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组织工程与细胞培养

中国红豆杉细胞经重复诱导和蔗糖饲喂后云南紫杉烷 C 生产的相应基因表达变化

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摘 要：红豆杉悬浮培养细胞具有可持续生产抗癌药物紫杉醇及其他紫杉烷的潜力。在中国红豆杉悬浮培养细胞中，云南紫杉烷 C(Tc) 是主要的次生代谢产物。为促使代谢前体由生成其他紫杉烷的代谢支路转到生产紫杉醇，实验采用实时定量 PCR 技术 (RQ-PCR) 揭示细胞培养过程中紫杉醇及紫杉烷合成关键基因的动态变化。在细胞培养的第 7 天和第 12 天，以 100 μmol/L 2,3-二羟丙基茉莉酸 (DHPJA) 进行诱导，同时在第 7 天饲喂 20 g/L 的蔗糖，在此过程中考察 6 个关键基因 (*TASY*，*TDAT*，*T5αH*，*TαH*，*T10βH* 和 *T14βH*) 的表达变化。上述联合调控手段使得 Tc 产量在第 1 次诱导 8 d 后达 (554.46±21.28) mg/L，第 2 次诱导 9 d 后高达 (997.72±1.51) mg/L。代谢早期基因 *TASY* 和 *TDAT* 在第 1 次诱导后表达量分别提高了 182 和 98 倍，在第 2 次诱导后表达量分别提高了 208 和 131 倍。在每次诱导后基因表达量提高约持续 24 h，之后下降。其他 4 个基因 (*T5αH*、*TαH*、*T10βH* 和 *T14βH*) 的情况有所不同。基因 *TαH* 在 2 次诱导后表达量分别提高了 3 061 和 1 016 倍。其他 3 个基因 *T5αH*、*T10βH*、*T14βH* 在第 1 次诱导后表达量分别提高 13、38、20 倍，在第 2 次诱导后分别提高 7、16、6 倍。RQ-PCR 结果表明基因表达和 Tc 积累之间存在紧密相关性：基因表达的变化与 Tc 产量的变化相一致，诱导可提高 6 个基因的表达量。基因的高表达随着培养过程逐渐衰减，再次诱导可再次促使基因的高表达。

关键词：中国红豆杉，云南紫杉烷 C，2,3-二羟丙基茉莉酸，实时定量 PCR

Expression profiling of genes involved in Taxuyunnanine C biosynthesis in cell suspension cultures of *Taxus chinensis* by repeated elicitation with a newly synthesized jasmonate and sucrose feeding

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Abstract: Taxus suspension cell culture has the potential to provide a sustainable source of anticancer drug paclitaxel (Taxol®) and other taxoids. In the cell culture of *Taxus chinensis*, Taxuyunnanine C (Tc) is the primary taxoid. To design a rational strategy for redirecting the precursor fluxes from other taxoids into paclitaxel production, we employed Real-time Quantitative PCR (RQ-PCR) to understand the dynamic profiling of key biosynthetic pathway genes of palcitaxel and taxoids during the culture process. Six genes (*TASY*, *TDAT*, *T5αH*, *TαH*, *T10βH* and *T14βH*) were quantified under the process condition of double elicitation by 2,3-dihydroxylpropanyl jasmonate (DHPJA) (100 μmol/L on day 7 and day 12), and sucrose feeding (20 g/L) on day 7. This process treatment led to a high accumulation of Tc at (554.46±21.28) mg/L 8 days after the first elicitation. Then 9 days after the second elicitation, Tc production was as high as (997.72±1.51) mg/L. The early pathway genes *TASY* and *TDAT* were significantly up-regulated by 182-fold and 98-fold, respectively for the first DHPJA elicitation and by 208-fold and 131-fold, respectively for the second elicitation. The induction occurred after each elicitation lasted for about 24 h before their abundances decreased. Things are somewhat different in the case of the other four genes *T5αH*, *TαH*, *T10βH* and *T14βH*. For gene TαH, it was highly up-regulated by 3061-fold for the first DHPJA elicitation and by 1016-fold for the second elicitation. For the other three genes *T5αH*, *T10βH*, *T14βH*, they were up-regulated by 13-fold, 38-fold and 20-fold, respectively for the first DHPJA elicitation and by 7-fold, 16-fold and 6-fold, respectively for the second elicitation. The RQ-PCR results showed that there is tight correlation between gene expression and Tc accumulation. Gene expression was in accordance with Tc yield. Elicitation could improve expression of six genes. While along with culture course, high expression of the genes weakened. Elicitation for the second time would promote high expression of the genes again.

