Pak. J. Bot., 51(2): 689-697, 2019. DOI: 10.30848/PJB2019-2(34)

TRICHODERMA ASPEREULLM ACCC30536 INOCULATION DIFFERENTLY REGULATES THE TIME-COURSE EXPRESSION OF FIVE INDOLE-3-ACETIC ACID AMIDO SYNTHETASE GENES AND THE LEVELS OF IAA, SA AND JA IN *POPULUS DAVIDIANA* × *P. ALBA* VAR. *PYRAMIDALIS*

TONGTONG ZHAI[1Θ], YUEFENG WANG[2Θ], ABDUL MAJEED BALOCH[1], ABDUL WAHID BALOCH[3], ZHAOYING LIU[1], CHUANYING JIANG[1] AND RONGSHU ZHANGR[1,4*]

[1]College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China;
[2]Photosynthesis Research Center, Key Laboratory of Photobiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China;
[3]Department of Plant Breeding and Genetics, Sindh Agriculture University Tandojam, Pakistan;
[4]State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, No. 2, Hexing Road, Xiangfang, Harbin, Heilongjiang 150040, China
[*]Corresponding author's email: zrs_nefu@163.com
[Θ]Authors contributed equally to this work

Abstract

Some indole-3-acetic acid amido synthetases (GH3 proteins) were confirmed to catalyze the connection between IAA, SA or JA with amino acid to regulate hormone dynamic balances in promoting plant growth; they can also improve plants' resistance to stress through activating SA, JA and ethylene signaling pathways. *Trichoderma* spp. are widely-known beneficial biocontrol agents. However, the expression pattern of plant *GH3* genes induced by *Trichoderma* remained unexplored. In this study, five *GH3* genes in *Populus davidiana* × *P. alba* var. *pyramidalis* (named *PodaGH3-1*, *PodaGH3-2*, *PodaGH3-5*, *PodaGH3-9* and *PodaGH3-10*) were cloned. Their characteristic analysis showed that the five *PodaGH3s* genes were highly conservative and closely related at the residues linked to ATP binding with IAA or JA, and their expression levels changed obviously under *Trichoderma* inoculation. Moreover, IAA, JA and SA contents showed a fluctuating yet increasing trend during the 72 hours when poplar seedling rhizosphere was colonized by *Trichoderma*. In summary, all the five *PodaGH3s* could be activated by the interaction between biotrophic *Trichoderma* and poplar seedlings.

Key words: *Populus davidiana* × *P. alba* var. *pyramidalis*, indole-3-acetic acid amido synthetase, RT-qPCR, *Trichoderma aspereullm.*

Introduction

P. davidiana × *P. alba* var. *pyramidalis* (Poda, Shanxin poplar) is an ideal landscape tree species in northern China. It is a female-sterile cultivar that does not produce airborne catkin pollutant since its infructescence hardly falls off in spring. Shanxin poplar grows fast and has narrow crown, making it an excellent tree choice for urban/rural greening, windbreak afforestation and timber production, which brings significant economic and ecological value. *Trichoderma* spp. are widely-known as beneficial fungi and biocontrol agents (Faisal *et al*., 2017). Studies suggest that *Trichoderma* colonization in the host plants' rhizosphere could promote growth and improve disease resistance through influencing the biosynthesis and transport of plant hormones (Sofo *et al*., 2011).

The plant hormone IAA (Indole-3-acetic acid) can modulate plant growth, including plant apical dominance, root formation, adversity stress adaptation and physiological aging process, by means of inducing faster and higher expression of primary response genes (Hagen & Guilfoyle, 2002). SA (Salicylic acid) is a signal molecule of plant defense reactions to abiotic stresses (Farag *et al*., 2017). JA (Jasmonic acid) is one of the key signal molecules inducing resistance responses of plants. Both of them play significant roles in regulating plant growth (Thaler & Bostock, 2004; Hohmann *et al*., 2017).

Previous studies demonstrated that *T. harzianum* colonized in *Cucumis melo* rhizosphere caused the contents of ZT (Zeatin), IAA, ACC (1-aminocyclopropane-1-carboxylic acid) and ABA (abscisic acid) in stem to increase by 30%, 37.1%, 32.3% and 120.9% respectively, thus promoting the growth of stems (Martínez-Medina *et al*., 2011). Previous researches have shown that concentrations of IAA and JA increased significantly, together with a significantly lower concentration of SA in the roots of tomato after treated with *T. harzianum* (Fiorini *et al*., 2015). The expression of the genes in SA and JA signal transduction pathways was up-regulated in varying degrees in *Nicotiana tabacum* induced by peptaibols purified from *T. pseudokoningii* (Luo *et al*., 2010). *T. virens* and its secretion of IAA, IAAld (indole-3-acetaldehyde) and IEt (indole-3-ethanohad) had effected on gene expression regarding auxin transport/response in *A. thaliana* seedlings (Contreras-Cornejo *et al*., 2009).

According to protein amino acid sequence, auxin primary response genes can be divided into three main categories: *GH3* (Gretchen Hagen 3), *SAUR* (small auxin up RNA) and *Aux/IAA* (Aelel & Theologis, 1996). *GH3* gene, a kind of auxin early response gene family, was found in soybeans (*Glycine max*) first in 1987 (Wright *et al*., 1987), then discovered gradually in *Oryza sativa* (Jain *et al*., 2006), *A. thaliana* (Staswick *et al*., 2005), *P. trichocarpa* and other plants. *A. thaliana* has 19 *GH3* family members that can be divided into three subclasses by the function and sequence similarity (Staswick *et al*., 2005), proteins encoded by some genes in 1st and 2nd subclass were involved in light signaling pathway. Moreover, some proteins can activate SA, JA and ethylene signaling pathways through catalyzing the connection of

plant hormones (IAA, SA and JA) with amino acid, thus regulate the dynamic balance of hormones and promote adversity resistance in plant (Zhang *et al*., 2007; Nobuta *et al*., 2007). Therefore, it is necessary to study the regulation of GH3 genes during plant growth and development under the induction of *Trichoderma*.

In order to study the expression of *GH3* genes in plants induced by *Trichoderma* and further reveal the mechanism of how *Trichoderma* promotes the growth and development of woody plant, five *GH3* genes were cloned in Shanxin poplar and their expression patterns under the induction of *Trichoderma* were analyzed. Furthermore, contents of IAA, SA and JA in the leaves and roots of the induced poplar seedlings were detected to analyze the correlation between IAA, SA and JA levels and the differential expressions of five *GH3* genes.

