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IMPROVEMENT IN BIOMASS, IAA LEVELS AND AUXIN SIGNALING- RELATED GENE EXPRESSION IN SHANXIN POPLAR SEEDLINGS (*POPULUS DAVIDIANA × P. ALBA VAR. PYRAMIDALIS*) INDUCED BY TRICHODERMA ASPERELLUM

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Abstract

Trichoderma spp. in the rhizosphere has been proposed to considerably increase growth and yield of some plant species. These fungi are potentially involved in changing IAA production via interactions between the host plant and rhizosphere microflora. The aim of present study was to elucidate the direct plant growth promoting activity of live *T. asperellum* ACCC30536 (Ta30536) conidia. The roots and shoots of tissue-cultured poplar (*Populus davidiana* × *P. alba var. pyramidalis*) seedlings were significantly affected by 60 days of treatment with Ta30536 under field conditions, with increases of 30.1% and 20.9% in total fresh and dry weight, respectively, and enhances of moisture content, compared to the control group (Con). Moreover, endogenous IAA in the leaves of poplar seedlings was consistently maintained at higher levels in treated plants than in control, reaching their highest level (1.461 μg/mL FW) before noonon the first day of continuous 15-day Ta30536 treatment. Simultaneously, RT-qPCR analysis showed that the auxin signaling-related genes *PodaARF*, *PodaAUX/IAA*, *PodaGH3* and *PodaSAUR* were differentially expressed in treated seedlings compared to control within 48 h of Ta30536 treatment. In addition, Pearson correlation analysis revealed that the correlation among the expression of these four genes was greatly altered in the treated plants versus control. All differences between treated and Con plants likely resulted from exposure to *T. asperellum*conidia. The results of current study provide valuable insights into the effects of growth stimulation by *T. asperellum*ACCC30536, which likely involve an auxin-related molecular mechanism.

Key words: *Populus davidiana* × *P. alba* var. *pyramidlis*, *Trichoderma asperellum*, Gene expression, IAA; LC-MS/MS, RT-qPCR.

Introduction

Trichoderma spp. are beneficial fungi that are known to penetrate plant roots, colonize the epidermis and outer cortex, and have beneficial effects on plant metabolism, promoting growth, increasing nutrient availability and improving crop productivity (Tripathi *et al.,* 2013; Chagas *et al.,* 2016; Jalali *et al.,* 2017). To date, numerous studies have demonstrated that *Trichoderma* strains significantly increase plant growth, biomass production and yield in plants such as bean (Hoyos-Carvajal *et al.,* 2009; Saima *et al.,* 2013), cucumber (Yedidia *et al.,* 2001), oilseed rape (Maag *et al.,* 2014), tomato (Gravel *et al.,* 2007) and cacao (Tchameni *et al.,* 2011). Some studies have revealed that *Trichoderma* not only enhance the solubilization of soil nutrients (Kashyap *et al.,* 2017), but they also increased the area of soil available to the plant by increasing root length and the number of root hairs available for absorbing nutrients (Zhang *et al.,* 2013). In addition, the growth promoting mechanism underlying *Trichoderma* is thought to be due to increase generation of plant hormones (indole-3-acetic acid [IAA], cytokinin, gibberellins, zeatin and so on) (Gravel *et al.,* 2007; Zhang *et al.,* 2013; López-Coria *et al.,* 2016).

Phytohormones play a vital role in regulating development and defensive responses of plants, as they can transduce signals among plant organs, and integrate them to produce adequate and concerted responses to environmental stimuli (Sofo *et al.,* 2011; Hermosa *et al.,* 2012). Auxin (IAA), a vital morphogenetic signal implicated in the control of cell identity throughout the plant development and growth (Shani *et al.,* 2017; Van *et al.,* 2017). IAA exerts pleiotropic effects on various types of development processes, such as differentiation of organs, initiation of lateral roots, elongation of leaves, embryogenesis and the development of shoots and fruits. Various researchers proposed that *T. atroviride* is able to synthesize IAA and its analog, and found that microbial IAA may be implicated in the growth stimulation of tomato plants via inoculation with *T. atroviride* (Gravel *et al.,* 2007; Mun *et al.,* 2012; Shani *et al.,* 2017). In addition, Sofo *et al.,* (2011) found that inoculation with *T. harzianum* strain T-22 significantly increases the IAA contents in both leaves and roots of cherry, and Martínez-Medina *et al.,* (2011) reported that melon shoots exhibit significant varies in IAA contents in response to inoculation with *T. harzianum*. Furthermore, auxin signaling's vital role in the plant growth promotion induced by *T. virens* was illustrated by Contreras-Cornejo *et al.,* (2009), who found that wild-type *Arabidopsis* seedlings inoculated with *Trichoderma* show the traits auxin-related phenotypes, and mutations of genes implicated in transport or signaling of auxin (including *aux1*, *big*, *eir1* and *axr1*) reduced the growth-promoting and root developmental effects of *T. virens* inoculation.

