 1A). The bases, which encode 248 amino acids, were composed of 28.2% adenine (A), 24.2% thymine (T), 19.4% cytosine (C) and 28.1% guanine (G) (Fig. 1A). Only one conserved domain, pfam02309, was found in the predicted PodaAUX/IAA protein and it was located between the 13^{th}-240^{th} of the PodaAUX/IAA amino acid sequence (Fig. 1B).

Table 1. Primers used for qRT-PCR analysis.

Gene	Primers	Sequences(5'-3')	Tm/°C	Size of product/bp
PodaAUX/IAA	*AUX/IAA*-Forward	ACCCTATCTTCGCAAGGTGGAC	58.1	221
	AUX/IAA-Reverse	GAACATCACCCACGAGCATCCA	59.0	
Podaactin	*Actin*-Forward	GCTGAGAGATTCCGTTGCCCTG	59.6	204
	Actin-Reverse	GGCGGTGATCTCCTTGCTCATT	59.0	
PodaEF1α	*EF*-Forward	TGGGTCGTGTTGAAACTGGTGT	58.6	212
	EF-Reverse	GGCAGGATCGTCCTTGGAGTTC	59.0	
Podaubiquitin	*UBQ*-Forward	TGTTGTGATCAACGCGAACTCG	58.2	203
	UBQ-Reverse	GAGGATGCCTAGTGCTACGCAT	58.3	

Table 2. Parameters of mass spectra for IAA and IBA.

	MRM (amu)	Declustering potential (V)	Collision energy (V)	Collision export potential (V)
IAA	174.1→130.0	−75	−16	−7
IBA	202.2→116.0	−105	−25	−6

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1   atggcaa cttct gtgct aggta ccgag cgaac tgatt tgaac ttcaag gagac tgagc tgtgt ctcgg attgc ccggt gctgt tggtg tc
    M  A  T  S  V  L  G  T  E  R  T  D  L  N  F  K  E  T  E  L  C  L  G  L  P  G  A  V  G  V
91  aag aatga agttg agaca cctaa taagg ctact gggaa aagag ggttt gctga gactg ttgac ttgaa gctta atctt caggc taaag ac
    K  N  E  V  E  T  P  N  K  A  T  G  K  R  G  F  A  E  T  V  D  L  K  L  N  L  Q  A  K  D
181 ggtgt catgg atctga atgat aatat caaga atatt acttc aaagg acaag aacca ccttc ctgct gctgc catca aggacc ctgct aag
    G  V  M  D  L  N  D  N  I  K  N  I  T  S  K  D  K  N  H  L  P  A  A  A  I  K  D  P  A  K
271 cc gccgg ccaagg cacaa gttgt aggtt ggcca ccagt tcgat cttac aggaa gaacg ttatg gctca gaaga acgcca gcgag gaagg t
    P  P  A  K  A  Q  V  V  G  W  P  P  V  R  S  Y  R  K  N  V  M  A  Q  K  N  A  S  E  E  G
361 gag aaggc aagcac tggcg gcagc agtgc agcat ttgtg aaggt ctgca tggat ggtgc accct atctt cgcaa ggtgga cttga agatg
    E  K  A  S  T  G  G  S  S  A  A  F  V  K  V  C  M  D  G  A  P  Y  L  R  K  V  D  L  K  M
451 ta cagga gctacc aagaa ttatc tgatg ccttg gccaa aatgt tcagt tcctt cacca tgggt aatta tggag cccagg gaatg ataga c
    Y  R  S  Y  Q  E  L  S  D  A  L  A  K  M  F  S  S  F  T  M  G  N  Y  G  A  Q  G  M  I  D
541 ttt atgaa tgagag caagt tgatg gatct actta atagt tccga gtatg tgcca tccta cgaag acaag gatgg tgattg gatgc tcgtg
    F  M  N  E  S  K  L  M  D  L  L  N  S  S  E  Y  V  P  S  Y  E  D  K  D  G  D  W  M  L  V
631 g gtgat gttcc atggg agatg tttgt tgatt catgc aagcg cctgcg cataa tgaaa ggatc tgaag ctgtt ggact tgcac caaga gca
    G  D  V  P  W  E  M  F  V  D  S  C  K  R  L  R  I  M  K  G  S  E  A  V  G  L  A  P  R  A
721 a tggag aaatg caaga gcaga acctga 747
    M  E  K  C  K  S  R  T  *
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Fig. 1. Protein coding sequence of PodaAUX/IAA and prediction of conservative domain
A. Coding sequence, B. Prediction of conservative domain