Materials and Methods

Strains and plant materials: *T. asperellum* ACCC30536 was obtained from the Agricultural Culture Collection of China. The concentration of water suspension of *T. asperellum* conidia was prepared as 5×10^5 cfu/mL for inoculation. In this study, tissue cultured Shanxin poplar seedlings, which have similar growth status with similar height (10-12 cm) and similar number of leaves (8-9 leaves), were rooted for 12 days in liquid woody plant medium (WPM) at 24ºC. Then, poplar seedlings which had rooted were placed in *T. asperellum* conidia water suspension for 0, 2, 6, 12, 24, 48 and 72 hours under sterile condition. Meanwhile, sterile water was used as mock inoculum to treat the control (CK) seedlings. Each group (treatment) contained ten plants as biological repeats and three technical repeats were performed. The leaves and roots of the treated seedlings were collected respectively and immediately frozen in liquid nitrogen for RNA extraction and IAA, SA and JA level detections.

Cloning of five *PodaGH3* genes in *P. davidiana* × *P. alba* var. *Pyramidalis*: The full-length cDNA sequences of five *PodaGH3* genes were acquired from a transcriptome database of leaves, stems and roots of Shanxin poplar established previously (unpublished), in accordance with BLAST search results. The primers used for RT-PCR are in Table 1. The PCR conditions were 94ºC for 3 min; 35 cycles of 94ºC for 30 sec (for different *GH3* genes) and 72ºC for 120 sec; then 72ºC for 7 min. The amplified fragments were purified and ligated in the vector pGEM-T (Promaga) following the manufacturer's instructions and sequenced (Shanghai Sangon Co., China). Five *PodaGH3* cDNA complete sequences (named *PodaGH3-1*, *PodaGH3-2*, *PodaGH3-5*, *PodaGH3-9* and *PodaGH3-10*) were obtained and deposited in GenBank.

Bioinformatic analyses of five *PodaGH3* genes: *PodaGH3* genes were analyzed using ExPASy-ProtParam tool (http://web.expasy.org/protparam//). The candidate *GH3s* were verified by Conserved Domain Database (Marchler-Bauer *et al*., 2015) search to ensure the presence of pfam03321 super family and GH3 domain. A multiple sequence alignment and phylogenetic tree of 5 PodaGH3s, 5 PoptrGH3s and 5 AtGH3s (*A. thaliana*) were built through the ClustalX program (http://www.ebi.ac.uk/Tools/clustalw2/) and the neighbor-joining method in MEGA 5.10 program, respectively. The structures of the PodaGH3s were predicted by IntFOLD Integrated Protein structure and function prediction server (Version 3.0) (http://www.reading.ac.uk/bioinf/IntFOLD/IntFOLD3_form.html).

Table 1. Primers for qRT-PCR of PodaGH3 genes.

Gene	GenBank accession number	Primer	GC%	Tm/ºC	Product size/bp
PodaGH3-1	KP893249	F---CTCGTCTAGAATGGCTATTGAGTCAGTACT	43.3	63.6	1794
		R---CGATTCTAGATCAACTTCGTCTTTCTGG	42.9	65.4	
		qF---ATCACCTCCTCGTCTCCTGGAC	59.1	59	212
		qR---GCAACTCAGCCTCATCGGTCTT	54.5	59	
PodaGH3-2	KP893243	F---ATCGTCTAGAATGGCTGTTGATAATGCTC	41.4	66.4	1791
		R---AGATTCTAGATCACCTTCTCCTTTCCGG	46.4	66.8	
		qF---CCAGCCAACTCTCCTAGTGACG	59.1	58.5	215
		qR---TTGACGCACCTTGGAACCTTGT	50	59	
PodaGH3-5	KP893244	F---CTAGTCTAGAATGCCTGAGGCACCAA	50.0	65.9	1836
		R---CGAGTCTAGATCAATTCTTGTTCCATTGC	41.4	66.9	
		qF---CCACTACCATTCCCGGCCACTA	59.1	59.8	191
		qR---AGTCCCTGGCTCAACGACCTT	57.1	59.2	
PodaGH3-9	KP893245	F---CTCGTCTAGAATGGATGGAAAGAAATTGG	41.4	67.5	1791
		R--- AGATTCTAGATCAAGGGATCCAAGCAGG	46.4	67.5	
		qF---ACCTTTGCTGGGCTGTATCGTT	50	58.6	200
		qR---ACGAGGAGGGCATCATAGGGTT	54.5	58.9	
PodaGH3-10	KP893246	F---ACTGTCTAGAATGGAACCAAAAAACACC	39.3	64.6	1788
		R---CAGCTCTAGACTAGCTATAAGCAGTGCTGT	46.7	63.9	
		qF---GGGATGTAGTGGAGGTTGCTGG	59.1	58.7	205
		qR---TGTGTTTCTAGCTCAGCGTGGC	54.5	59.5	
Podaactin1	KP973950	F---GCTGAGAGATTCCGTTGCCCTG	59.1	59.6	204
		R---GGCGGTGATCTCCTTGCTCATT	54.5	59	
PodaEF1-α	KP973951	F---TGGGTCGTGTTGAAACTGGTGT	50	58.6	212
		R--- GGCAGGATCGTCCTTGGAGTTC	59.1	59	
PodaUBQ	KP973952	F---TGTTGTGATCAACGCGAACTCG	50	58.2	203
		R---GAGGATGCCTAGTGCTACGCAT	54.5	58.3	

Quantitative real-time-PCR: RT-qPCR was performed using Light Cycler 96 real-time PCR detection system (Roche Co.). Genes *Podaactin1*, *PodaEF1-α* and *Podaubiquitin* were used as internal controls. The primers used for RT-qPCR are given in Table 1. Amplification was performed according to Fast Start Essential DNA Green Master Mix (Roche) protocol: 95°C for 600s for preincubation, followed by 45 cycles of 95°C for 5s and 59°C for 15s, 72°C for 10s and 95°C for 10s, 65°C for 60s and 97°C for 1s for melting. All experiments were performed with three biological repeats, each containing three technical repeats. Gene expression level was calculated from the threshold cycle by the $2^{-\Delta\Delta CT}$ method (Livak *et al*., 2001).

IAA, SA and JA extraction and LC-MS/MS procedures: Standards of IAA, IBA (indole-3-butyric acid), SA, JA, and H_2JA ((±)-dihydrojasmonic acid) were purchased from Sigma-Aldrich. HPLC-grade methanol and formic acid were purchased from Merck. IBA (50 ng/mL) and H_2JA (100 ng/mL), as the double internal standard, were prepared in methanol solution and stored at 4°C for extraction assays.

