Induction Expression of P450 by Tannic Acid in *Micromelalopha troglodyta* (Lepidoptera: Notodontidae) Larvae[1]

Xiaoling Shi[2], Renjie Fu[2], and Fang Tang[3]

Co-Innovation Center for the Sustainable Forestry in Southern China, College of Forestry, Nanjing Forestry University, Nanjing 210037, China

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Abstract Cytochromes P450, a gene family generally associated with detoxification, was analyzed by real-time fluorescent quantitative PCR (qRT-PCR) in *Micromelalopha troglodyta* (Graeser) (Lepidoptera: Notodontidae). Our studies showed that the nine p450 genes had different levels of response to tannic acid, and the mRNA expression of six p450s reached the maximum level at the concentration of 1 mg/ml in the midgut of *M. troglodyta*. Furthermore, the mRNA expression level of Unigene27074_All was 370 times higher than that observed in the controls. Also, the mRNA expressions of CL3273.Contig4_All and Unigene27074_All were induced by 10 mg/ml tannic acid. These findings indicate that p450s have an effect on the metabolism of plant secondary substances by *M. troglodyta*.

Key Words *Micromelalopha troglodyta*, tannic acid, Cytochrome P450s, mRNA expression, plant secondary substance

Micromelalopha troglodyta (Graeser) (Lepidoptera: Notodontidae) is an important leaf-feeding pest of poplar trees, *Populus* spp., in many parts of China. *Micromelalopha troglodyta* typically exhibits a large number of generations, high fecundity rates, and high egg hatching rates, which contribute to rapid population increases. The foliar pests often devastate poplar trees by stripping their leaves, thus, reducing photosynthesis, growth, production, and value resulting in economic losses (Zhang et al. 2009). Actively feeding larvae may consume tannic acid, which is produced by host plant trees as a secondary metabolite to provide a direct defense against herbivores (Cheng et al. 2015). Many insects possess detoxification enzymes that enable them to metabolize toxic phytochemicals produced and accumulated by plants (Gatehouse et al. 2002, Mazumdar-Leighton and Broadway 2001, Wittstock et al. 2004). Cytochrome P450s are among those enzymes, with p450s, glutathione S-transferases, and carboxylesterases being three important detoxifying enzymes of insects, and are considered to be primary mechanisms of resistance to insects (Després et al. 2007).

Cytochrome P450s constitute one of the largest gene superfamilies in all living organisms, including animals, plants, insects, and bacteria, and possess a diversity of physiological and biochemical functions. Insect cytochrome P450s function as oxidase

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[2]These authors contributed equally to this study and article.
[3]Corresponding author (Email: tangfang76@sohu.com).

and monooxygenase, which not only are involved in the synthesis of many endogenous physiological substances but also catalyze exogenous substances in various structures (Feyereisen 2012). Thus, insect cytochrome P450s play an important role in enabling insects to adapt to plant defense compounds and in developing resistance to insecticides (Hodgson et al. 1995, Waters et al. 1992, Li et al. 2002).

P450 is an effective and efficient multifunctional enzyme system. Some insect P450 genes can be induced by exogenous and endogenous compounds. However, although their importance in insect physiology and toxicology is widely recognized, there are gaps in our knowledge of insect P450s and their functions (Liu and Kang 2011). The induction mechanism of p450s by tannic acid has not yet been demonstrated; therefore, our goal in this study was to characterize the tannic acid induction mechanism of P450s and the expression by tannic acid in *M. troglodyta*. We also hoped to provide insights into the evolution and functions of the p450s in *M. troglodyta*.

Materials and Methods

Insects. *Micromelalopha troglodyta* larvae used in these assays were collected from poplar trees in Nanjing of Jingsu Province, China. In the laboratory, they were supplied fresh poplar leaves in a room maintained at 26 ± 1°C and 70–80% relative humidity with a photoperiod of 16:8 h (light: dark).

Treatment of test insects. Tannic acid (Sigma Chemical, St. Louis, MO) was dissolved in a small aliquot of ethanol and then serially diluted with distilled water to concentrations of 10, 1, 0.1, 0.01, and 0.001 mg/ml. Freshly collected poplar leaves were immersed in these various solutions and then allowed to air dry. Once dried, the treated leaves were placed individually into triangle bottles with newly molted third instars and placed in the rearing room. Controls consisted of larvae fed on leaves immersed in distilled water. Larvae were permitted to feed on treated leaves for 96 h when they were removed for dissection. All experiments were performed in triplicate. Midguts were removed from individual larvae by dissection on ice, peritrophic membrane-associated midgut contents were eliminated, and then midguts were gently shaken to free all contents and rinsed in 1.15% ice-cold KCl. The midgut body by dissection was immediately placed in liquid nitrogen and then placed in −80°C until assayed.

Extraction of total RNA. Total RNA was extracted from 50 mg of midgut tissues by using Trizol reagent (Invitrogen™, Thermo Fisher Scientific, Inc., Waltham, MA), according to the manufacturer's instruction, dissolved in 30 μl double-distilled water. The integrity and quality of total RNA were tested by running 1% agarose gel electrophoresis and measuring absorbance at 260 and 280 nm by using a Thermo Scientific NanoDrop 2000 instrument. Then, RNA samples were treated with a PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara Biotechnology Co. Ltd., Dalian, China), which is special reverse transcription kit for real-time fluorescent quantitative PCR (qRT-PCR). The synthesized cDNA was kept at −80°C for determination of p450 mRNA expression.