At present, partial genes involved in auxin signal transduction pathway has been proclaimed on KEGG pathway (http://www.genome.jp/kegg/pathway.html). On this pathway, *ARF*s (auxin transcription factor genes) and *Aux/IAA*s (indole-3-acetic acid-inducible gene) were two crucial related gene families for regulating auxin-modulated genes expression (Tiwari *et al.,* 2003). Most ARFs share conserve *N*-terminal B3-like DNA-binding domain (DBD); conserve C-terminal dimerization domain (CTD) resembling domains III and IV of Aux/IAA proteins, serving for homo- and heterodimerization with other ARFs or Aux/IAA proteins. They also share a changeable middle region (MR) located between DBD and CTD conferring activator or repressor activity (Boer *et al.,* 2014). ARFs, using the DBD, specifically objective to auxin-response elements in promoters of early auxin responsive genes, *viz.*, *Aux/IAA*, *GH3* and the small auxin up RNA (*SAUR*), and heterodimerize with Aux/IAAs in answering auxin stimulus. ARF and Aux/IAA proteins form a fine network with other co-regulators and trigger organism growth and development (Yang *et al.,* 2014; Wu *et al.,* 2017). The IAA-amidosynthetase is encoded by *GH3* (Gretchen Hagen 3) genes, and it helps to maintain homeostasis of auxin by conjugating the excess IAA to amino acids, either for storage or degradation (Peat *et al.,* 2012; Feng *et al.,* 2015). Thus, the expression of *GH3*s can be used to monitor auxin activity (Staswick *et al.,* 2005). *SAUR*s (small auxin up RNA) are major auxin responsive genes that encode extremely unstable mRNAs (Wu *et al.,* 2012). Active auxin can induce their expression within 2-5 min, which demonstrates that important role is played by the auxin in the transcriptional regulation of *SAUR*s (Chen *et al.,* 2014).

Although the contribution of *Trichoderma* strain inoculation to the improvement of plants' growth and development has been observed in many crops and forest species (Gravel *et al.,* 2007; Hoyos-Carvajal *et al.,* 2009; Nawrocka *et al.,* 2018), not many are known about the effect of these fungi on growth promotion and hormonal profiles of poplar seedlings. Shanxin poplar (*P. davidiana* × *P. alba var. pyramidlis*) was an improved poplar variety that was fast growing, cold tolerant and has a narrow crown, was widely used in the plain regions of China for landscaping, sand storm prevention and timber production (Guo *et al.,* 2009). In this study, the expression of auxin-mediated genes *PodaARF1*, *PodaGH3-2*, *PodaAUX/IAA14* and *PodaSAUR30* was analyzed by RT-qPCR in host poplar plants inoculated with *T. asperellum* ACCC30536 (Ta30536). The weights and moisture contents of the roots, shoots and leaves of poplar seedlings in response to Ta30536 treatment were measured. In addition, the endogenous IAA levels in young leaves of poplar seedlings were detected by LC-MS/MS in response to 15 days of Ta30536 treatment. Meanwhile, the expression of auxin-mediated genes *PodaARF1*, *PodaGH3-2*, *PodaAUX/IAA14* and *PodaSAUR30* was analyzed by RT-qPCR in host poplar plants inoculated with Ta30536. The purpose of this study was to know the effects of the microorganism *Trichoderma* on the growth of poplar seedlings and to explore the different expression of auxin-related genes underlying the plant's response to this beneficial microorganism.

Materials and Methods

Preparation of *Trichoderma* strain and its conidia: *T. asperellum* ACCC30536 was used in this study. It was grown on the PDA (Potato Dextrose Agar) medium in Petri Dish at 28°C for 6 days for spore production. Conidia were scraped from the surfaces of colonies and mixed with tap water to obtain conidia solution. The concentration of conidia in the solution was calculated with a hemocytometer. The solution was prepared for pouring into potting soil of potted Shanxin poplar seedlings at a concentration of 5×10^3 cfu/cm^3 soil.

Preparation of tissue-cultured poplar seedlings: Shanxin poplar seedlings were grown through tissue culturing according to patented methods of Liu *et al.,* (2012). The poplar seedlings were subcultured on solidified medium (WPM) (Lloyd & McCown, 1981) supplemented with 6-BA (0.5 mg/L) and NAA (0.1 mg/L). Rooting of seedling was carried out on solidified WPM medium added IBA (0.4 mg/L). When the seedlings reached approximately 10 cm in height, they were transplanted into liquid WPM rooting medium under sterile conditions. After the seedlings developed strong roots, they were dipped in clear plastic cups containing sterilized water for 48 h to remove residual agar on their roots. The poplar seedlings were then transplanted into pots filled with sterile soil collected from croplands around Harbin city, located at 45°44′ N and 126°36′ E of China. After these tissue-cultured seedlings adapted to the soil, they were transported to the nursery garden in summer and grown under field conditions in Harbin.