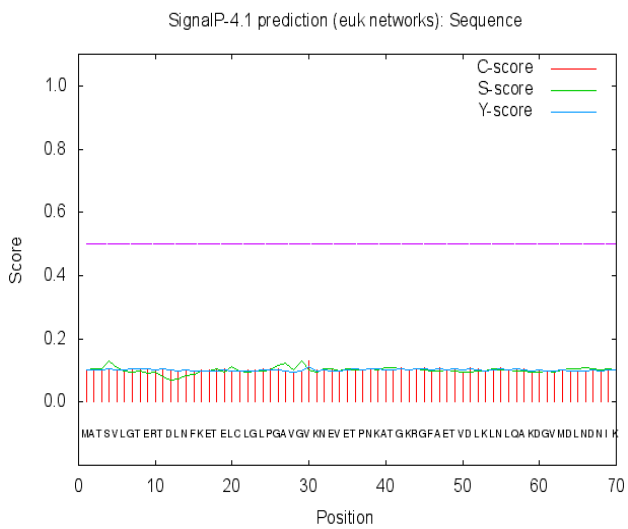

Fig. 2. Signal peptide prediction of PodaAUX/IAA.

Predicted physicochemical property of PodaAUX/IAA: The molecular formula of PodaAUX/IAA protein was predicted as C_{1187} H_{1906} N_{326} O_{369} S_{18}.by using Protaram tools. Amino acids whose frequency of occurrence is more than 5% are lysine, alanine, leucine, serine, glycine, valine, glutamic acid, aspartic acid, methionine and asparagine. The total number of atoms was 3806 and the predicted molecular mass of the protein was 27 kda. Estimated half-life of PodaAUX/IAA was within the range of 10-30 h, within stability index of 39.819 (<40) that belongs to stable protein. Its theoretical pI was found to be 8.21; the aliphatic index was 69.64 and the grand average of hydropathicity (GRAVY) was determined as 0.456, thus showing that PodaAUX/IAA was hydrophilic in nature. No signal peptides on membrane was predicted in PodaAUX/IAA by the Signalp 4.1 Server program; it was a non-secretory cytoplasmic protein and a nucleo protein (Fig. 2).

Sequence alignment andphylogenetic analysis: The search results of bBLASTp by NCBI suggested that query cover of protein sequences was 100%; identity between two AUX/IAA protein sequences, AMB61330.1 of Shanxin poplar and AFZ78617.1 of *P. tomentosa* was 98%. While the identity of AMB61330.1 with two AUX/IAA protein sequences, XP_002311658. 1 and XP_002315736. 1 of *P. trichocarpa* were 96% and 98%. The identity of AMB61330.1 with four AUX/IAA protein sequences, X2 (XP_011027555.1) and X1 (XP_011027545.1) of two auxin-responsive protein IAA14 isoforms and X2 (XP_011030586.1) and X1 (XP_011030585.1) of two auxin-responsive protein IAA14-like isoforms of *P. euphratica* were 96%, 95%, 95% and 96%, respectively. Identity of AMB61330.1 and CAC84710.1, auxin-responsive protein sequence of *P. tremula* × *P. tremuloides*, was 84%. Analysis of multiple sequence alignment showed that all of the nine AUX/IAA protein amino acid sequences of five *Populus* species contained four highly conservative structural domains (Fig. 3). Four conserved domains are underlined as red. Between Domain I and Domain II, the red-frames indicate conserved KR residues which are crucial for protein degradation. The yellow frames signify putative nucleus localization signals; the conserved residues in blue-frames are crucial for protein–protein interaction and the green frame indicates conserved W residue in OsIAA23 is also vital for protein-protein interaction. These results revealed that PodaAUX/IAA (AMB61330.1) is a highly conserved AUX/IAA protein, which is highly identical with proteins of the other eight AUX/IAA proteins (Fig. 3).