IAA, SA and JA were extracted from the samples using the standard addition method (Zhang *et al*., 2011). Then, 10 µl of the obtained extract solution was injected into a liquid chromatography-tandem mass spectrometry (LC-MS) system to determine the contents of IAA, and 10 µl of each sample was injected to detect SA and JA levels. In each case, the contents of IAA, SA and JA were determined for three parallel samples.

LC column system and gradient elution profile were used for IAA detection (Zhang *et al*., 2011). SA and JA level detection were performed with 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) mixed by the ratio 1:1 at a constant flow rate of 1 ml per min. The column temperature was constantly kept at 25°C.

An API 3000 Triple tandem quadrupole mass spectrometer (Applied Biosystems, USA) with a TurboIon-Spray interface in the negative electrospray ionization mode (ESI-) was used. Ionization took place in ESI- conditions shown in Table 2. Mass spectrometer was operated in the multiple reaction monitoring (MRM) mode to heighten the selectivity and specificity for testing the low-content hormones in poplar seedlings. Data were processed with Analyst 1.4.0 software.

Statistical analysis: Acquired data were processed using Excel 2007 (Microsoft Company, USA) and SPSS 22.0 (SPSS Inc., IBM company, USA). Data showed in the figures are means ± standard deviation (SD) of replications.

Results

Cloning and predicted characteristics of five *PodaGH3* genes: *PodaGH3-1, PodaGH3-2, PodaGH3-5, PodaGH3-9* and *PodaGH3-10* were cloned and obtained in GenBank with the accession numbers of KP893249, KP893243, KP893244, KP893245 and KP893246 (Table 1).

The predicted proteins encoded by the full ORFs of the five *PodaGH3s* varied from 575 (PodaGH3-10) to 611 amino acids (PodaGH3-5), with corresponding molecular mass ranging from 64.61 to 69.07 kDa and predicted isoelectric point ranging from 5.31 (PodaGH3-1) to 6.45 (PodaGH3-5) (Table 3). The indole-3-acetic acid-amido synthetase domains of the *PodaGH3s* were matched separately to PLN02249 (PodaGH3-1 and PodaGH3-2), PLN02620 (PodaGH3-5 and PodaGH3-10) and PLN02247 (PodaGH3-9), and their GH3 auxin-responsive promoter domain was specifically matched to pfam03321.

Sequence alignment, phylogenetic analysis, structure and function prediction of PodaGH3s: During comparison of the conservative domains of the five PodaGH3s with five PoptrGH3s and five AtGH3 proteins, we found that they shared highly conservative sites including Ser-108, Thr-312, Tyr-336, Asp-416 and Phe-432, which were closely related to the residues linked to ATP binding for combination of IAA. Meanwhile, there were relatively high consistencies at the sites Arg-115, Met-335, Val-172, Leu-173, Ala-337 and Tyr-342. On the other hand, an obvious difference between IAA-conjugating and benzoate- or JA- conjugating GH3 enzymes was observed on the four residues at the positions 172, 173, 337, and 342 (Fig. 1I).

Table 2. Mass spectrometric parameters for hormones.

	MRM(amu)	IS(v)	DP(v)	FP(v)	EP(v)	CE(v)	CXP(v)	CUR(psi)	NEB(psi)	TEM(℃)
IAA	174.0→130.0	-4500	-75	-375	-10	-16	-7	12	10	300
IBA	202.1→116.0	-4500	-105	-375	-10	-25	-6	12	10	300
SA	136.9→93.0	-4500	-35	-375	-10	-20	-6	12	10	300
JA	209.3→59	-4500	-37	-375	-10	-25	-23	12	10	300
H_2JA	211.1→59.2	-4500	-90	-375	-10	-24	-7	12	10	300

Abbreviations: IS: ionspray voltage; DP: declustering potential; FP: focal potential; EP: entrance potential; CE: collision energy; CXP: collision export potential; CUR: Curtain gas; NEB: Nebulize gas

Table 3. Characteristics of 5 PodaGH3s with full ORFs of Shanxin poplar.

Gene	Identity (%)	AA	pI	MW (kDa)	CDS	
					K14487	PF03321
PodaGH3-1	96/with *PoptrGH3-1*	597	5.31	67.70	1-1788	76-1701
PodaGH3-2	98/with *PoptrGH3*	596	5.60	67.68	1-1785	79-1698
PodaGH3-5	97/with *PoptrGH3-5*	611	6.45	68.99	1-1833	82-1728
PodaGH3-9	98/with *PoptrGH3-9*	596	5.48	67.40	40-1788	46-1713
PodaGH3-10	97/with *PoptrGH3-10*	595	6.31	67.01	61-1722	58-1773

Abbreviations: AA: number of amino acids; pI, isoelectric point; MM, molecular mass; (+), plus strand; (−), minus strand; exons, number of exons; introns, number of introns; CD, putative conserved domain