Randomized block design was used for the experiment and each group (consisting of five replicates) with three replications. The prepared conidia solution was poured into potting soil around the bases of potted seedlings at certain concentration under the natural condition. For the controls, poplar seedlings were treated with same volume of tap water. These plants were watered every day with same volume of tap water and cultured for 12 weeks, including 4 weeks for plants' adaptation to field conditions and 8 weeks for observing the effects of Ta30536 on poplar growth promotion.

Young leaves (the first and second leaf near the shoot tip, which were less than 1 cm long) of poplar seedlings were harvested after *Trichoderma* induction for 1, 2, 4 h, as well as 1, 2, 5, 8, 11 and 15 d, before noon on each day. Liquid nitrogen was immediately used to keep harvested young leaves for the IAA content determination and analysis of differential expression of auxin response genes.

Biomass measurement and sample collection: After 8 weeks of Ta30536 treatment, plants were harvested by removing intact plants from the pots. The roots were washed to clean remaining soil and wiped dry with blotting paper. The fresh weights of roots, shoots and leaves of each plant were measured separately. After measuring the fresh weights, each part of the plant was dried according to the method of Cornelissen *et al.,* (2003) and the dried weights were measured. The moisture content was calculated by the following formula:

Moisture content % = (W_{FW}-W_{DW})/ W_{FW} % (W_{FW}: fresh weight of sample, g; W_{DW}: dry weight of sample, g.)

Sample extraction and LC-MS/MS procedures: Leaves treated by Ta30536 for 15 days were employed for the detection of IAA levels. IAA and IBA (indole-3-butyric acid) standards were purchased from Sigma-Aldrich. Methanol and formic acid [high-performance liquid chromatography (HPLC)-grade] were obtained from Merck. IBA (50 ng/mL), as the internal standard, was arranged in the methanol solution and stored at 4°C for the extraction experiments.

IAA was extracted from the samples employing the standard addition method of Zhang *et al.,* (2011). Then, 10 µL of the obtained extract solution was injected into an LC-MS (Liquid chromatography-tandem mass spectrometry) system to determine the IAA contents.

Mass spectrometer was operated in MRM (multiple reaction monitoring) mode to increase the selectivity and specificity of detection of hormones present at very low concentrations in poplar seedlings. The liquid chromatography (LC), column system and gradient elution profile for IAA detection were as the same described in our previous work Zhang *et al.,* (2011). In each case, IAA content was determined in three parallel samples (biological repeats). Analyst 1.4.0 software was utilized to process the data.

Differential expression analysis of genes implicated in auxin signaling pathway: Genes of *PodaARF1*, *PodaAUX/IAA14*, *PodaGH3-2* and *PodaSAUR30* implicated in auxin signaling pathway were obtained from previous study. These sequences were submitted to GenBank and their accession numbers are given in Table 1. Expression levels of these genes were assayed using leaves treated by Ta30536 for 48 h.

Total RNAs were extracted from young leaves using the cetyltrimethyl ammonium bromide (CTAB) according to method of Zhang *et al.,* (2011) and digested with DNase I (Promega) to remove any residual DNA. Total RNA (used 1 µg) from each sample was reverse-transcribed into cDNA in a 10 μL volume using oligodeoxythymidine according to the PrimeScript RT reagent kit (TaKaRa) protocol. The synthesized cDNAs were diluted to 100 µL with ddH_2O water and utilized as the template for real-time fluorescence quantification PCR (RT-qPCR).

RT-qPCR was performed employing a LightCycler 96 real-time PCR detection system (Roche Co.). Genes for *Podaactin1*, *PodaEF1-α* (elongation factor 1-α) and *Podaubiquitin* were used as the internal controls to normalize the amount of total RNA present in each reaction. Primers utilized for qPCR are given in Table 1. Reaction mixture (20 µL) contained 10 µL of FastStart Essential DNA Green Master Mix (Roche), each forward and reverse primers (0.5 µM) and cDNA template (2 µL, equivalent to 100 ng of total RNA). Amplification was set as follows: 95°C for 600 s for preincubation followed by 45 cycles at 95°C for 5 s, 59°C for 15 s, 72°C for 10 s and 95°C for 10 s, 65°C for 60 s and 97°C for 1 s for melting. To ensure reproducibility of our results, all the experiments were performed with three biological repeats (each biological repeat performed with three technical repeats). Expression level of each gene was calculated from the threshold cycle according to $2^{-\Delta\Delta CT}$ method Wu *et al.,* (2012).

Statistical analysis: Data of correlation among the expression of four genes examined were analyzed by Pearson correlation analysis employing SPSS 19.0 (SPSS Inc., IBM Company, USA). Data presented in the Figures were means ± standard deviation (SD) of replications.