Keywords: *Taxus chinensis*, Taxuyunnanine C, 2,3-dihydroxylpropanyl jasmonate, Real-time Quantitative PCR

Paclitaxel (Taxol®, Bristol-Myers Squibb), a famous anti-cancer drug, is one side-chain taxane, which is structurally more complex and representative of more than 350 taxoids[1-2]. At present, semi-synthesis is the main source of paclitaxel: 10-deacetylbaccatin-III, a paclitaxel precursor that is harvested from Taxus needles, is converted to paclitaxel *via* chemical synthesis. Another source approved by FDA was through plant cell culture, which is environment friendly and is expected to contribute significantly for the sustainable supply of paclitaxel[3].

Introduction

A noticeable phenomenon is that considerable precursors fluw to taxoid rather than paclitaxel in both intact Taxus tissues[4] and derived cell cultures[5]. Among the taxoids, 14β-hydroxy taxoids (Tc and its relatives) are most usual. C-14 hydroxylation is probably the early sideway of taxane synthesis. Taxol® has no oxygen substitute at C-14. Tc is a novel compound which has a neuron growth factor (NGF)-like activity[6].

A clear understanding of paclitaxel biosynthetic pathway regulation is necessary to develop a superior strain for the supply of paclitaxel or regulate (either up or down) the genes that encode Taxol® or taxoid pathway steps. Although the taxane biosynthetic pathway is not fully clarified, the first three steps of paclitaxel biosynthesis have been elucidated. Derived from isoprenyl diphophate (IPP) and dimethylallyl diphosphate (DAPP), geranylgeranyl pyrophosphate (GGPP) is synthesized by geranylgeranyl pyrophosphate synthase (GGPPS)[7]. GGPP is converted to taxa 4(5),11(12)-diene by taxadiene synthase (TASY) to establish the taxane ring[8-10] and then to taxa 4(20),11(12)-dien-5α-ol by taxadiene 5α hydroxylase (T5αH)[11]. The first hydroxylation at the C 5α-position, with allylic migration of the double bond occurred. A branch occurred at this point[12-13]. Taxadiene 13α-hydroxylase (TαH) converts taxa 4(20), 11(12)-dien-5α-ol to taxa 4(20), 11(12)-dien-5α,13α-diol[14]. The alternate branch in the pathway implements taxadiene 5α-ol O-acetyltransferase (TDAT) to form 4(20), 11(12)-dien-5α-yl acetate from 4(20), 11(12)-dien-5α-ol, which is then further converted to 4(20), 11(12)-dien-5α-acetoxy-10β-ol by taxane 10β hydroxylase (T10βH). Only taxanes with

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C-5 acetyl oxygen substitute and C-10 hydroxylation can be catalyzed by Taxane 14β-hydroxylase (T14βH) to form C-14 hydroxyl substitute. The cytochrome P450 taxoid 14β-hydroxylase, principally utilizes the C_5-acetate esters of 5α-hydroxytaxadiene and 5α, 10β-dihydroxytaxadiene as preferred substrates[15]. The steps leading from the acetate or the diol intermediates to functionalized taxanes are unknown.