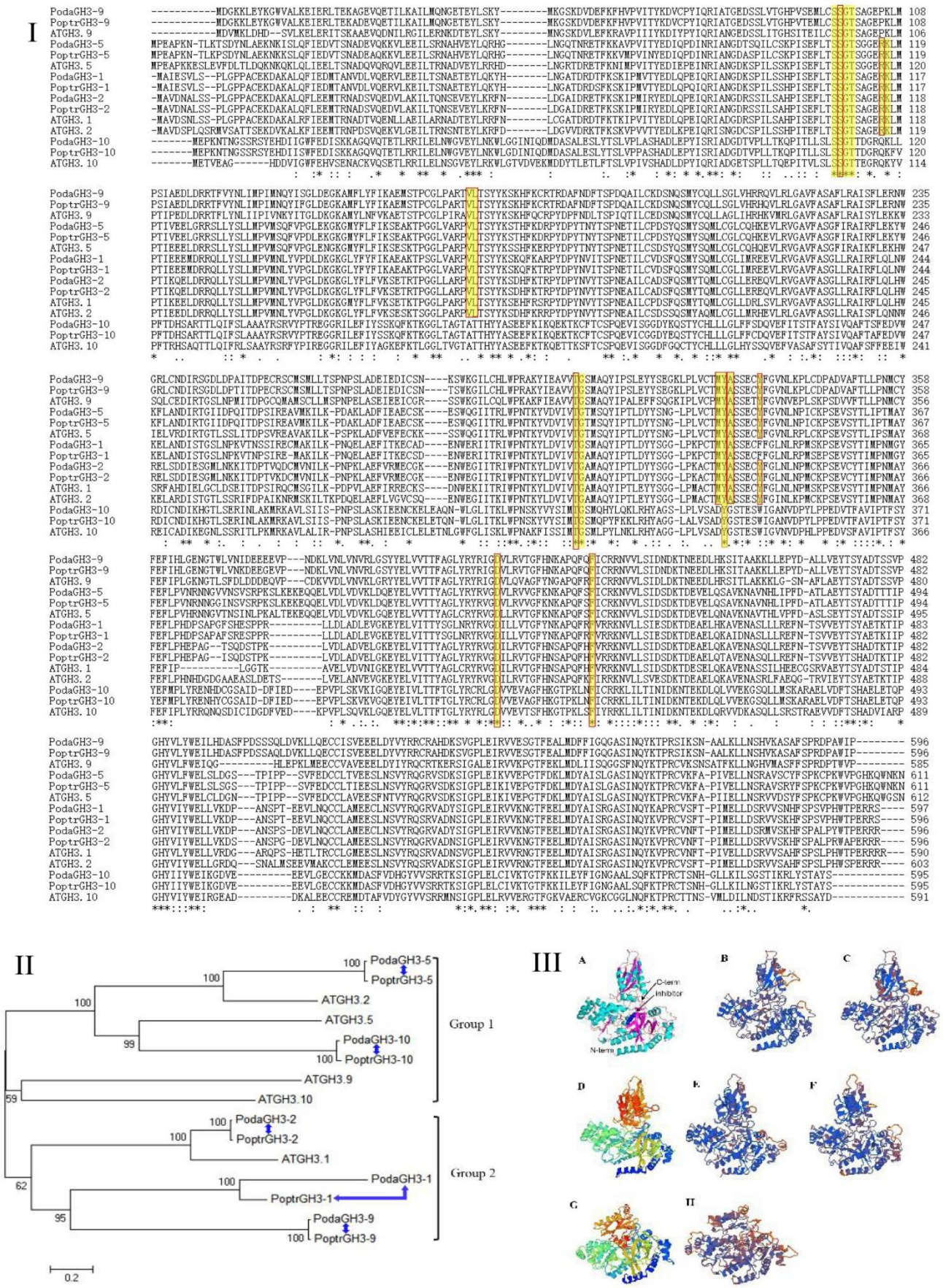

Fig. 1. Characteristics analysis of PodaGH3s. I Sequence alignment of 15 GH3s. II Phylogenetic relationships of the 15 GH3 genes. Five GH3s of Poda- and Potri- showing close relationships marked severally with blue bold arrows. III Three-dimensional structure models of model proteins and PodaGH3s. A VvGH3-1; B PodaGH3-1; C PodaGH3-2; D AtGH3.5; E PodaGH3-5; F PodaGH3-9; G FIN219-FIP1; H PodaGH3-10.

A phylogenetic tree based on the 15 GH3s was constructed (Fig. 1II). The 15 GH3s could be divided into two groups: eight were clustered in group 1, including *PodaGH3-5*, *PoptrGH3-5*, *PoptrGH3-10*, *PodaGH3-10*, *AtGH3.2*, *AtGH3.5*, *AtGH3.9* and *AtGH3.10*, and the others were in groups 2. The *PodaGH3s* had close relationships with the *PoptrGH3s*, with one-to-one correspondences in the phylogenetic tree, suggesting that they were orthologous genes.

Protein structure and function prediction showed that PodaGH3-1 or PodaGH3-2 had the highest coverage (100%) and identify (79.53% and 86.91%) with single crystal *VvGH3-1,* an indole-3-acetic acid amido synthase derived from *Grapevine* (Table 4 and Fig. 1III). PodaGH3-5 or PodaGH3-9 was predicted to have the highest coverage (100%, 98% respectively) and identity (84.94%, 55.99% respectively) with the GH3.5 acyl acid amido synthetase in *A. thaliana*. PodaGH3-10 was predicted to have the highest coverage (96%) and identity with FR insensitive 219 (FIN219), one of the GH3 proteins in *Arabidopsis* that belongs to the adenylate-forming family of enzymes (Table 4 and Fig. 1III).

Rhizosphere colonization of *T. aspereullm*: Scanning electron microscope observation showed that *T. aspereullm* had colonized intensely and wrapped the roots of Shanxin poplar (Fig. 2) at 72 hours post-inoculation. The colonization of *Trichoderma* in plant roots might be due to some chemicals in favor of conidia germination secreted by the roots (Dastogeer *et al*., 2017).

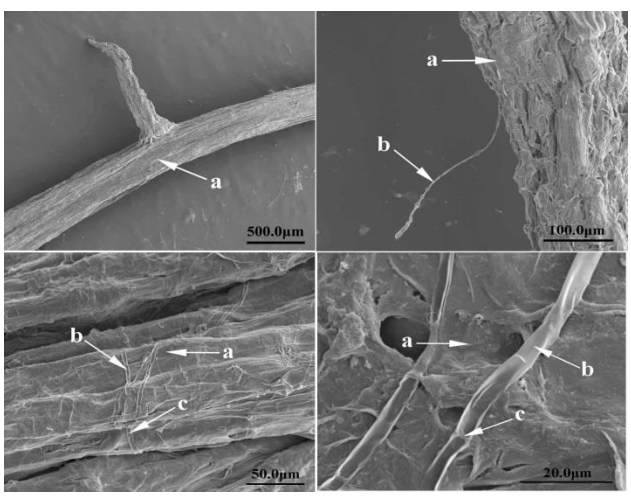

Fig. 2. *Trichoderma* colonized on the roots of poplar seedlings at 72 h. a: Root of Shanxin poplar; b: Hyphae of *T. aspereullm*; c: Septate hyphae.

Expression profiles of the five *PodaGH3* genes in poplar leaves and roots: In the leaves of Shanxin poplar, after treated with *Trichoderma*, the changes in gene expression of *PodaGH3-1* compared to CK was opposite to *PodaGH3-2*. The expression of *PodaGH3-1* was downregulated, then upregulated and downregulated again over a 24-hour period; they had no significant change at 48 h, then its expression increased again to 6.97 times of CK at 72 h. On the other hand, the expression level of *PodaGH3-2* was upregulated first and then downregulated within 0-24 h, with no significant change at 24 h; then the gene expression was downregulated again. The expression of *PodaGH3-5* was downregulated due to *Trichoderma* treatment within the first 72 hours, during which it was downregulated to 0.24 times of CK at 48 h, reaching the minimum. The expression levels of *PodaGH3-9* and *PodaGH3-10* were upregulated: the expression of *PodaGH3-9* was upregulated to 29.1 times of CK at 6 h, reaching its maximum; *PodaGH3-10* expression was 9.48 times of CK at 2 h, which was its maximum.