Results

Biomass yield of *Populus*: The measured results revealed that the mean root FW of *Trichoderma* treated group (T) was 17.42 g, which was 47.50% higher than that of control group. The mean shoot FW of the T group was 8.28 g, which was increased by 18.12% compared to control, while the mean leaf FW of the T was 8.73 g, which was only 14.27% higher than control (Table 2). One the other hand, the mean root DW of T was 6.69 g, increasing by 31.95% compared to control. Meanwhile, the mean shoot DW of T was 4.29 g, which was 16.26% higher than that of Con (Table 2). However, mean leaf DW values of the two groups were approximately equal; it could be due to insect damage and leaves senescence under field conditions. The calculated moisture contents of poplar seedlings were increased by 7.9, 1.7 and 3.5% in roots, shoot and leaves, respectively. Ta30536 clearly improved the moisture contents in various tissues of Shanxin poplar seedlings. The results demonstrated that Ta30536 promoted the growth of poplar seedlings in our experiments.

Changes in endogenous IAA levels during 15 days: From LC-MS/MS analysis, negative ion MS/MS spectra of IAA and IS is given in Fig. 1a and b. And under MRM scan mode, typical LC-MS/MS chromatogram of a mixed standard solution is given in Fig. 1c and d, which present better peak symmetry and sharper peak shape. Retention times of IAA and IS were 7.25 ± 0.04 and 12.10 ± 0.03 min, respectively. Calibration curve was constructed as $Y = 0.4137x + 2.7341$ (Y: AU_{IAA}/AU_{IS}; x: C_{IAA}/C_{IS}; AU: absorbance unit; C: concentration of solution), $R^2 = 0.9989$. Superior linearity was discovered in the range of 16-48,600 ng/ml. Quantity of IAA presented in poplar samples from different time points was calculated using following equation: $M_{IAA} = (C_{IAA} * V_{sample})/W_{sample}$ (M_{IAA}: amount of IAA in sample, ng/g FW; C_{IAA}: IAA concentration in sample, ng/mL; V_{sample}: sample volume, mL; W_{sample}: fresh weight of sample, g).

To investigate the effects of Ta30536 on the endogenous IAA levels in the leaves of vigorous poplar seedlings, the IAA concentrations of the T-group were detected and compared to that of control. The endogenous IAA levels in the leaves of T and control poplar seedlings (Con) were detected continuously for 15 days (Fig. 2). During the interval from 11:00 to 14:00 of the first day after Ta30536 treatment, the endogenous IAA levels in the seedling leaves of T showed an increasing trend compared with that of Con-group. By 14:00, the IAA levels in T-group seedling leaves increased by 59.07% compared to that of Con. Meanwhile, the IAA concentration reached its highest level (1.456 µg/mL FW) at the time point after treated 24 h of the 15-day induction. Furthermore, the IAA levels were throughout higher in T-group than in Con (Fig. 2).

Table 1. Primers of genes used for RT-qPCR analysis.

Gene	GenBank accession number	Primers	GC%	Tm/°C	Size of product /Bp
ARF1	KM113035.1	*F*---TGCCATTGCGACTGGAACCC	60	59	229
		R---CCACTCAGAATCAGCCCATCCC	59.1	58.8	
AUX/IAA14	KP165071	*F*---ACCCTATCTTCGCAAGGTGGAC	54.5	58.1	221
		R---GAACATCACCCACGAGCATCCA	54.5	59	
GH3-2	KP893243	*F*---CCAGCCAACTCTCCTAGTGACG	59.1	58.5	214
		R---TTGACGCACCTTGGAACCTTGT	50	59	
SAUR30	KP893248	*F*---ACTACCTCAAACAGCCGTCCTT	50	57.6	211
		R---TTCCTCTTCTGCTCGTTGAAGC	50	57	
actin1	KP973950	*F*---GCTGAGAGATTCCGTTGCCCTG	59.1	59.6	204
		R---GGCGGTGATCTCCTTGCTCATT	54.5	59	
EF1-α	KP973951	*F*---TGGGTCGTGTTGAAACTGGTGT	50	58.6	212
		R--- GGCAGGATCGTCCTTGGAGTTC	59.1	59	
ubiquitin	KP973952	*F*---TGTTGTGATCAACGCGAACTCG	50	58.2	203
		R---GAGGATGCCTAGTGCTACGCAT	54.5	58.3	

Table 2. The mean FW[a], DW[b] and moisture contents of different seedling tissues.

Samples	Control			*Trichoderma* treatment		
	FW (g)	DW (g)	Moisture contents (%)	FW (g)	DW (g)	Moisture contents (%)
Root	11.81 ± 2.89b	5.07 ± 0.95b	0.571 ± 0.02b	17.42 ± 3.42a	6.69 ± 1.17a	0.616 ± 0.01a
Shoot	7.01 ± 1.56b	3.69 ± 0.93b	0.474 ± 0.02a	8.28 ± 1.29a	4.29 ± 0.79a	0.482 ± 0.02a
Leaf	7.64 ± 1.86b	3.25 ± 0.52b	0.575 ± 0.04a	8.73 ± 2.13a	3.54 ± 0.87a	0.595 ± 0.03a
Total	26.46	12.01		34.43	14.52	