The last steps have been already characterized by direct cloning methods. 10-deacetylbaccatin III, the important semi-synthetic precursor of paclitaxel, is produced *via* taxane 2α-O-benzoyltransferase (DBBT). Then 10-DAB is converted to baccatin-III by 10-deacetylbaccatin-III-10-O-acetyltransferase (DBAT). Baccatin III: 3-amino, 3-phenylpropanoyltransferase (BAPT) ligates phenylisoserine (derived from phenylalanine *via* phenylalanine amin-omutase (PAM)) to baccatin III to produce 3’-N-debenzoyl-2-deoxytaxol. 3’-N-debenzoyl-2-deoxytaxol-N-benzoyltransferse (DBTNBT) ligates a benzoyl CoA group to 3’-N-debenzoyl-2-deoxytaxol to produce 2’-deoxytaxol. Finally, benzamidation of 2’-deoxytaxol yields paclitaxel[16-19].

Methyl jasmonate (MJA) is commonly used to stimulate paclitaxel or taxane accumulation in Taxus plant cell culture. 2,3-dihydroxylpropanyl jasmonate (DHPJA), a MJA derivative synthesized by Qian[20], has been proved to have the ability to greatly enhance Tc accumulation[21].

In this paper, we examined the regulation of Tc biosynthetic pathway using a DHPJA responsive, well-characterized *T. chinensis* cell line. DHPJA was used twice to induce Tc synthesis in combination with sucrose feeding. The expression of 6 known pathway genes was examined by RQ-PCR. Tc was quantified *via* HPLC. We found that unelicited cultures produce low levels of Tc while DHPJA-elicited cultures accumulate much more Tc. The regulation of taxane biosynthetic pathway is proved to occur at the level of mRNA and that there is a tight correlation between steady-state transcript abundance and respective taxane accumulation.

1 Materials and methods

1.1 Plant cell line and suspension subcultures

T. chinensis cell line was used for all experiments and obtained as a gift from Prof. Jianjiang Zhong of Shanghai Jiaotong University. Suspensions were subcultured every 2 weeks in Murashige and Skoog medium basal salts with 30 g/L sucrose, supplemented with 0.5 mg/L of 6-benzyladenine (6-BA), 0.2 mg/L of 2,4-dichlorophenoxy-acetic acid (2,4-D), 0.5 mg/L of naphthaleneacetic acid (NAA) and 100 mg/L of ascorbic acid. The pH was adjusted to 5.8 before autoclaving. Cultures were maintained in a 500-mL Erlenmeyer flask containing 100 mL medium and cultured on a rotary shaker at 110 r/min and kept at (25±1) °C in the dark.

1.2 Double elicitation and sucrose feeding

For shake-flask cultures, 2 g fresh cells were inoculated in a 100-mL Erlenmeyer flask containing 20 mL medium with the same culture conditions as that of subcultures. On day 7, DHPJA was added to the cultures in 1 μL of ethanol per mL of culture medium at 100 μmol/L after being sterilized by filtering through 0.22 μm polyvinylidenedifluoride (PVDF) syringe filters (Millipore). The same volume of ethanol was added to the control. Simultaneously twenty grams per liter sterile sucrose were feeded on day 7. On day 12, DHPJA was added for the second time at 100 μmol/L. The cells were collected on day 0, 7, 7.5, 8, 9, 12, 12.5, 13, 15, 18, 21, 24 and 30 for analysis. All experiments were performed in triplicate and the data are expressed as the means of three samples with standard deviation.

1.3 Taxane extraction from culture samples and quantification *via* HPLC

Taxane extraction and determination by HPLC were done according to the methodology described by Zhang[22].

For the extraction of taxane, 100 mg of powdered dry cells were soaked in 4-mL methanol and dichloromethane (1:1, *V*/*V*), then ultrasonicated for 30 minutes for six times. After centrifugation at 4 000 r/min for 10 min, the extract was evaporated to dryness at 25 °C by a rotary evaporator. The residue was dissolved in 4 mL dichloromethane and 1 mL distilled water and extracted for four times. After sufficient mixing, the mixture was centrifuged at 4 000 r/min for 10 min. The organic phase (the bottom layer) was collected and evaporated to dryness at 25 °C by a rotary evaporator. The residue was dissolved in 1 mL chromatograph- pure methanol and filtered through a

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0.22 μm PVDF syringe filter (Millipore).