To analysis the evolutionary relationships of AUX/IAAs, a phylogenetic tree was constructed based on nine AUX/IAA protein sequences from *Populus*. Phylogenetic tree was analyzed by MEGA6.0 and evolutionary distances were calculated in Poisson correction method. The results displayed that all AUX/IAA proteins could be obviously classified into groups I and II (Fig. 4). PodaAUX/IAA was classified into group I, which included AFZ78617.1, XP_002311658.1, XP_011027555.1, XP_011027545.1, XP_011030586.1 and XP_011030585.1; meanwhile, AFZ78617.1 is the closest relative of AMB61330.1. XP_002315736.1 and CAC84710.1 belonged to group II.

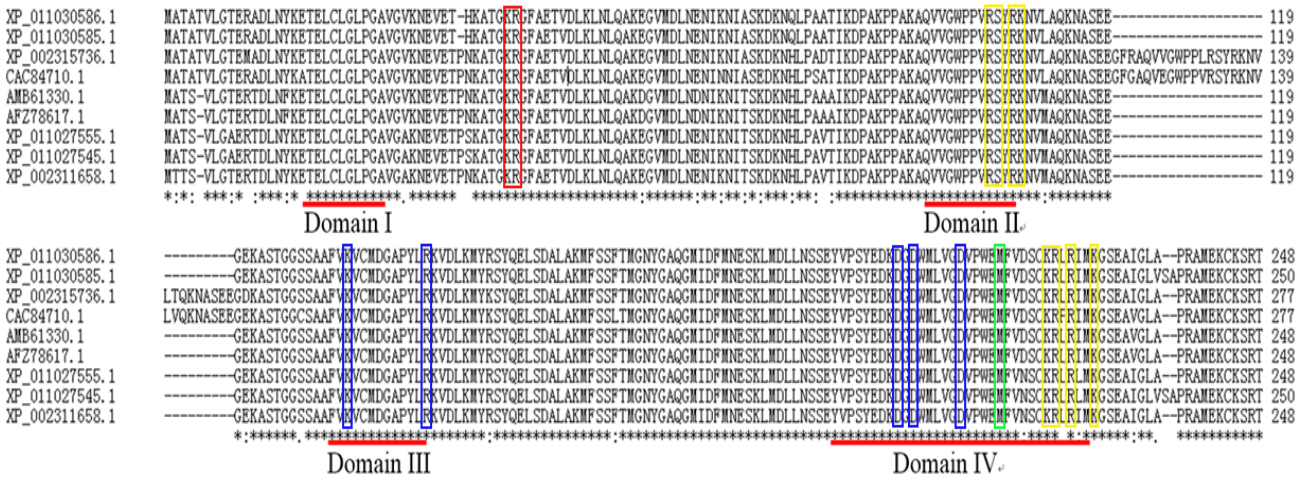

Fig. 3. Nine amino acid sequence alignments of AUX/IAA Protein of Populus species.