[a]FW: Fresh weight; [b]DW: Dry weight

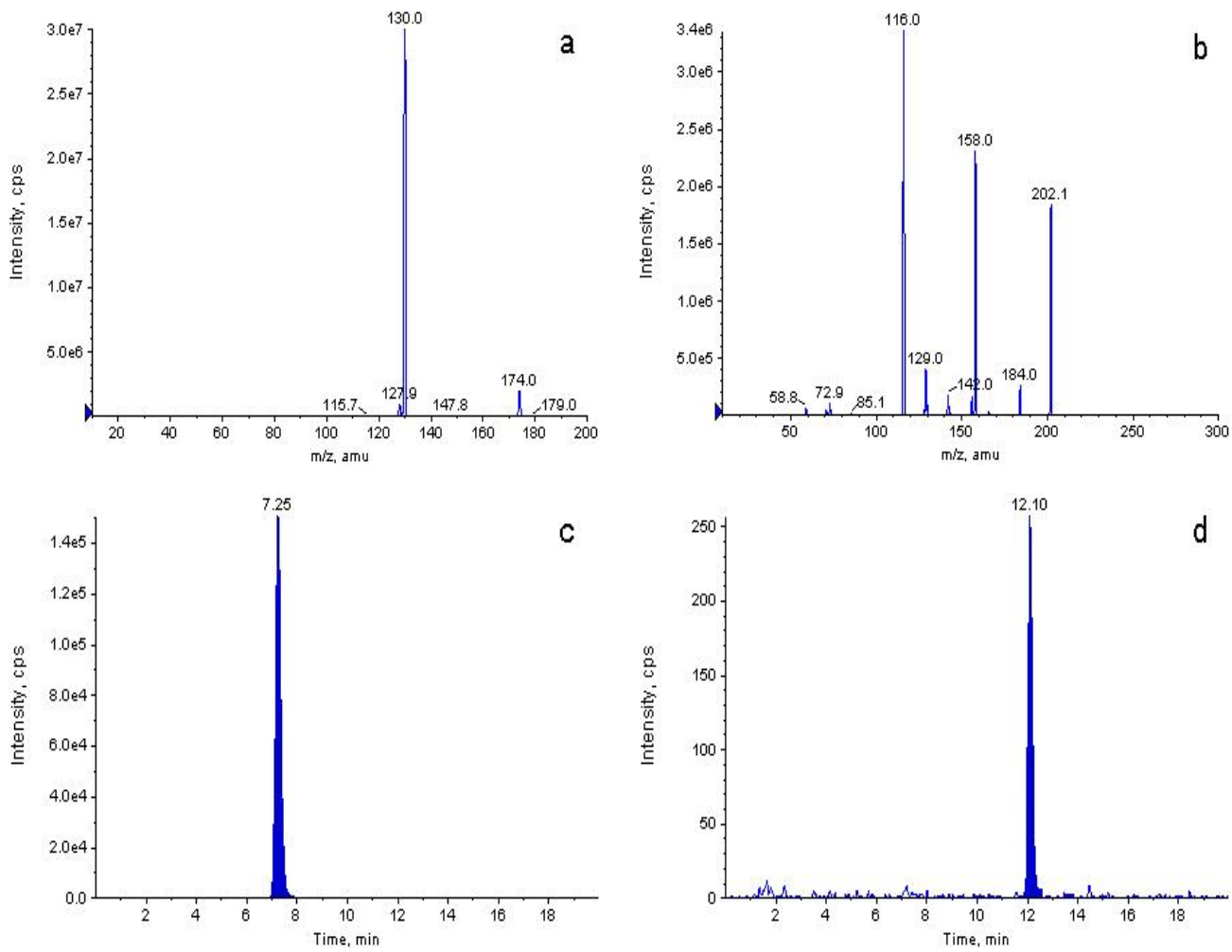

Fig. 1. Negative ion MS/MS spectra from a single LC–MS/MS analysis and representative LC-MS/MS chromatogram of a mixed standard solution containing IAA and IS. a, c IAA; b, d IS.

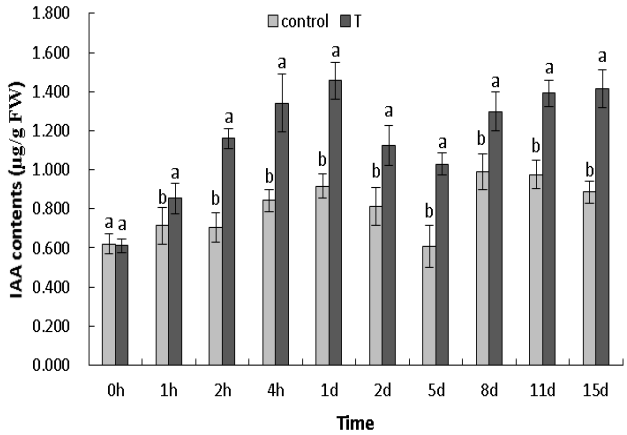

Fig. 2. Time-course of changes in IAA levels in plants by Ta-treating for 15 d.