In the roots, there had also been significant changes in the expression levels of the 5 *GH3* genes after *Trichoderma* inoculation. The expression of *PodaGH3-1* was upregulated during 0-72 h except the downregulation at 2 h; and its expression reached maximum at 6 h, which was 1.92 times of CK. The expression of *PodaGH3-2* was upregulated within 0-72 h except the downregulation at 24 h. Expression levels of *PodaGH3-5*, *PodaGH3-9* and *PodaGH3-10* were all upregulated within 0-24 h, reaching the maximums at 2 h, which were 2.28, 9.30 and 4.13 times of CK, respectively. At 48 h, the expression of *PodaGH3-5* and *PodaGH3-10* were downregulated slightly, while *PodaGH3-9* was still upregulated. At 72 h, the expression of *PodaGH3-5* and *PodaGH3-9* was downregulated while *PodaGH3-10* was upregulated to 1.90 times of CK (Fig. 3).

Hormone levels in leaves and roots of Shanxin poplar seedlings: IAA levels in the leaves and roots: IAA contents in poplar roots and leaves were measured separately. In general the IAA contents were lower in the roots than in the leaves. In the leaves, after inoculation with *Trichoderma*, the contents of IAA were higher than CK except at 6 h; IAA level reached maximum at 72 h, which was 499.7 mol/g FW, 57.0 mol/g FW higher than CK. The fluctuation of IAA levels in the roots was more apparent than that in the leaf during 0-72 h. The contents of IAA in the roots varied as leaves, reaching the maximum at 24 h, which was 208.1 mol/g FW, 38.6 mol/g FW higher than CK (Fig. 4).

Table 4. The results of the IntFOLD integrated protein structure.

Protein name	Domains	Title	Coverage/%	Identity	Method	Oligo state	Ligands
PodaGH3-1	4b2g.1.A	*GH3-1* auxin conjugating Enzye	100	79.53	X-ray, 2.4Å	monomer	1×V1N
PodaGH3-2			100	86.91			
PodaGH3-5	5kod.2.A	Indole-3-acetic acid-amido synthetase *GH3.5* from Arabidopsis thaliana	100	84.94	X-ray, 2.2 Å	monomer	1×AMP, 1×IAC
PodaGH3-9	5kod.2.A		98	55.99			
PodaGH3-10	5eco.2.A	Jasmonic acid-amido synthetase JAR1	96	49.12	X-ray, 1.8Å	hetero-oligomer	2×GSH, 1×LEU, 1×JAA

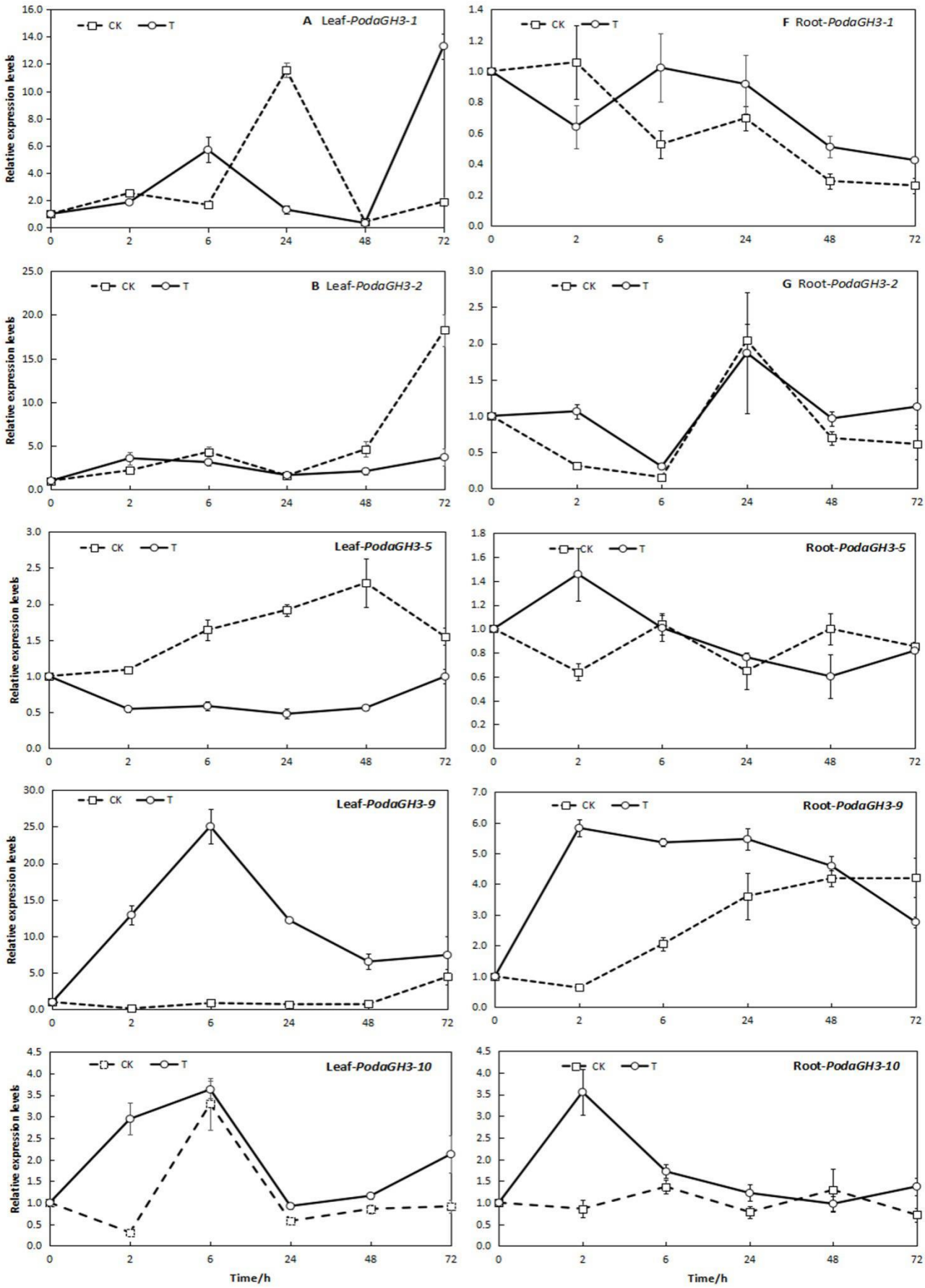

Fig. 3. Expression analysis of five PodaGH3s in response to *Trichiderma*. Relative expression levels were the transcription level by *Trichiderma* inoculation or not. All relative expression levels were log2 transformed and error bars (SD) obtained from multiple replicates (n=9) of RT-qPCR.