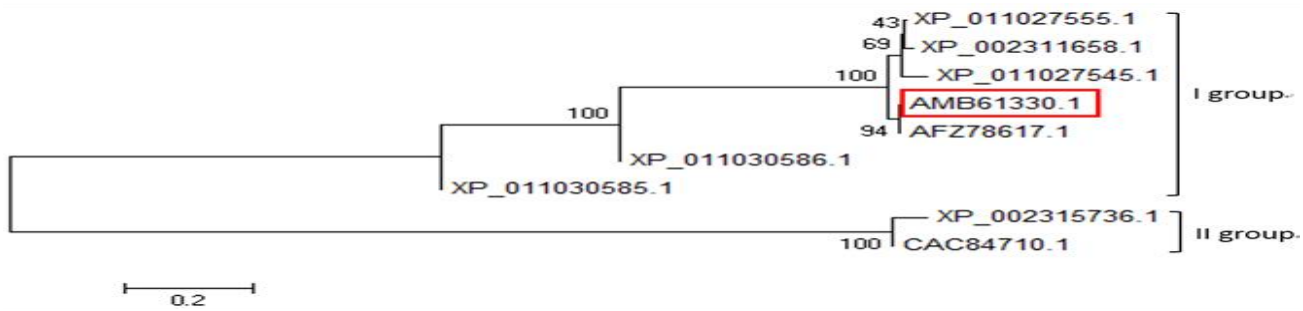

Fig. 4. PodaAUX/IAA Protein homology and the phylogenetic tree
Note: PodaAUX/IAA was marked by red box

TBLASTn was used in aligning of sequences between Shanxin poplar PodaAUX/IAA (AMB61330.1) from group I with AFZ78617.1, XP_002311658.1, XP_011027555.1 and XP_011027545 whose relationship were near with it using *P. trichocarpa* v3.0. The e-value acceptable in the tBlASTn analysis was "1E-30" and the results showed that all of them had the highest consistency with *PtIAA14.1* (estExt_Genewise1_v1. C_LG_VIII2464 *Potri. 008G161200*) mapped on Chromosome VIII, and score values were 160.2, 160.2, 192.6, 187.6 and 188.0, respectively. It is speculated that these 5 *AUX/IAA* genes maybe play a similar regulating role as *PtrIAA14.1*.

Quantitative Real-Time RT-PCR: *Podaactin1*, *PodaEF1-α* and *Podaubiquitin* genes were employed as internal reference genes, and their accession numbers in GenBank were KP973950, KP97395 and KP973952, respectively. The results of qReal-Time RT-PCR suggested that *PodaAUX/IAA* gene was expressed in both roots and leaves of Shanxin poplar. However, there were differences in its expression level when it comes to different tissues, different *Trichoderma* strain treatments or different inducing time.

Expression levels of *PodaAUX/IAA* genes were down-regulated first and then up-regulated in the roots of control group (Con) within 24 h. The expression was induced to 1.39 times, reaching its maximum after 4 h. Expression was then down-regulated to 0.74 at 72 h. Expression levels of the *PodaAUX/IAA* gene were significantly changed by *Trichoderma* treatment. The expression level of *PodaAUX/IAA* of the T1 (Ta492) group was induced to 2.21 times compared to Con over a 0.5 h period ($P< 0.05$). After 4 hours' treatment, the expression level of *PodaAUX/IAA* was down-regulated to 0.26 times compared to Con and then up-regulated to 4.23 times. After 0.5 h of T2 (Ta536) treatment, the expression level of *PodaAUX/IAA* was raised sharply to 1.51 times ($P< 0.05$), then down-regulated; the changes in expression varied from 0.59 to 0.63 times compared to Con at 2, 4 and 24 h. The expression level of *PodaAUX/IAA* was raised again to 2.73 times after 72 hours of treatment ($P< 0.05$). After 0.5 h of T3 treatment (Ta4), the expression level was increased sharply to 2.49 times compared to Con ($P< 0.05$) and then gradually down-regulated and reached a minimum of 0.17 times after 4 hours of treatment. After that the expression levels were up-regulated again, when the treatment reached 24 h and 72 h, the expressions of *PodaAUX/IAA* were raised to 1.68 and 1.08 times compared to Con, respectively (Fig. 5A).