The sequences of nine P450s were found through the transcription group sequencing data. The coding region was found by DNAMAN 6 software. Then, the primers of P450s and the actin gene (Table 1) were designed by Primer 5 and synthesized by Shanghai General Biotechnology Co. Ltd.

Table 1. Detail of primers used for qRT-PCR.

P450 Gene	Gene Description	Primer*	Sequences (5′-3′)
Actin		F	GCGGCGCGACTCACCGACTAC
		R	GGGAAGAGAGCCTCAGGGCAAC
CL1404.Contig3_All	Cytochrome P450 6AE32	F	ATTACTTCAGCGGCAGGGAG
		R	GCGTGCAGTCTTGGATTAGG
CL1922.Contig2_All	Cytochrome P450 6B45	F	GGAAGTGGTGCTAAGACGGA
		R	ACTTGGCGTTGTCATCCTGT
CL1922.Contig3_All	Cytochrome P450 6B45	F	ATCTACAGGATGACAACGCCA
		R	TTCCTCAACGCTCCCCAA
CL3273.Contig4_All	Cytochrome P450	F	AGATGTGGTGCCTGTTCTTTCC
		R	CCTCCTTGTATGTTTTCCCGTC
CL336.Contig1_All	Cytochrome p450 CYP337B1	F	TTACGATGGAAACTGATGCG
		R	GGAAGGCTTTGTCCTAAACTCT
CL441.Contig4_All	Cytochrome P450 CYP9	F	TTATCGTCCGTAAAGGAACTGG
		R	GAGAAACGCTCAGGATCAAACT
CL441.Contig5_All	Cytochrome P450 CYP9	F	GGATGGTTCTCCCCTTATTTG
		R	CGAGCTTGGATTGATCTTATGC
Unigene27074_All	Cytochrome P450	F	CATTGAGTCCAGCATTTACCAG
		R	GCCTTGATCCTTCCGTTGA
Unigene8938_All	Cytochrome P450 CYP332A1	F	TGGCTTCAAATGAGACACGA
		R	GTTTGTTGCCGTCGATGTAGT

* F, forward; R, reverse.

qRT-PCR. The advantages of the qRT-PCR are that they are relatively easy to perform and require small amounts of template, as quantification of a sample can be performed in a single tube. In our experiments, the qRT-PCR was performed on a 7500 Real-Time PCR system (Applied Biosystems, Foster, CA) to compare the mRNA expression of p450 in the midguts of *M. troglodyta* larvae exposed to tannic acid. The p450 mRNA expression was studied by using the SYBR® Premix Ex Taq™ (Tli RNaseH Plus) (Takara Biotechnology Co. Ltd., Dalian). The amplification of cDNA by the qRT-PCR was performed in a 20-μl mixture containing about 2 μl cDNA, 10 μl of SYBR Premix Ex Taq, 0.4 μl of Rox Reference Dye II, 0.4 μl of both sense and antisense primers of P450, and 6.8 μl double-distilled water. As an endogenous control, the P450 gene primers were replaced by a pair of actin gene primers (0.4 μl for each) in the amplification reaction by using the same cDNA as the template. The qRT-PCR reaction conditions were as follows: the reaction mixture was first kept at 95°C for 30 s, then 40 cycles of 95°C for 5 s and 60°C for 34 s. To confirm the amplification of specific products, melting-curve cycles were continued under the following conditions: 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s. All experiments were performed in triplicate. The relative mRNA expression level of P450 was calculated by the $2^{-\Delta\Delta Ct}$ method (Giulietti et al. 2001).

Statistical analysis. Data collected from these assays were subjected to analysis of variance using InStat software (GraphPad, San Diego, CA) with significance defined as $P < 0.05$.

Results

The mRNA expressions of nine p450s in the midgut of *M. troglodyta* were clearly induced by different concentrations of tannic acid at 96 h; quantitative real-time PCR results are shown in the figures. The induction of mRNA expression of CL1404.Contig3_All, CL1922.Contig2_All, CL1922.Contig3_All, CL336.Contig1_All, and Unigene8938_All by tannic acid was dose dependent in the range of 0.001 to 10 mg/ml (Figs. 1, 2, 3, 4, 5). The mRNA expression of CL1404.Contig3_All was significantly upregulated at a concentration of 0.01 mg/ml, whereas other concentrations of tannic acid caused significant downregulation ($F = 684.40$; df = 5,12; $P < 0.0001$) (Fig. 1). The mRNA expression of CL1922.Contig2_All was induced by 0.01–1 mg/ml tannic acid ($F = 2077.2$; df = 5,12; $P < 0.0001$) (Fig. 2), and the mRNA expression of CL1922.Contig3_All was induced by 0.1–1 mg/ml tannic acid ($F = 755.88$; df = 5,12; $P < 0.0001$) (Fig. 3). Moreover, the expression of CL336.Contig1_All mRNA was significantly decreased by 0.001 mg/ml tannic acid, whereas the expression of CL336.Contig1_All was induced by 1.0–10 mg/ml tannic acid ($F = 180.31$; df = 5,12; $P < 0.0001$) (Fig. 4). The mRNA expression of Unigene8938_All was upregulated by 0.001 to 0.1 mg/ml tannic acid ($F = 31.017$; df = 5,12; $P < 0.0001$) (Fig. 5).