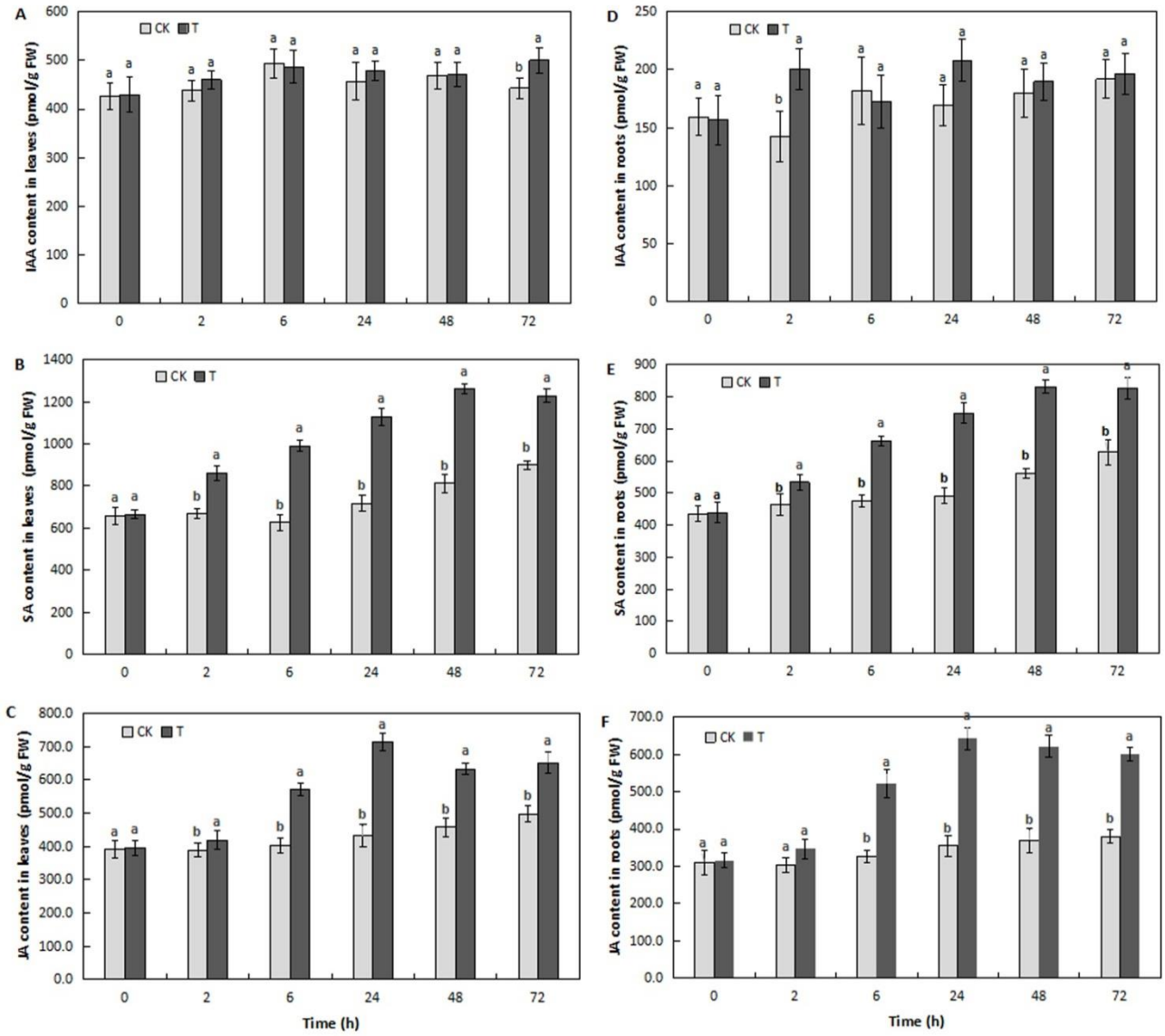

Fig. 4. IAA, SA, JA levels in the leaves and roots of poplar seedlings after inoculated with *Trichiderma*.

SA level in the leaves and roots: After inoculation with *Trichoderma*, SA levels became higher than CK during 0-72 h in both leaves and roots. They reached maximum at 48 h, which were 1260.2 mol/g in leaves and 831.4 mol/g in roots, higher than CK by 448.2 mol/g and 269.1 mol/g, respectively. The results reflected a slightly rising trend in SA level during 0-72 h in both the roots and the leaves of CK, and they showed clear rising trends in treated poplar seedlings after inoculation with *Trichoderma* (Fig. 4).

JA level in the leaves and roots: There was no significant fluctuation in JA levels in the roots and leaves of CK during 0-72 h. However, JA levels in the seedlings inoculated with *Trichoderma* were constantly higher than CK during 0-72 h in both leaves and roots, and both reached maximum at 24 h, which were 713.7 mol/g in leaves and 641.9 mol/g in roots, respectively 282.2 mol/g and 287.4mol/g higher than CK. Moreover, in both roots and leaves, the SA levels showed weak increase in CK during 24-72 h, while it was slightly decreased during 24-72 h after inoculation with *Trichoderma* (Fig. 4).

Discussion

Many studies have been carried out on the biological functions of *GH3* family genes. So far 20 *AtGH3* family genes had been identified in *A. thaliana*, 14 *OsGH3s* in *Oryza sativa* and 9 *VvGH3s* in *V. vinifera*. 14 *PoptrGH3*s have been identified which were respectively located in the chromosomes LG I, LG II, LG III, LGVII, LG IX, LG XI, LG XIII, LG XIV and LGXVIIII. Evolution analyses between these *PoptrGH3*s with the five *PodaGH3s* showed that there were close evolutionary relationships between *PodaGH3-1*, *2*, *5*, *9* and *10* with Potri. 007G050300.1, Potri. 009G092900.1, Potri.011G129700.1, Potri. 002G206400.1 and Potri. 013G144300.1 (alias as *PotriGH3-1, 2, 5, 9* and *10*), respectively, though they were differently annotated as auxin responsive GH3 gene family, IAA-amido synthetase and/or DWARF IN LIGHT.