Differential expression analysis of genes implicated in auxin signaling pathway: To investigate the expression variation of genes implicated in auxin signaling pathway after Ta30536 treatment, transcription of 4 genes (*PodaARF1*, *PodaGH3-2*, *PodaAux/IAA14* and *PodaSAUR30*) were analyzed by RT-qPCR. Results demonstrated that expression levels of *PodaARF1* of T were upregulated to 1.44- and 1.71-fold of control at 1 and 4 h, respectively. Maximum expression level of *PodaARF1* of T was 1.97-fold of control at 24 h. Then expression of *PodaARF1* was decreased to 1.03-fold of control at 48 h (Fig. 3a). Not alike the expression levels of *PodaARF1*, the expression of *PodaAux/IAA14* of T firstly was downregulated to 0.89-fold of control at 1 hour, then displayed rapid upregulation to 1.25- and 1.52-fold of control at 4 and 24 h, respectively. Expression of this gene was again downregulated, reaching 1.12-fold of control at 48 hours (Fig. 3b). Meanwhile, expression levels of *PodaGH3-2* was consistently upregulated during the 48 h time period. However, the changed folds of *PodaGH3-2* were significantly different, which were 1.62-, 1.89-, 1.91- and 2.10-fold of control at 1, 4, 24 and 48 h, respectively (Fig. 3c). Besides, the expression level of *PodaSAUR30* was significant upregulated by Ta30536 treatment at 1 h (3.98-fold of control). This *PodaSAUR30* gene was rapidly downregulated to only 0.18-fold of control at 4 h. However, this gene was upregulated again at 24 h, with 2.46-fold expression compared to Con-group. Subsequently, its expression in T plants was 2.16-fold to that of Con (Fig. 3d).