A volume of 10 μL was analyzed by reverse-phase HPLC, using a Hewlett-Packard series 1100 HPLC system (Agilent). An alkyl phenyl column (250 mm× 4.6 mm, 5 μm) was used at 25 °C. The mobile phase consisted of acetonitrile and water (58:42, *V*/*V*), and the flow rate was 1 mL/min. Taxane was monitored at 227 nm by using authentic standards as the reference.

1.4 RNA extraction and cDNA synthesis

Cells were collected by filtration from the media at low pressure and stored at −80 °C in polypropylene tubes. RNA was extracted using TRIZOL® reagent (Invitrogen life technologies) according to their handbook instructions. RNA quality detection was *via* formaldehyde denaturation agarose gel electrophoresis.

1.5 Probe creation

Probes for RQ-PCR were created by amplifying gene fragments from *T. chinensis* genomic DNA. Primers were designed from previously cloned cDNAs in regions conserved amongst *T. brevifolia* and are listed in Table 1. Amplification of fragments *via* PCR was performed using PCR Thermal Cycler (TaKaRa, Japan).

1.6 RQ-PCR

Quantification of total RNA was performed with Rotor-Gene 3000 Realtime PCR (Corbett Research). PCR reagents were as follows: 2.5 mmol/L dNTP (dATP, dGTP, dCTP and dTTP, 2.5 mmol/L each) (HyTest Ltd); 10×PCR buffer (Promega); 25 mmol/L $MgCl_2$ (Promega); *Taq* polymerase (Promega); 100 bp DNA ladder (Tiangen Biotech Co., LTD, Beijing.); 10 000× Sybergreen (Molecular Probes).

PCR reaction system consisted of 25 μL, conditions were set as follows: 40 PCR cycles (94 °C, 20 s; annealing temperature, 20 s; 72 °C, 30 s); 72 °C elongated 5 min.

RQ-PCR conditions of housekeeping gene GAPDH and the other six genes are listed in Table 2. In order to establish melting curve of PCR products, temperature was raised slowly from 72 °C to 99 °C (1 °C increment every 5 seconds) after the completion of amplification reactions.

Table 1 Primers used to amplify gene fragments from *T. brevifolia*

Gene	GenBank Accession No.	Primer (5′−3′)	Denaturing temperature (°C)	Length (bp)
GAPDH		F: CGGAGACAGTCGATCAAGC R: CCCATCCTCAACCCAATAA	58	210
TASY	U48796	F: AACGGGCGGTTCTTCTCCTC R: GCATCGTCCATAGCTCCTTCG	58	113
T5αH	AY289209	F: TCCTATGACACCACCACTTCG R: GTGATTTCTTCGCCCTCCTC	59	131
TDAT	AF190130	F: GGAGCCGATTGGGATTTG R: CGACGCACTTTCATACTTTAGC	59	240
T10βH	AF318211	F: CCCTTACCCTCGCACCTAT R: AGGCCCGCAGAGTACAAC	59	242
TαH	AY056019	F: CGGGAATCCAACGCCACAT R: TGCGACGGAGAAGACGAGGT	62	92
T14βH	AY188177	F: CTGTCGGTGCATCATTTTGTT R: CTTGACCTGGTTGCCTGTG	59	159

Table 2 RQ-PCR conditions of housekeeping gene *GAPDH* and six selected genes

Gene	Denaturing temperature and time	Cycles	Denaturing temperature and time	Annealing temperature and time	Elongating temperature and time	Fluorescence collecting temperature and time
GAPDH	95 °C, 4 min	35	95 °C, 10 s	58 °C, 15 s	72 °C, 20 s	79 °C, 15 s
TASY	95 °C, 4 min	35	95 °C, 10 s	58 °C, 15 s	72 °C, 20 s	83 °C, 15 s
T5αH	95 °C, 4 min	35	95 °C, 10 s	59 °C, 15 s	72 °C, 20 s	79 °C, 15 s
TDAT	95 °C, 4 min	35	95 °C, 10 s	59 °C, 15 s	72 °C, 20 s	81 °C, 15 s
T10βH	95 °C, 4 min	35	95 °C, 10 s	59 °C, 15 s	72 °C, 20 s	81.5 °C, 15 s
TαH	95 °C, 4 min	35	95 °C, 10 s	62 °C, 15 s	72 °C, 20 s	80 °C, 15 s
T14βH	95 °C, 4 min	35	95 °C, 10 s	59 °C, 15 s	72 °C, 20 s	81 °C, 15 s