These *GH3s* were highly conserved in the sites linked to ATP binding. Regardless of species and acyl substrate, the residues of all selected proteins were consistent at Arg-115, Ser-108, Tyr-336, Asp-416, and Phe-432 as in *Vv GH3-1*. *VvGH3-1* had been verified to contain a long

N-terminal domain (437 amino acids) which has catalytic activities, a short C-terminal domain (161 amino acids) and an ATP-binding domain (with at least one magnesium ion on the active sites) (Peat *et al*., 2012). And the four residues which likely contribute to IAA binding in *VvGH3-1* (Val-172, Leu-173, Ala-337, and Tyr-342) showed significant difference between IAA-conjugating with benzoate- or JA-conjugating *GH3* enzymes (Westfall *et al*., 2016). In our study, PodaGH3-1 and PodaGH3-2 were predicted to have higher consistency in crystal structure with Vv GH3-1. Their highly conserved amino acid residues may be related to their physiological functions. GH3-5 and GH3-9 (though GH3-9 had slightly lower identity) were matched with *GH3.5* acyl acid amido synthetase from *A. thaliana*. *AtGH3.5* protein, a multifunctional acetyl-amido synthetase, has the activities for adenosine acylation of IAA and SA. Furthermore, it is involved in the regulation of disease resistance mediated by SA and susceptibility response mediated by IAA (Staswick *et al*., 2002; Westfall *et al*., 2016). *GH3-10* is a putative JA-amido synthetase (though the identity was slightly lower) (Table 3) named FIN219, which directly controls the biosynthesis of jasmonoyl-isoleucine in JA-mediated defense responses and the rotated C-terminal domain of FIN219 changes ATP binding and the core structure of the active site (Chen *et al*., 2016). These previous findings suggested homologous function difference/redundancy of these *PodaGH3* genes.

In this study, we found that the five *PodaGH3* genes were all expressed in the leaves and roots of poplar seedlings. After inoculation with *Trichoderma*, these genes were upregulated in roots during the early 6 hour (except *PodaGH3-1*), and then their expressions showed dramatic changes. They showed different expression trends in leaves: during the early interaction at 2 h and 6 h, the expression patterns of *GH3-1*, *GH3-2* and *GH3-9*, which were group I in the phylogenetic tree, were opposite; while *GH3-5* and *GH3-10* in group II displayed distinct expression patterns in leaf and consistent expression patterns in root. With the increase on time after inoculation, the five genes displayed various regulation patterns. Previous study suggested that GH3 proteins in *Arabidopsis* were involved in plant growth and development, photomorphogenesis, light-and auxin-signaling, and auxin homeostasis (Nakazawa *et al*., 2001; Takase *et al*., 2004; Staswick *et al*., 2005). The distinction in inducibility of individual *GH3* genes, especially when the time after inoculation extended, were probably due to various factors including kinetics of induction, tissue-specific auxin reception, cell-type-dependent and differential regulation of free auxin concentrations, or different mechanisms of auxin-dependent transcriptional and posttranscriptional regulations. It was also likely to be associated with the elicitors produced by *Trichoderma*. Researches also found that *Trichoderma* was able to synthesize antagonistic compounds including proteins, enzymes and antibiotics, and micro-nutrients such as vitamins, hormones and minerals to boost the biocontrol activity (Wijesinghe *et al*., 2011; Prasad *et al*., 2013; Björkman, 2004). It could also be caused by the genetic redundancy of *GH3* gene family.

Studies demonstrated that interactions between *Trichoderma* and plants can induce systemic resistance of plants by jasmonic acid-ethylene signal pathways. We found that the contents of JA and SA in poplar seedlings had increased during the first 72 hours after treated by *Trichoderma*. These results agreed with previous studies that interaction between *T. harzianum* T6776 and tomato increased the content of JA but decreased SA, which might be partly due to the different time of treatment (Fiorini *et al*., 2015). We found that the increment of IAA content in roots was larger than leaves 24 hours after induced by *Trichoderma*. It might be related with the balance of IAA affected by *Trichoderma* but still needs further proof.

Above all, the interaction between biotrophic *Trichoderma* and poplar seedling activated the five *PodaGH3s*, and largely changed the relationships between the expression of the five *GH3* genes and the IAA, JA and SA levels. It is worth exploring what product secreted by *Trichoderma* can affect the protein function of GH3s, thus influence the growth and development of plants.

Acknowledgments

This work were supported by State Key Laboratory of Tree Genetics and Breeding (Northeast Forestry University) (201202), the National Natural Science Foundation of China (NSFC: 31370642) and the Natural Science foundation of Heilongjiang Province of China (C201216).

References

Aelel, S. and A. Theologis. 1996. Early genes and auxin action. *Plant Physiol*., 111(6): 9-17.

Björkman, T. 2004. Effect of *Trichoderma colonization* on auxin-mediated regulation of root elongation. *Plant Growth Regul*., 43:89-92.

Chen, C.Y., S.S. Ho, T.Y. Kuo, H.L. Hsieh and Y.S. Cheng. 2016. Structural basis of jasmonate-amido synthetase FIN219 in complex with glutathione S-transferase FIP1 during the JA signal regulation. *Proc. Natl. Acad. Sci. U. S. A*, 114(10): 1815-1824.

Contreras-Cornejo, H.A., L. Macı´as-Rodrı´guez, C. Corte´s-Penagos and J. Lo´pez-Bucio. 2009. *Trichoderma* virens, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. *Plant Physiol.*, 149: 1579-1592.

Dastogeer, K.M.G., H. Li, K. Sivasithamparam, M.G.K. Jones, X. Du, Y.L. Ren and S.J. Wylie. 2017. Metabolic responses of endophytic Nicotiana benthamiana plants experiencing water stress. *Environ. & Exp. Bot.*, 143: 59-71.

Faisal, H., A. Muhammad, A. Iram us, F. Muhammadi, A. Muhammad and H. Alamdar. 2017. Occurrence of fruit-rot of chilli in sindh and their biomanagement under laboratory conditions. *Pak. J. Bot.*, 49(4): 1593-1597.

Farag, M.A., D.A. Al-Mahdy, A. Meyer, H. Westphal and L.A. Wessjohann. 2017. Metabolomics reveals biotic and abiotic elicitor effects on the soft coral *Sarcophyton* ehrenbergi terpenoid content. *Scientific Reports*, 7. DOI:10.1038/ s41598-017-00527-8.