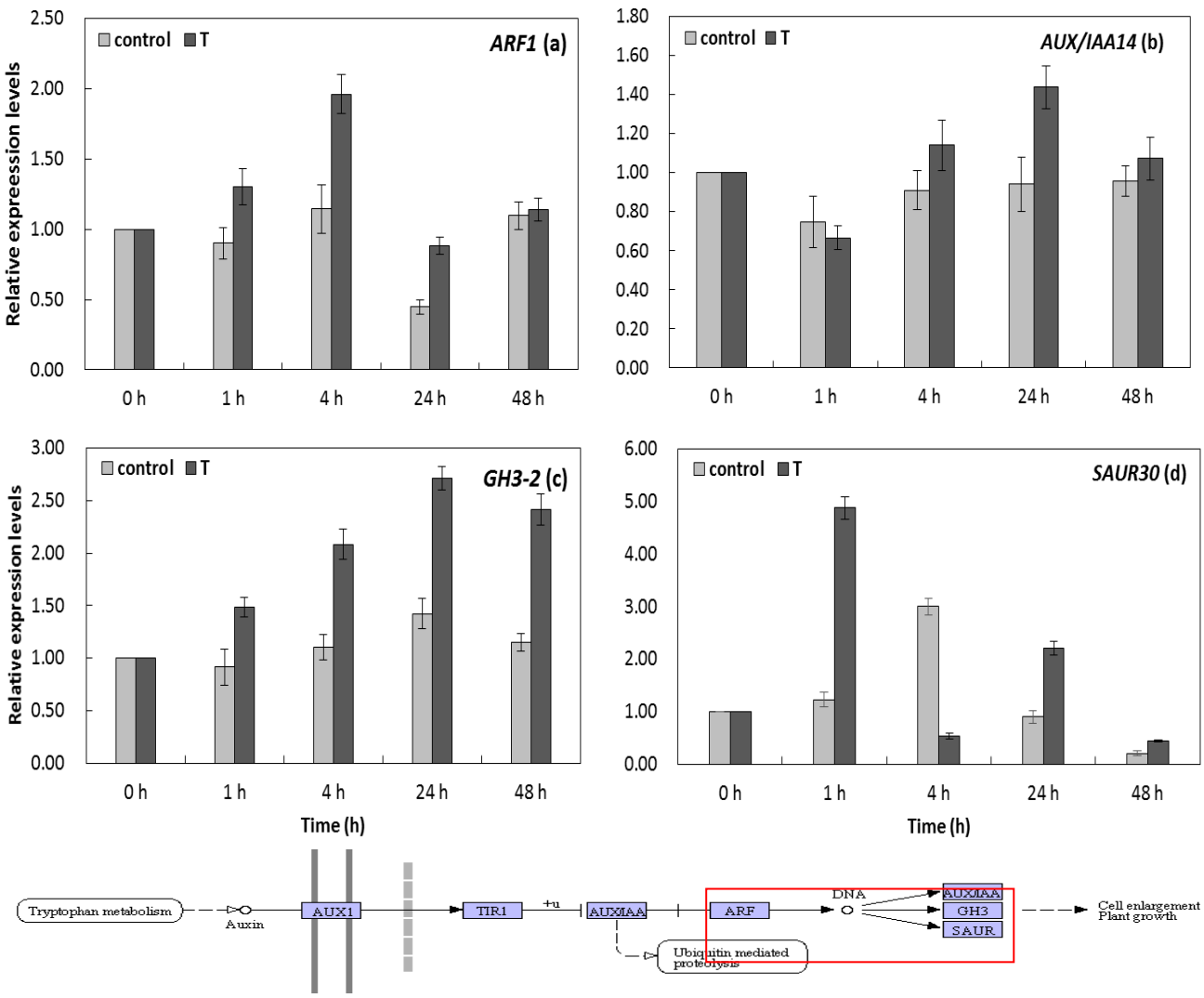

Fig. 3. Differential expression of four genes during a 48-h period and auxin signal transduction pathway (http://www.genome.jp/kegg/pathway.html). X-axis, time points; Y-axis, relative expression level, i.e., expression level of treatment/expression level at 0 h. T, treatment group. The part of our research is marked by red box in the pathway.

Table 3. Pearson correlation analysis for IAA levels and gene expression of the two test groups.

		Pearson coefficient			*SAUR*	*ARF*	*P* value		
		ARF	***AUX/IAA***	***GH3***			***AUX/IAA***	***GH3***	***SAUR***
Control	IAA	-0.384	0.159	0.630**	0.118	0.079	0.285	0.006	0.337
	ARF		0.123	-0.625**	0.288		0.331	0.006	0.149
	AUX/IAA			0.144	-0.127			0.305	0.326
	GH3				-0.176				0.265
T	IAA	0.274	0.686**	0.898**	-0.210	0.187	0.002	0.000	0.227
	ARF		-0.137	-0.042	-0.163		0.313	0.440	0.281
	AUX/IAA			0.651**	-0.516*			0.004	0.025
	GH3				-0.225				0.211

**Correlation is significant at 0.01 level (1-tailed). *Correlation is significant at 0.05 level (1-tailed)

Correlation analysis of IAA contents and expression levels of four genes implicated in auxin signal pathway: Relationship among the endogenous IAA level with expression levels of 4 genes implicated in auxin signaling pathway were determined by performing Pearson correlation analysis (Table 3). Firstly, in the Con-group, the analysis result displayed that a negative correlation of endogenous IAA levels with the expression folds of *PodaARF1* gene and positive correlation of IAA levels with the expression levels of *PodaAUX/IAA14*, *PodaGH3-2* and *PodaSAUR30*. However, the correlation of IAA levels with the expression folds of the 4 genes had altered significantly under the treatment of Ta30536. IAA levels showed a significant positive correlation with the expression of *PodaARF1*, *PodaAUX/IAA14* and *PodaGH3-2*, with the *P* values were 0.187, 0.002 ($p<0.01$) and 0.000 ($p<0.01$) respectively. Correlation of IAA levels with the expression levels of *PodaSAUR30* altered to the negative correlation. Meanwhile, significant positive correlation was exhibited between expression levels of *PodaAUX/IAA14* and *PodaGH3-2* in the T-groups, with the Pearson coefficient of 0.651 ($p<0.01$). It revealed that increased IAA contents inducing by Ta30536 triggered the changes of the expression of 4 genes implicated in the auxin signaling.

Discussion

Trichoderma species have been successfully used as the potential biological control agents against certain phytopathogenic fungi. Several strains of *Trichoderma* have the ability to enhance root growth and plant development, because they are potentially involved in changes in IAA production caused by interaction between host plant and rhizosphere microflora (Sharma *et al.,* 2003; Gravel *et al.,* 2007; Kashyap *et al.,* 2017). In the present study, Shanxin poplar seedlings growing under field conditions were used to find the mechanism of *T. asperellum* ACCC30536 promoting tree growth and development.

To obtain experimental materials with identical genetic backgrounds, tissue-cultured poplar seedlings were used in the present study. Results revealed that Ta30536 significantly affects the growth of poplar seedlings. In particular, fresh and dry weights of plant roots under Ta30536 treatment significantly increased (by 47.50% and 31.95%, respectively) compared to control after 60 days of growth in potting soil containing Ta30536. Root system is crucial for the fitness of plants as it provides anchorage, also helps to water use effectiveness and makes possible acquisition of mineral nutrients from soil (Contreras-Cornejo *et al.,* 2009). Results of the study also displayed that the moisture contents of plant roots and leaves under Ta30536 treatment increased by 7.9 and 3.5%, respectively, compared with Con, which suggests that Ta30536 may improve water absorption by increasing number of roots in poplar plants.