Tannic acid caused significant downregulation of CL3273.Contig4_All mRNA expression at concentrations of 0.001, 0.01, and 0.1 mg/ml; however, tannic acid caused significant upregulation at 10 mg/ml ($F = 207.72$; df = 5,12; $P < 0.0001$) (Fig. 6). Furthermore, the mRNA expression of CL441.Contig4_All was significantly upregulation by 1 mg/ml tannic acid ($F = 5168.9$; df = 5,12; $P < 0.0001$), and the expression level was about 7 times higher than that observed in the controls (Fig.

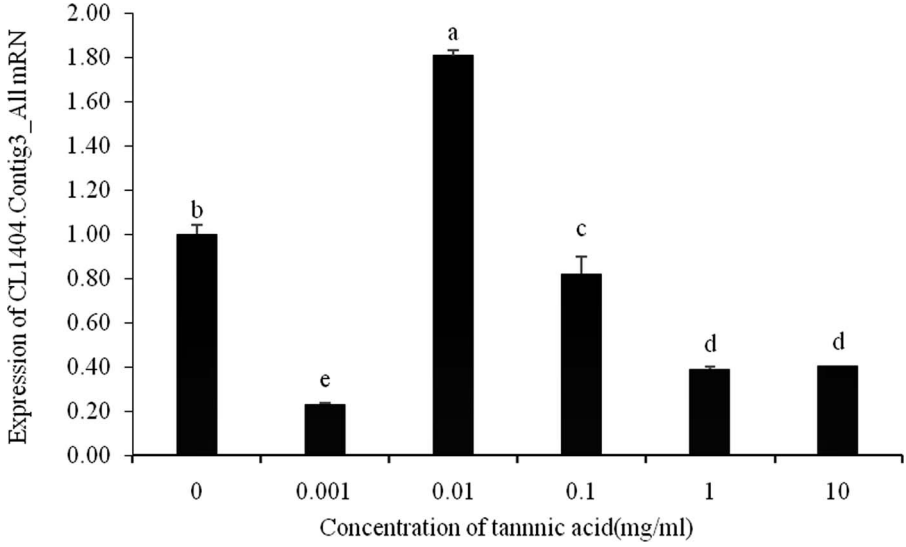

Fig. 1. The mRNA expressions of CL1404.Contig3_All in the midgut of *M. troglodyta* exposure to various concentrations of tannic acid. Means with the same lowercase letter are not significantly different (Tukey's test, $P < 0.05$). Data were analyzed using InStat software (GraphPad, San Diego, CA, USA). All experiments were performed in triplicate.

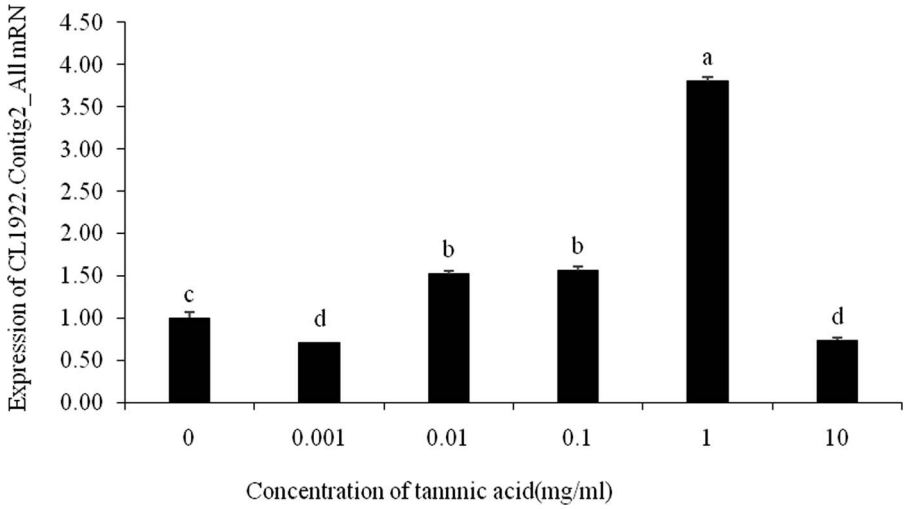

Fig. 2. The mRNA expressions of CL1922.Contig2_All in the midgut of *M. troglodyta* exposure to various concentrations of tannic acid. Means with the same lowercase letter are not significantly different (Tukey's test, $P < 0.05$). Data were analyzed using InStat software (GraphPad, San Diego, CA, USA). All experiments were performed in triplicate.