Fiorini, L., L. Guglielminetti, L. Mariotti, M. Curadi, P. Picciarelli, A. Scartazza, S. Sarrocco and G. Vannaci. 2015. *Trichoderma harzianum* T6776 modulates a complexmetabolic network to stimulate *tomato* cv. *Micro-Tom* growth. *Plant & Soil*, 400: 351-366.

Hagen, G. and T. Guilfoyle. 2002. Auxin-responsive gene expression: genes, promoters and regulatory factors. *Plant Mol. Biol.*, 49: 373-385.

Hohmann, P. and M.M. Messmer. 2017. Breeding for mycorrhizal symbiosis: focus on disease resistance. *Euphytica*, 213(5): 113. DOI:https://doi.org/10.1007/s10681-017-1900-x

Jain, M., N. Kaur, A.K. Tyagi and J.P. Khurana. 2006. The auxin-responsive *GH3* gene family in rice (*Oryza sativa*), Funct. Lntegr. *Genomics*, 6(1): 36-46.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25: 402-408.

Luo, Y., D.D. Zhang, X.W. Dong, P.B. Zhao, L.L. Chen, X.Y. Song, X.J. Wang, X.L. Chen, M. Shi and Y.Z. Zhang. 2010. Antimicrobial Peptaibols induce defense responses and systemic resistance in tobacco against tobacco mosaic virus. *FEMS Microbiol. Letl.*, 313(2): 120-126.

Marchler-Bauer, A., M.K. Derbyshire, N.R. Gonzales, S.N. Lu, F. Chitsaz, L.Y. Geer, R.C. Geer, J. He, M. Gwadz, D.I. Hurwitz, C.J. Lanczycki, F. Lu, G.H. Marchler, J.S. Song, N. Thanki, Z.X. Wang, R.A. Yamashita, D.C. Zhang, C.J. Zheng and S.H. Bryant. 2015. CDD: NCBI's conserved domain database. *Nucl. Acids Res.*, 43: 222-226.

Martínez-Medina, A., A. Roldán, A. Albacete and J.A. Pascual. 2011. The interaction with arbuscular mycorrhizal fungi or *Trichoderma harzianum* alters the shoot hormonal profile in melon plants. *Phytochem.*, 72: 223-229.

Nakazawa, M., N. Yabe, T. Ichikawa, Y.Y. Yamamoto, T. Yoshizumi, K. Hasunuma and M. Matsui. 2001. DFL1, an auxin responsive *GH3* gene homologue, negatively regulates shoot cell elongation and lateral root formation, and positively regulates the light response of hypocotyl length. *The Plant J.*, 25(2): 213-221.

Nobuta, K., R.A. Okrent, M. Stoutemyer, N. Rodibaugh, L. Kempema, M.C. Wildermuth and R.W. Innes. 2007. The *GH3* acyl adenylase family member PBS3 regulates salicylic acid-dependent defense responses in *Arabidopsis*. *Plant Physiol.*, 144(2): 1144-1156.

Peat, T.S., C. Böttcher, J. Newman, D. Lucent, N. Cowieson and C. Davies. 2012. Crystal structure of an indole-3-acetic acid amido synthetase from *Grapevine* involved in auxin homeostasis. *The Plant Cell*, 24: 4525-4538.

Prasad, A., A. Mathur, A. Kalra, M.M. Gupta, R.K. Lal and A.K. Mathur. 2013. Fungal elicitor-mediated enhancement in growth and asiaticoside content of Centella asiatica L. shoot cultures. *Plant Growth Regul.*, 69: 265-273.

Sofo, A., A. Scopa, M. Manfra, M.D. Nisco, G. Tenore, J. Troisi, R.D. Fiori and E. Novellino. 2011. *Trichoderma harzianum* strain T-22 induces changes in phytohormone levels in cherry rootstocks (*Prunus cerasus* × *P. canescens*). *Plant Growth Regul.*, 65: 421-425.

Staswick, P.E., B. Serban, M. Rowe, I. Tiryaki, M.T. Maldonado, M.C. Maldonado and W. Suza. 2005. Characterization of an *Arabidopsis* enzyme family that conjugates amino acids to indole-3-acetic acid. *Plant Cell*, 17(2): 616-627.

Staswick, P.E., I. Tiryaki and M.L. Rowe. 2002. Jasmonate response locus JAR1 and several related *Arabidopsis* genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acid in an assay for adenylation. *Plant Cell*, 14: 1405-1415.

Takase, T., M. Nakazawa, A. Ishikawa, M. Kawashima, T. Ichikawa, N. Takahashi, H. Shimada, K. Manabe and M. Matsui. 2004. ydk1-D, an auxin-responsive *GH3* mutant that is involved in hypocotyl and root elongation. *The Plant J.*, 37: 471-483.

Thaler, J.S. and R.M. Bostock. 2004. Interactions between abscisic-acid-mediated responses and plant resistance to pathogens and insects. *Ecol. Soc. Amer.*, 85(1): 48-58.

Westfall, C.S., A.M. Sherp, C. Zubieta, S. Alvarez, E. Schraft, R. Marcellin, L. Ramirez and J.M. Jez. 2016. *Arabidopsis thaliana GH3.5* acyl acid amido synthetase mediates metabolic crosstalk in auxin and salicylic acid homeostasis. *Proc. Natl. Acad. Sci*. U.S.A. 113(48): 13917-13922.

Wijesinghe, C.J., R.S. Wilson Wijeratnam, J.K.R.R. Samarasekara and R.L.C. Wijesundera. 2011. Development of a formulation of *Trichoderma asperellum* to control black rot disease on pineapple caused by (*Thielaviopsis paradoxa*). *Crop Prot.*, 30: 300-306.

Wright, R.M., G. Hagen and T. Guilfoyle. 1987. An auxin-induced polypeptide in dicotyledonous plants. *Plant Mol. Biol.*, 9(6): 625-634.

Zhang, R.S., Y.C. Wang, C. Wang, Z.G. Wei, D.A. Xia, Y.F. Wang, G.F. Liu and C.P. Yang. 2011. Time-course analysis of levels of indole-3-acetic acid and expression of auxin-responsive *GH3* genes in *Betula platyphylla*. *Plant Mol. Biol. Rep*., 29(4): 898-905.

Zhang, Z., Q. Li, Z. Li, P.E. Staswick, M. Wang, Y. Zhu and Z. He. 2007. Dual regulation role of *GH3.5* in salicylic acid and auxin signaling during *Arabidopsis-Pseudomonas syringae* interaction. *Plant Physiol.*, 145: 450-464.

(Received for publication 11 December 2017)