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2 Results

2.1 Time course of dry cells and Tc accumulation

Fig. 1 and Fig. 2 showed the time courses of dry cells and Tc accumulation. As to dry cell weight, before day 18, the dry cell weight of double elicitation and sugar feeding was lower than that of control. While after day 18, due to sugar feeding on day 7, the dry cell weight of double elicitation and sugar feeding was higher than that of control.

A high accumulation of Tc was obtained at (554.46±21.28) mg/L 8 days after the first elicitation. And 9 days after the second elicitation, Tc production was as high as (997.72±1.51) mg/L.

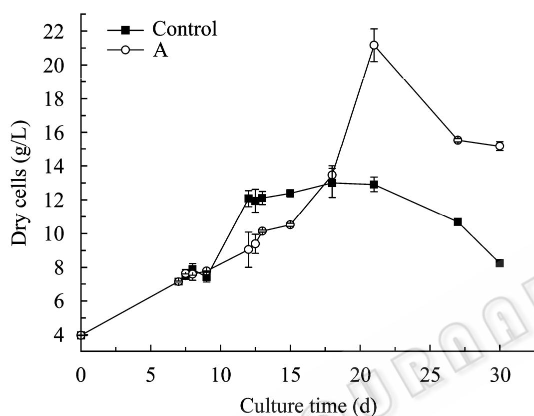

Fig. 1 Dry cell weight of *T. chinensis* cell suspension cultures under control and double DHPJA elicitation with sucrose feeding (A). Control: day 7 20 μL EtOH+0.5 mL H_2O, day 12 20 μL EtOH; A: day 7 100 μmol/L DHPJA+20 g/L sucrose, day 12 100 μmol/L DHPJA.

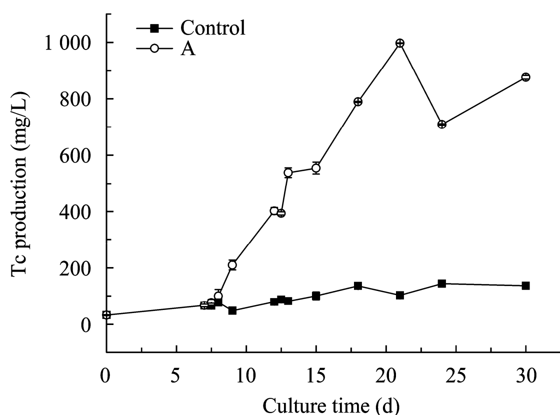

Fig. 2 Tc production of *T. chinensis* cell suspension cultures under control and double DHPJA elicitation with sucrose feeding (A).

2.2 Time courses of mRNA accumulation

As shown in Fig. 3 and Fig. 5, the early pathway genes *TASY* and *TDAT* were significantly up-regulated by 182-fold and 98-fold, respectively for the first DHPJA elicitation and by 208-fold and 131-fold, respectively for the second elicitation. The induction after each elicitation lasted for about 24 h before their abundances decrease. The mRNA levels of *TASY* and *TDAT* increased upon DHPJA elicitation at day 7, by reaching maximal levels at 24 h, then declined till another elicitation. The second peak was reached 24 h after DHPJA second elicitation at day12, then declined sharply.