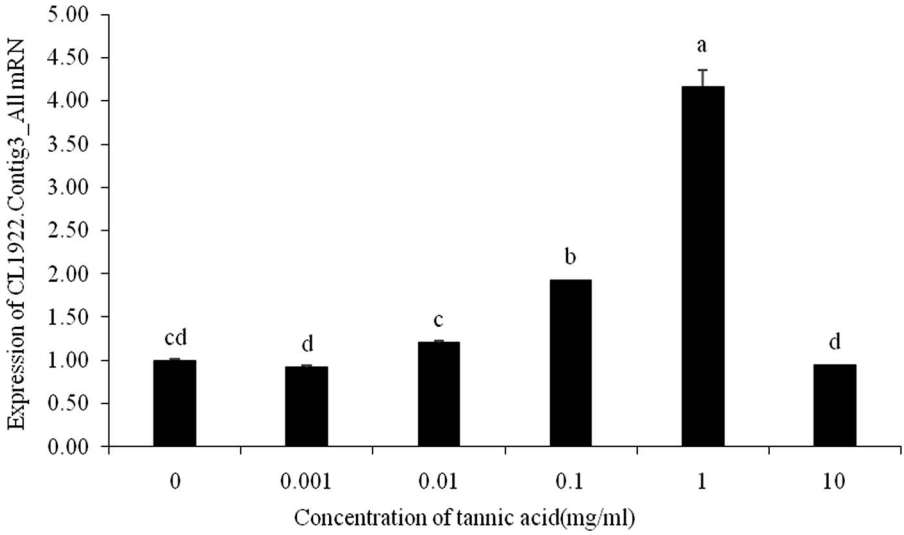

Fig. 3. The mRNA expressions of CL1922.Contig3_All in the midgut of *M. troglodyta* exposure to various concentrations of tannic acid. Means with the same lowercase letter are not significantly different (Tukey's test, $P < 0.05$). Data were analyzed using InStat software (GraphPad, San Diego, CA, USA). All experiments were performed in triplicate.

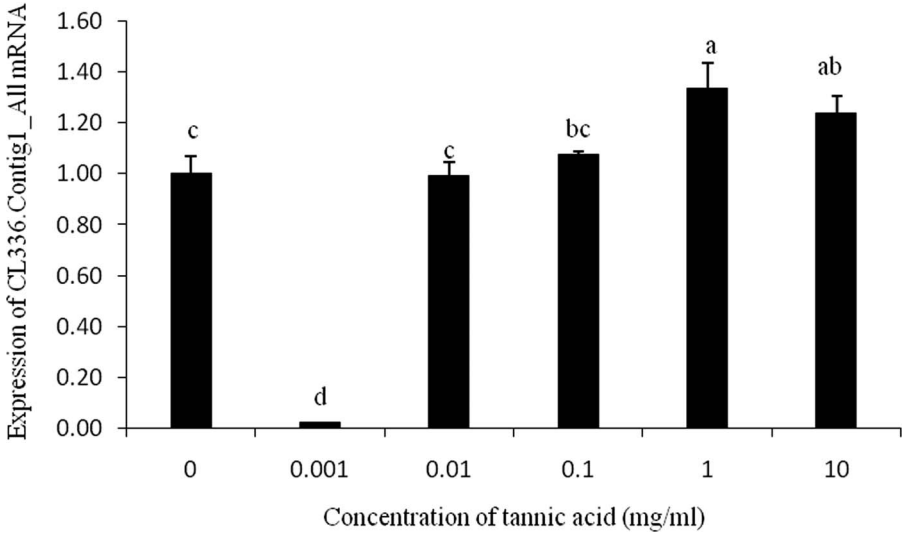

Fig. 4. The mRNA expressions of CL336.Contig1_All in the midgut of *M. troglodyta* exposure to various concentrations of tannic acid. Means with the same lowercase letter are not significantly different (Tukey's test, $P < 0.05$). Data were analyzed using InStat software (GraphPad, San Diego, CA, USA). All experiments were performed in triplicate.

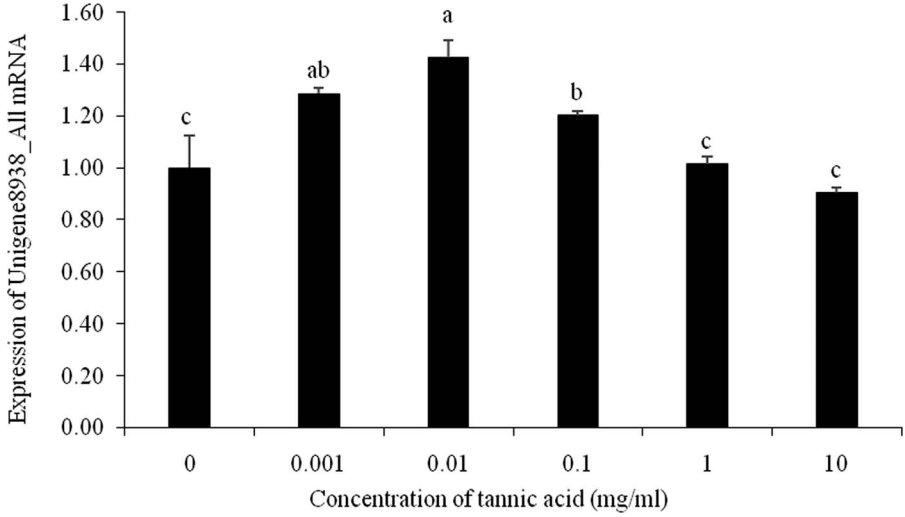

Fig. 5. The mRNA expressions of Unigene8938_All in the midgut of *M. troglodyta* exposure to various concentrations of tannic acid. Means with the same lowercase letter are not significantly different (Tukey's test, $P < 0.05$). Data were analyzed using InStat software (GraphPad, San Diego, CA, USA). All experiments were performed in triplicate.