The expression levels of *PodaAUX/IAA* were up-regulated first and then down-regulated in the leaves of Con within 24 h; the expression level was induced to 2.52 times, reaching the maximum at 4 h ($P< 0.05$). The expression level was down-regulated to 0.34, reaching the minimum at 24 h, then again up-regulated to 1.13 at 72 h. The expression levels of *PodaAUX/IAA* were changed by *Trichoderma* treatment. The expression levels were up-regulated, down-regulated and up-regulated again over a 24-hour period of T1 (Ta492) treatment. After 0.5 h of treatment, the expression level was raised sharply to 1.73

times compared to Con ($P< 0.05$) then down-regulated to 0.27 times, reaching this minimum at 4 h. The expression was raised sharply to 2.70 times after 24 h of treatment, then down-regulated to 0.83 times again at 72 h. After 0.5 h of T2 (Ta536) treatment, the expression level was raised to 1.73 times compared to Con ($p< 0.05$), down-regulated to 0.39 times, reaching the minimum at 4 h, then the expression level was down-regulated again. After 24 h and 72 h of treatment, the expression levels of *PodaAUX/IAA* were raised to 3.27 and 1.38 times compared to Con, respectively. In the T3 (Ta4) treatment group, the expression levels of *PodaAUX/IAA* showed a down-regulated trend within 4 h and was down-regulated to 0.25 times compared to Con at 4 h, reaching its minimum; it was then up-regulated to 4.00 times, reaching the maximum, at 24 h ($p< 0.05$). However, the expression levels were down-regulated again at 72 h (Fig. 5B).

IAA content detection: Results of IAA content measured in roots and leaves of Shanxin poplar showed a similar trend of IAA content change between the roots and the leaves. The IAA contents in the leaves were 4-5 times higher than those in roots. The IAA contents in the roots of Con was up-regulated first, then down-regulated and up-regulated again. IAA content reached a maximum of 121 ng/g fresh weight (FW) at 0.5 h and a minimum of 97 ng/g FW at 4 h; after 7 h, the IAA content was reduced to 0.90 times of 0 h, variation between the contents was not distinct. The IAA content in T1 group was reduced to 97 ng/g FW first, which was distinctly lower than Con ($p<0.05$). IAA content was in an 113-131 ng/g FW range, higher than Con during the period of 2 h to 72 h. The IAA content reached a minimum of 93 ng/g FW at 5 h of Ta536 (T2) treatment, significantly lower than Con ($p<0.05$). IAA contents, which were within an 114-126 ng/g FW range, increased compared to Con at 2 h, 4 h, 24 h, 72 h. In T3 (treated with Ta4) group, the contents of IAA fluctuated between 117-131 ng/g FW while maintaining higher than Con, and the IAA content reached its highest (131 ng/g FW) at 4 h, which was significantly higher Con ($p< 0.05$) (Fig. 6a).

IAA content in the leaves of Shanxin poplar (Con) reached its maximum of 525 ng/g FW at 0.5 h, then it started to decrease and was reduced to 397 ng/g FW after 4 h. Besides, at 24 h and 72 h the contents were 1.05 and 1.06 times respectively. IAA content in T1 was decreased to 451 ng/g FW first, which was 0.86 times that of Con and significantly lower than Con ($p< 0.05$). The IAA content was 651 ng/g FW at 2 h, which was significantly higher than Con ($p< 0.05$). Content levels were apparently higher than Con ($p< 0.05$) at 4 h, 24 h and 72 h. In T2, content of IAA was 443 ng/g FW, which was 0.84 times of Con. Then there was an ascendant trend, the content reached 664 ng/g FW, the highest level, at 4 h; it was significantly higher than Con ($p< 0.05$) even at 24 h and 72 h. Under T3 (Ta4) treatment, the content of IAA was significantly higher than Con ($p< 0.05$) except for that content was 1.02 times (531 ng/g FW) of Con at 2 h (Fig. 6b).

Correlation between the *PodaAUX/IAA* expression and IAA levels: To confirm whether the *PodaAUX/IAA* gene expression and endogenous IAA levels were correlated during the period of *Trichoderma* treatment, we analyzed the relationship between the gene expression of *PodaAUX/IAA* and IAA levels by performing Pearson correlation analysis. Results showed that the expressions of *PodaAUX/IAA* were negatively correlated with IAA accumulation in roots and leaves of Con (significant negative correlations were found in leaves, with $p<0.01$), suggesting that *PodaAUX/IAA* gene expression may be regulated by IAA level through negative feedback. The relevant significance among these samples treated by *Trichoderma* was relatively reduced (except T1 group in the roots). It was probably because that the three strains of *Trichoderma* affect IAA content in roots and leaves of poplar saplings differentially (Table 3).