In this work, the temporal changes in endogenous IAA levels were examined in young leaves of poplar seedlings by LC-MS/MS. The results reveal significant changes in IAA levels in plants under treatment with Ta30536 fungus. The IAA levels rapidly increased during the 4 hours period after Ta30536 treatment compared to the untreated group. Moreover, the treated plants consistently maintained higher levels of IAA at 11:00 a.m. throughout the 15-day treatment period. IAA is the most important natural auxin, which is implicated in initiation and emergence of lateral and adventitious root, as well as development of shoot, through regulating various processes including cell division, expansion and differentiation (Wan *et al.,* 2006; Zhang *et al.,* 2013). The increased biomass and IAA production of poplar seedlings indicate that the growth promotion activity of Ta30536 may be mediated through the IAA biosynthetic and signal transduction pathway. Our data are consistent with those of Contreras-Cornejo *et al.,* (2009) and Martínez-Medina *et al.,* (2011), who detected increased production of biomass and endogenous IAA in the tissues of melon and *Arabidopsis* in response to inoculation with *Trichoderma* spp.

Several genes related to auxin signal transduction pathways, including *PodaARF1*, *PodaGH3-2*, *PodaAUX/IAA14* and *PodaSAUR30*, were cloned and their different expression levels were analyzed by RT-qPCR in the current study. Numerous researches have suggested that *Aux/IAA* genes play a central role in the signaling of auxin. *Aux/IAA* encode short-lived nuclear proteins that act as repressors of auxin-regulated transcriptional activation and regulate auxin-mediated gene expression by controlling the activity of ARFs (Woodward & Bartel, 2005; Zouine *et al.,* 2014). Our results demonstrated that there was significant positive correlation between *PodaAUX/IAA14* expression and IAA levels of treated group, which means IAA levels had influence on the expression of *PodaAUX/IAA14*. However, expression levels of *PodaAUX/IAA14* was firstly downregulated at 1 hour, then rapidly upregulated after 4 hours of Ta30536 treatment, but expression levels of *PodaARF1* was gradually upregulated at the same time. Reed (2001) suggested that the activity of ARFs was negatively regulated by heterodimerization with Aux/IAA proteins. *PodaAUX/IAA14* gene expression showed

instant decrease possibly due to its heterodimerization with *PodbARF1* or with the other *PodbARF*s, however the molecular specificity that caused the expression alteration of *PodaARF1* and *PodaAUX/IAA14* at this time point needs further verification in the future experiments. Molecular genetics and biochemical studies indicated that *AtARF1* in *Arabidopsis* acted in a partially redundant manner with *AtARF2*, *arf1* mutations enhanced many *arf2* phenotypes. In the flowers of *Arabidopsis arf1* mutants the transcription of Aux/IAA genes increased, thus showed that the *ARF1* acted as a transcriptional repressor (Ellis *et al.,* 2005). Our result showed that expression levels of *PodaARF1* was all negative correlation with that of *PodaAUX/IAA14* and *PodaGH3-2* of Ta30536 treated group by performing Pearson correlation analysis.

Fukaki *et al.,* (2002; 2006) found that *SOLITARY-ROOT/IAA14* gene in *Arabidosis* encodes a key nucleus protein as transduction regulator in auxin-regulated growth and development by repression *AtARF7* and *AtARF19*, particularly in lateral root formation of *Arabidopsis*. Higher expression of *PodaAUX/IAA14* after 4 hours treatment was probably related to the enhanced dry weights of the roots of *Populus*. In addition, the expression levels of *PodaAUX/IAA14* and *PodaGH3-2* were significantly positive correlated in the study. *PodaGH3-2* gene also had positive correlation with IAA levels assayed by using Pearson correlation analysis, which could have been caused by its function. Staswick *et al.,* (2005) proposed that *AtGH3.2* in *Arabidopsis* implicated in maintaining auxin homeostasis by conjugating excess IAA to amino acids, either for storage or degradation. Expression levels of *PodaGH3-2* in our study was significantly changed and consistently upregulated bit by bit in keeping with the increased IAA levels in the samples treated for 48 h with Ta30536. Besides, several findings have suggested that *SAUR* genes encode short-lived proteins, which may exert a role in auxin-mediated cell elongation (Hagen & Guilfoyle, 2002; Knauss *et al.,* 2003). Results of the rapidly upregulated or downregulated expression of *PodaSAUR30* during 48 h of the treatment group also revealed that *PodaSAUR30* gene responded the inducing of Ta30536 and caused changes in expression. The auxin response transcription factor and three early auxin responsive family genes all exhibited concerted responses to Ta30536 treatment that accompanied the increase in IAA levels and enhanced biomass yields.

In conclusion, our results suggest that *T. asperellum* ACCC30536 significantly promote the growth and development of tissue-cultured poplar seedlings under field conditions. Higher fresh weights, dry weights and moisture contents in the roots, shoots and leaves of poplar seedlings were achieved in our experiments, which likely resulted from higher IAA levels stimulation by Ta30536. The results provide valuable insights to help to clarify the effects of growth promotion from *T. asperellum* ACCC30536, probably through an auxin-related molecular mechanism. It will be important to further the specific mechanisms related to growth promotion by *T. asperellum*, and to better understand the close interaction between the host plant and microorganisms.

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Conflict of interest: All authors declare that they have no conflict of interest.

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