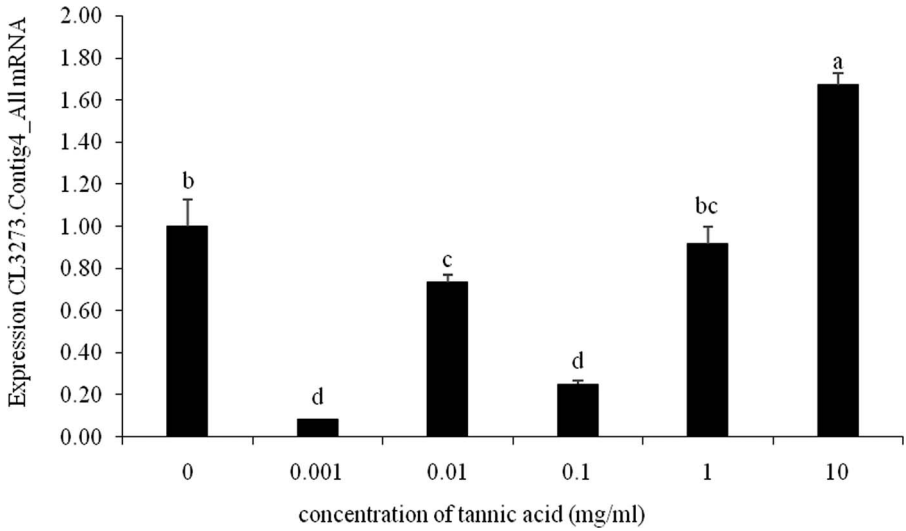

Fig. 6. The mRNA expressions of CL3273.Contig4_All in the midgut of *M. troglodyta* exposure to various concentrations of tannic acid. Means with the same lowercase letter are not significantly different (Tukey's test, $P < 0.05$). Data were analyzed using InStat software (GraphPad, San Diego, CA, USA). All experiments were performed in triplicate.

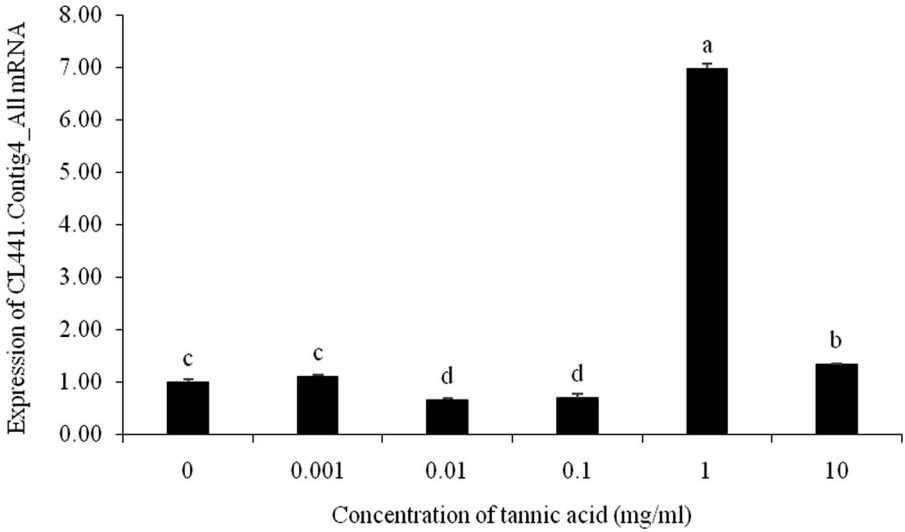

Fig. 7. The mRNA expressions of CL1441.Contig4_All in the midgut of *M. troglodyta* exposure to various concentrations of tannic acid. Means with the same lowercase letter are not significantly different (Tukey's test, $P < 0.05$). Data were analyzed using InStat software (GraphPad, San Diego, CA, USA). All experiments were performed in triplicate.

7). The mRNA expression of CL441.Contig5_All was induced by concentrations of 0.001, 0.1, and 1 mg/ml tannic acid, and it was restrained by 10 mg/ml ($F = 826.62$; df = 5,12; $P < 0.0001$) (Fig. 8).

The mRNA expression of Unigene27074_All was upregulated by tannic acid at all concentrations except the concentrations of 0.001 and 0.1 mg/ml ($F = 40905$; $df = 5,12$; $P < 0.0001$) (Fig. 9). Furthermore, the highest inducing multiple of Unigene27074_All mRNA was 367 in larvae exposed to 1 mg/ml tannic acid, and the expression level in larvae exposed to 10 mg/ml tannic acid was 72.6 times higher than that observed in the controls (Fig. 9).