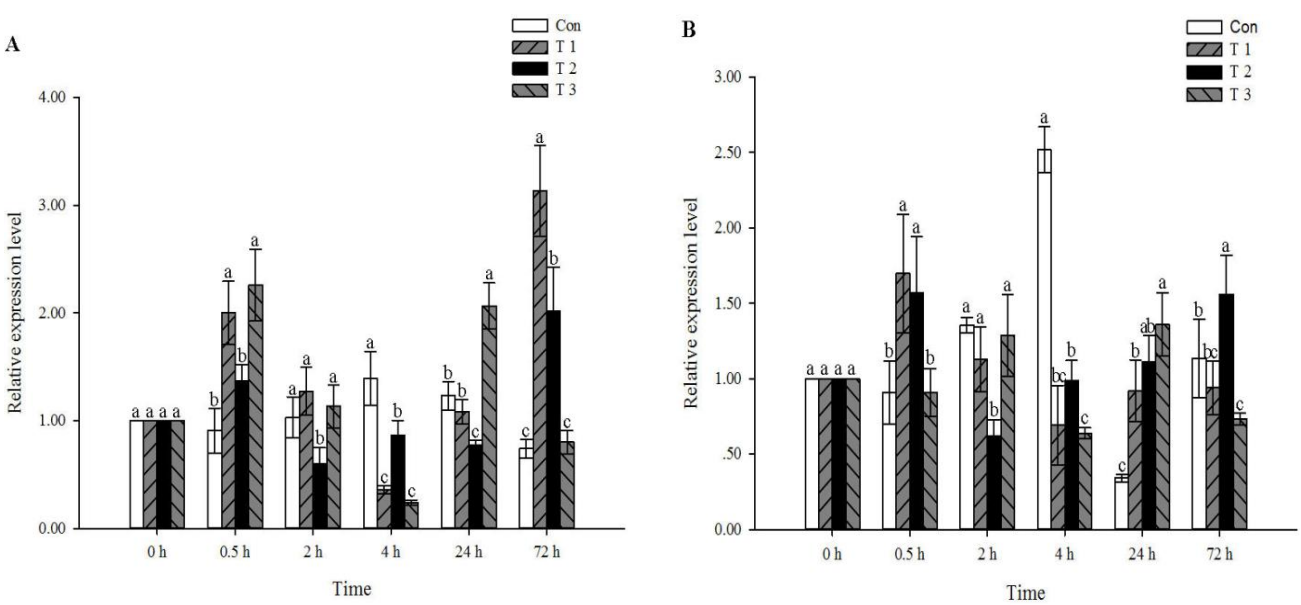

Fig. 5. Expression levels of *PodaAUX/IAA* in the roots and leaves of Shanxin poplar saplings
A in the root; B in the leaf