Discussion

Among the major insect defense mechanisms, metabolic detoxification was the most important and was mainly accomplished by three different enzyme systems. More specifically, P450s were among these enzymes (Li 2015). Cytochrome P450 is a heme–thiolate protein (Mansuy 1998) with a characteristic absorbance peak at 450 nm when its reduced form was combined with carbon monoxide (Omura and Sato 1964). Insect cytochrome P450s are known to play an important role in detoxifying insecticides and plant toxins, and some insect P450 genes could be induced by exogenous and endogenous compounds. Increased P450 protein levels and P450 activities resulting from transcriptional upregulation of P450 genes have been shown to be involved in the enhanced metabolic detoxification in insects, leading to the development of resistance to insecticides (Cariño et al. 1994, Kasai et

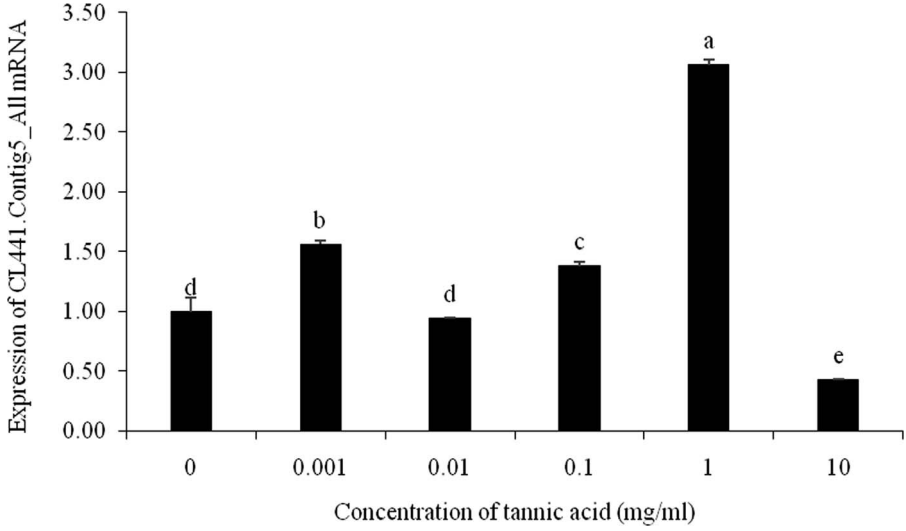

Fig. 8. The mRNA expressions of CL441.Contig5_All in the midgut of *M. troglodyta* exposure to various concentrations of tannic acid. Means with the same lowercase letter are not significantly different (Tukey's test, $P < 0.05$). Data were analyzed using InStat software (GraphPad, San Diego, CA, USA). All experiments were performed in triplicate.

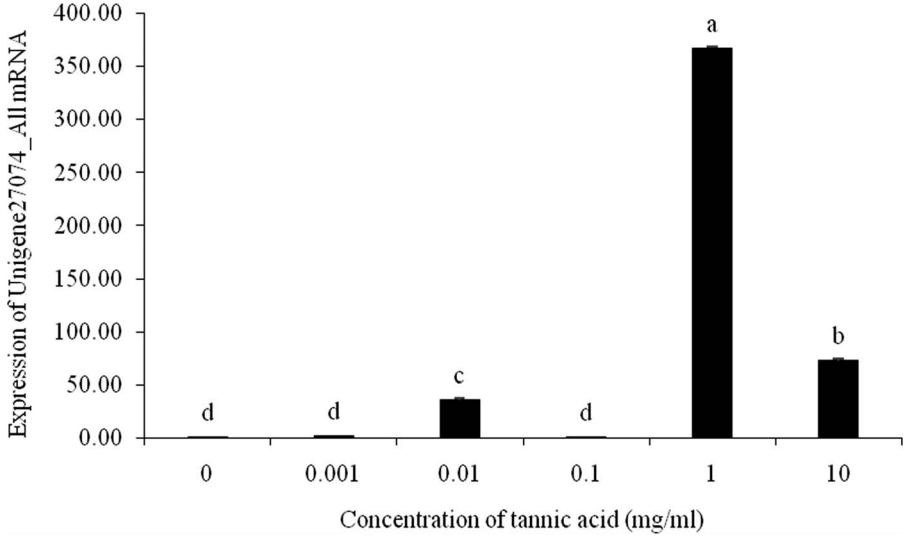

Fig. 9. The mRNA expressions of Unigene27074_All in the midgut of *M. troglodyta* exposure to various concentrations of tannic acid. Means with the same lowercase letter are not significantly different (Tukey's test, $P < 0.05$). Data were analyzed using InStat software (GraphPad, San Diego, CA, USA). All experiments were performed in triplicate.

al. 2000, Feyereisen 2005) and tolerance to plant toxins (Li et al. 2002, Wen et al. 2003).

An interesting characteristic of insect P450 genes is that the expression of some P450 genes could be induced by exogenous and endogenous compounds. This induction process appears to be an adaptation mechanism of organisms to counteract chemical stress (Chen 2003). The induction of cytochrome P450 by plant secondary metabolites has been confirmed, and the studies have been conducted at the molecular level (Danielson et al. 1997, Li et al. 2000, Snyder et al. 1995). Liu (2005) reported on plant allelochemical induction of insect cytochrome P450s by using furanocoumarin induction in *Papilio polyxenes* (Stoll), showing that the plant secondary metabolites significantly affected the expression of P450s. Tannic acid is characterized as a plant polyphenol and is commonly distributed in many plants. Our results suggest the mRNA expression of nine p450s in *M. troglodyta* was induced by tannic acid. We found that the mRNA expression of six p450s reached the maximum level in the midguts exposed to 1 mg/ml tannic acid, and the mRNA expression level of Unigene27074_All was 370 times higher than that observed in the controls. Furthermore, mRNAs of CL3273.Contig4_All and Unigene 27074_All were induced by tannic acid at the concentration of 10 mg/ml. These results were consistent with other reports, including the induction of *CYP6B6* mRNA in the midgut of *Helicoverpa armigera* (Hübner) by tannic acid (Liu 2005) and three p450 genes (*LmCYP6HL1*, *LmCYP6FG1*, and *LmCYP4C7*9) from *Locusta migratoria* (Meyen) by indole and 2-tridecanoe (Li 2015).