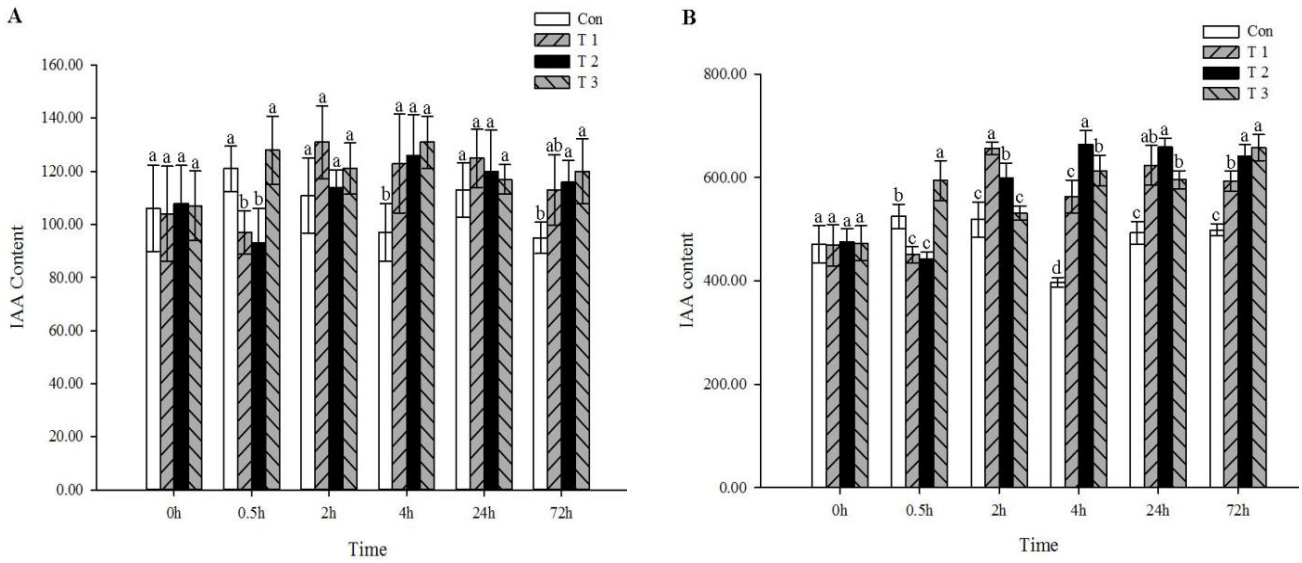

Fig. 6. IAA contents in the roots and leaves of Shanxin poplar saplings
A in the root; B in the leaf

Table 3. Correlation coefficient analysis of *PodaAUX/IAA* expression and IAA levels in the roots and leaves.

Expression levels of AUX/IAA		IAA levels in the roots				IAA levels in the leaves			
		IAA_{CON}	IAA_{T1}	IAA_{T2}	IAA_{T3}	IAA_{CON}	IAA_{T1}	IAA_{T2}	IAA_{T3}
AUX/IAA_{CON}	Pearson	-0.288				-0.656**			
	P value	0.247				0.003			
AUX/IAA_{T1}	Pearson		-0.292				-0.423		
	P value		0.240				0.080		
AUX/IAA_{T2}	Pearson			-0.125				-0.246	
	P value			0.622				0.325	
AUX/IAA_{T3}	Pearson				-0.124				-0.363
	P value				0.623				0.139

**P <0.01, significant

Discussion

It was demonstrated that *Trichoderma*'s colonizing the rhizosphere of host plants could change the host plant's hormone balance in root and leaf tissues and also the expression patterns of related genes thus influencing the biosynthesis and transportation of the hormones that promote growth and improve disease resistance (Contreras-Cornejo *et al*., 2009). For example, *T. harzianum*'scolonizing in the rhizosphere of Muskmelon (*Cucumis melo*) can promote stem growth by making zeatin (Ze), IAA, 1-aminocyclopropane-1-carboxylic acid (ACC) and abscisic acid (ABA), whose contents increased 30%, 37.1%, 32.3% and 120.9%, respectively (Martínez-Medina *et al*., 2011). *T. virens* and secretion of IAA, IAAld and IEt could affect auxin transport or response gene expression in *Arabidopsis* seedlings. Those secretions play an important role in auxin signaling. *T. virens* can also increase root fresh weight and number of lateral roots (Contreras-Cornejo *et al*., 2009). In this study we found that each of the 3 strains of *T. asperellum* (Ta492, Ta536 and Ta4) could increase the level of IAA in the roots and leaves of Shanxin poplar. The test began at 11 a.m, when there was high environmental temperature and light intensity; endogenous IAA level should have been higher in the case of *T. asperellum* inoculation for 0.5 h and 2 h. After being induced by *T. asperellum* for 0.5 h, the levels of IAA in roots and leaves were lower than those of the control group (except T3). It shows that endogenous IAA levels may be decreased in the early treatment stage. Then, as the inducing proceeds, the content of IAA in roots and leaves become significantly higher than the control group at 4 h, in the inoculated groups IAA levels were increased to different extents. Ellasson (Ellasson, 1971) proposed that the root tissue is the synthesis site of IAA. However, plant IAA mainly comes from the young leaves near the apical meristem and it is transported to the other parts of the plant to regulate growth and development. Our results showed that the IAA contents in Shanxin poplar leaves were higher by 4-5 times than roots, which were consistent with the results of Ellasson (1971).

The early auxin-responsive *AUX/IAA* gene encodes a protein that plays a critical role in the auxin signaling pathway and its regulation shown in Fig. 7 (http://www.kegg.jp/keggbin/show_pathway?map=ath040

75&show_description=show). In this study, we cloned a *PodaAUX/IAA* gene encoding an early-stage responsive protein to auxin in Shanxin poplar. Multi-sequence alignment analysis showed that PodaAUX/IAA shared high identity in four conserved domains with eight AUX/IAA proteins in other *Populus* species. The genome of *P. trichocarpa*, which was regarded as a model woody plant, has been sequenced and published (Kalluri *et al*., 2007). Liu *et al*. (2015) found in a research on *PtrIAA14.1* gene function that it restrained auxin responsive gene expression. In *Arabidopsis*, the expression of wild-type *PtrIAA14.1* could change auxin-related phenotypes, resulting in down-curling leaves, greatly reduced fertility and semi-dwarf with increased number of branches. But the expression of the *AtAux/IAA* genes was detected to have changed rarely in the transgenic plants. Furthermore, PtrIAA14.1 interacted with ARF5, not other ARFs. The results of this study suggest that the *PodaAUX/IAA* gene has the highest consistency with *PtrIAA14.1*,amember of *P. trichocarpa* IAA family.*PodaAUX/IAA* was expressed in roots and leaves, but the expression level was different due to distinct tissues, *T. asperellum* strains and induced time. Results of correlation analysis demonstrated a negative correlation between the expression levels of *PodaAUX/IAA* and the IAA levels in both leaves and roots of Shanxin poplar saplings. Compared to the leaves, this negative correlation were less significant in the roots. In addition, compared with Con, the correlation between the expression of *PodaAUX/IAA* and the IAA content was reduced except for the root of the saplings induced by T1.

It is known that *AUX/IAA* is a multi-member family, in different plants, places and times, each member plays various roles in regulating plant growth and development (Han *et al*., 2012; Wu *et al*., 2012). We only cloned one*AUX/IAA* gene in Shanxin poplar. Its expression pattern changed with IAA level, which both changed under the induction of *T. asperellum*. It should be studied in further details the number of *AUX/IAA* genes in Shanxin poplar, where these genes are distributed in the chromosomes, the changes in expression patterns when induced by *Trichoderma* and the interaction between *PodaAUX/IAA* and the *ARF* gene.

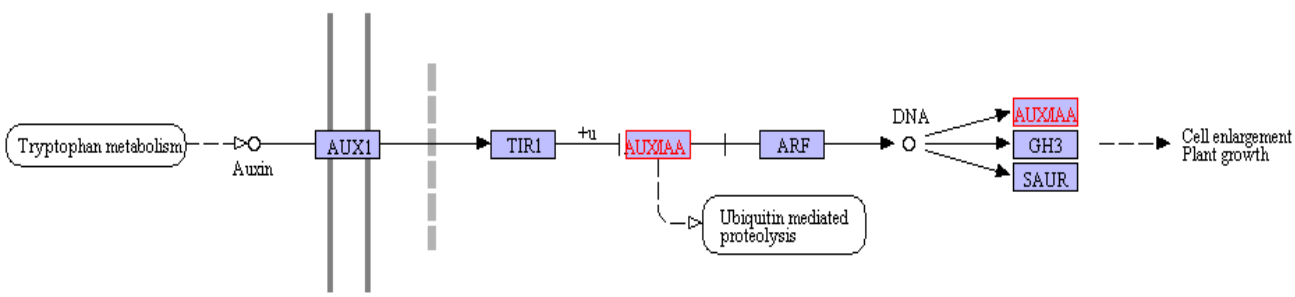

Fig. 7. Plant auxin signal transduction pathway

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