An important feature of P450s is the complexity of their substrate specificity due to existence of multiple forms that may have broad and overlapping substrates. As a consequence, P450s not only catalyze biosynthetic reactions to produce juvenile and ecdysteroid hormones that have essential physiological functions (Feyereisen 2005; Rewitz et al. 2006) but also catalyze detoxifying reactions with significant ecological and toxicological consequences (Scott and Wen 2001, Berenbaum 2002, Li et al. 2007). The first reports for the involvement of specific P450s in insecticide metabolism appeared in the 1990s showing that *CYP6D1*, *CYP6A1*, and *CYP12A1* of *Musca domestica* (L.) can detoxify the insecticides aldrin, heptachlor, and diazinon by *CYP6A1* (Andersen et al. 1994, Sabourault et al. 2001) and chlorpyrifos, chlorpyrifos oxon, deltamethrin, and cypermethrin by *CYP6D1* (Wheelock and Scott 1992, Korytko and Scott 1998), as well as aldrin, heptachlor, and diazinon by *CYP12A1* (Guzov et al. 1998). The expression of *Drosophila melanogaster* (Meigen) cytochrome P450 by a baculovirus expression system showed that cytochrome P450 was involved in the metabolism of pepper toxin (Cohen et al. 1992). Our results showed that P450s might have an effect on the metabolism of plant secondary substance.

In conclusion, we analyzed the mRNA expression of nine p450s in *M. troglodyta* fed tannic acid to characterize the induction effect of p450 by plant secondary compounds. Even though unprecedented progress has been made in the last decade, especially the last several years, in our understanding of the roles of individual P450 involved in insect interactions with insecticides, there is much to be learned to fully appreciate the complexity of P450 involvement in these interactions considering the large numbers of insect P450s involved and their complicated regulation schemes. Concerted research efforts in this area may provide improved

insight in developing specific strategies for the effective use of currently available insecticides or in developing new insect control agents (Liu and Kang 2011).

Acknowledgements

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References Cited

Andersen, J.F., J.G. Utermohlen and R. Feyereisen. 1994. Expression of house fly *CYP6A1* and ANDPH–cytochrome P450 reductase in *Escherichia coli* and reconstitution of an insecticide–metablizing P450 system. Biochem. 33: 2171–2177.

Berenbaum, M.R. 2002. Postgenomic chemical ecology: From genetic code to ecological interactions. J. Chem. Ecol. 28: 873–896.

Cariño, F.A., J.F. Koener, F.W. Plapp and R. Feyereisen. 1994. Constitutive overexpression of the cytochrome P450 gene *CYP6A1* in a house fly strain with metabolic resistance to insecticides. Insect Biochem. Mol. Biol. 24: 411–418.

Chen, F. J. 2003. Induction and gene cloning of glutathione S-transferases in *Helicoverpa armigera* (Hübner). PhD Diss., China Agricultural Univ., Beijing.

Cheng, H.P., F. Tang, W. Li and M. Xu. 2015. Tannic acid induction of a glutathione S-transferase in *Micromelalopha troglodyta* (Lepidoptera: Notodontidae) larvae. J. Entomol. Sci. 50: 350–362.

Cohen, M.B., M.A. Schuler and M.R. Berenbaum. 1992. A host-inducible cytochrome P450 from a host-specific caterpillar: Molecular cloning and evolution. Proc. Natl. Acad. Sci. USA. 89: 10920–10924.

Danielson, P.B., R.J. MacIntyre and J.C. Fogleman. 1997. Molecular cloning of a family of xenobiotic inducible drosophila cytochrome P450s: Evidence for involvement in host plant allelochemicals resistance. Proc. Natl. Acad. Sci. USA. 94: 10797–10802.

Després, L., J.P. David and C. Gallet. 2007. The evolutionary ecology of insect resistance to plant chemicals. Trends Ecol. Evol. 22: 298–307.

Feyereisen, R. 2005. Insect cytochrome P450. *In* Comprehensive Molecular Insect Science. Elsevier, Oxford.

Feyereisen, R. 2012. Insect CYP genes and P450 enzymes. *In* Insect Molecular Biology and Biochememistry. Elsevier, Oxford.

Gatehouse, J.A. 2002. Plant resistance towards insect herbivores: a dynamic interaction. New Phytol. 156: 145–169.

Giulietti, A., L. Oververgh, D. Valckx. B. Decallonne, R. Bouillon and C. Mathieu. 2001. An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. Methods 25: 386–401.

Guzov, V. M., G. C. Unnithan, A. A. Chernogolov and R. Feyereisen. 1998. CYP12A1, a mitochondrial cytochrome P450 from the house fly. Arch. Biochem. Biophys. 359: 231–240.

Hodgson, E., R. L. Rose, D.Y. Ryu, G. Falls, B.L. Blake and P.E. Levi. 1995. Pesticide-metabolizing enzymes. Toxicol. Lett. 82/83: 73–81.

Kasai, S., I.S. Weerashinghe, T. Shono and M. Yamakawa. 2000. Molecular cloning, nucleotide sequence and gene expression of a cytochrome P450 (*CYP6F1*) from the pyrethroid-resistant mosquito, *Culex quinquefasciatus* Say. Insect Biochem. Mol. Biol. 30: 163–171.

Korytko, P.J. and J.G. Scott. 1998. *CYP6D1* protects thoracic ganglia of houseflies from the neurotoxic insecticide cypermethrin. Arch. Insect Biochem. Physiol. 37: 57–63.

Li, W., R.A. Petersen, M.A. Schuler and M.R. Berenbaum. 2002. *CYP6B* cytochrome p450 monooxygenases from *Papilio canadensis* and *Papilio glaucus*: potential contributions of sequence divergence to host plant associations. Insect Mol. Biol. 11: 543–551.

Li, X.C., M.R. Berenbaum and M.A. Schuler. 2000. Molecular cloning and expression of *CYP6B8*: A xanthotoxin-inducible cytochrome P450 cDNA from *Helicoverpa zea*. Insect Biochem. Mol. Biol. 30: 75–84.

Li, X., M. A. Schuler and M. R. Berenbaum. 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu. Rev. Entomol. 52: 231–253.

Li, Y.H. 2015. Cytochrome P450s from the *Locusta migratoria*: Responses to plant allelochemicals and pesticides. MS Thesis, Shanxi Univ., Shanxi

Liu, T.X and L. Kang. 2011. Recent advances in entomological research: from molecular biology to pest management. Higher Education Press, Springer.

Liu, X.N. 2005. Cytoehrome P450s induced by allelochemieals, gene cloning and expression of *CYP6B6* in *Helicoverpa armigera* (Hübner). PhD Diss., China Agrieultural Univ., Beijing.

Mansuy, D. 1998. The great diversity of reactions catalyzed by cytochromes P450. Comp. Biochem. Phys. C. 121: 5–14.

Mazumdar-Leighton, S. and R.M. Broadway. 2001. Identification of six chymotrypsin cDNAs from larval midguts of *Helicoverpa zea* and *Agrotis ipsilon* feeding on the soybean (Kunitz) trypsin inhibitor. Insect Biochem. Mol. Biol. 31: 633–644.

Omura, T. and R. Sato. 1964. The carbon monoxide–binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J. Biol. Chem. 239: 2370–2378.

Rewitz, K.F., R. Rybczynski, J.T. Warren and L.I. Gilbert. 2006. The Halloween genes code for cytochrome P450 enzymes mediating synthesis of the insect moulting hormone. Biochem. Soc. Trans. 34: 1256–1260.

Sabourault, C., V.M. Guzov, J. F. Koener, C. Claudianos, F.W. Plapp and R. Feyereisen. 2001. Overproduction of a P450 that metabolizes diazinon is linked to a loss–of–function in the chromosome 2 ali–esterase (MdαE7) gene in resistant house flies. Insect Mol. Biol. 10: 609–618.

Scott, J.G and Z.M. Wen. 2001. Cytochromes P450 of insects: The tip of the iceberg. Pest Manag. Sci. 57: 958–967.

Snyder, M.J., J.L. Stevens, J.F. Andersen and R. Feyeerisen. 1995. Expression of cytochrome P450 genes of the CYP4 family in midgut and fat body of tobacco hornworm *Manduca sexta*. Arch. Biochem. Bipohys. 321: 13–20.

Waters, L.C., A.C. Zelhof, B.J. Shaw and L.Y. Chang. 1992. Possible involvement of the long terminal repeat of transposable element 17.6 in regulating expression of an insecticide resistance-associated P450 gene in *Drosophila*. Proc. Natl. Acad. Sci. USA. 89: 4855–4859.

Wen, Z.M., L.P. Pan, M.R. Berenbaum and M.A. Schuler. 2003. Metabolism of linear and angular furanocoumarins by *Papilio polyxenes CYP6B1* co-expressed with NADPH cytochrome P450 reductase. Insect Biochem. Mol. Biol. 33: 937–947.

Wheelock, G.D and J.G. Scott. 1992. The role of cytochrome $P450_{lpr}$ in deltamethrin metabolism by pyrethroid-resistant and susceptible strains of house flies. Pestic. Biochem. Physiol. 43: 67–77.

Wittstock, U., N. Agerbirk, E.J. Stauber, C.E. Olsen, M. Hippler, T. Mitchell-Olds, J. Gershenzon and H. Vogel. 2004. Successful herbivore attack due to metabolic diversion of a plant chemical defense. Proc. Natl. Acad. Sci. USA. 101: 4859–4864.

Zhang, X.B., F. Tang, Y.S. Liu and X.W. Gao. 2009. Induction of glutathione S-transferases by tannic acid in *Micromelalopha troglodyta*. China. Bull. Entomol. 46: 579–584.