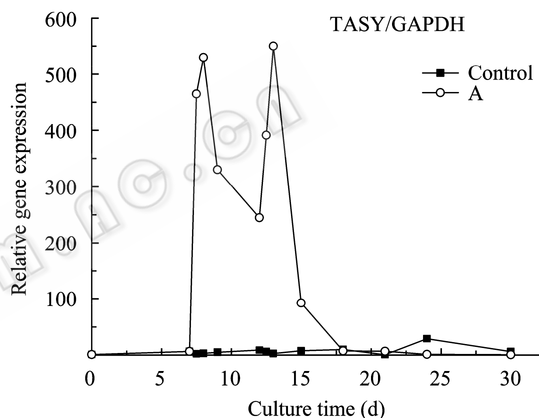

Fig. 3 TASY mRNA expression of *T. chinensis* cell suspension cultures under control and double DHPJA elicitation with sucrose feeding (A).

In the case of the other four genes *T5αH*, *TαH*, *T10βH* and *T14βH*, things are somewhat different. For gene TαH, it was greatly up-regulated by 3 061-fold for the first DHPJA elicitation and by 1016-fold for the second elicitation, as shown in Fig. 7. The first and second expression peak was reached 12 h and 24 h after each elicitation respectively. For the other three genes *T5αH*, *T10βH*, *T14βH*, they were up-regulated by 13-fold, 38-fold and 20-fold, respectively for the first DHPJA elicitation and by 7-fold, 16-fold and 6-fold, respectively for the second elicitation, as shown in Fig. 4, Fig. 6 and Fig. 8, respectively.

3 Discussion

The RQ-PCR results showed that there is tight correlation between gene expression and Tc accumulation. Changes of gene expression were in accordance with

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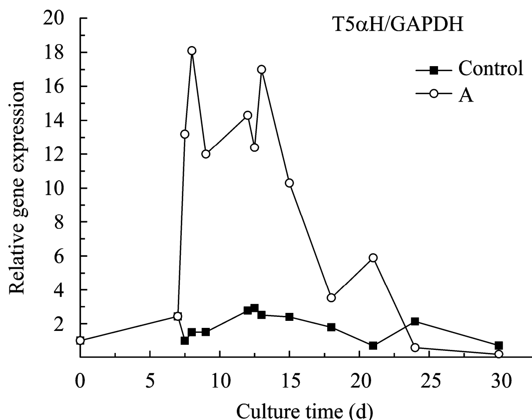

Fig. 4 T5αH mRNA expression of *T. chinensis* cell suspension cultures under control and double DHPJA elicitation with sucrose feeding (A).

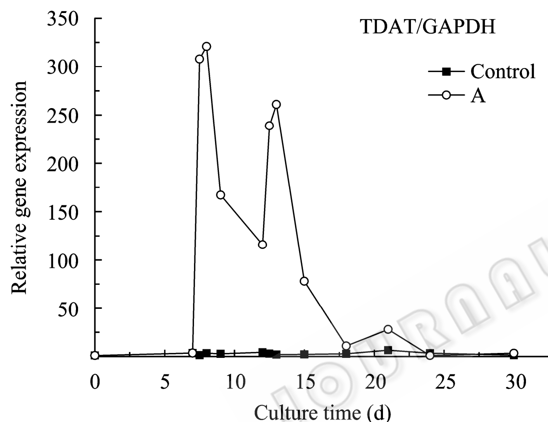

Fig. 5 TDAT mRNA expression of *T. chinensis* cell suspension cultures under control and double DHPJA elicitation with sucrose feeding (A).

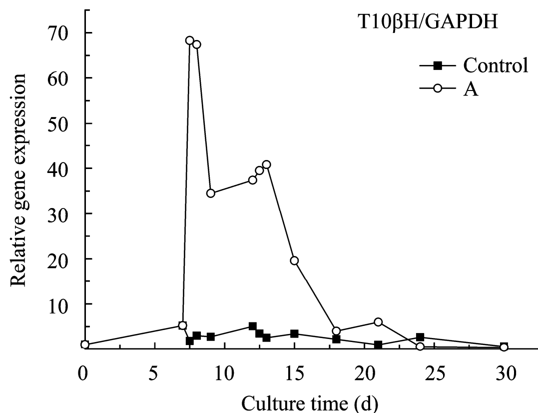

Fig. 6 T10βH mRNA expression of *T. chinensis* cell suspension cultures under control and double DHPJA elicitation with sucrose feeding (A).

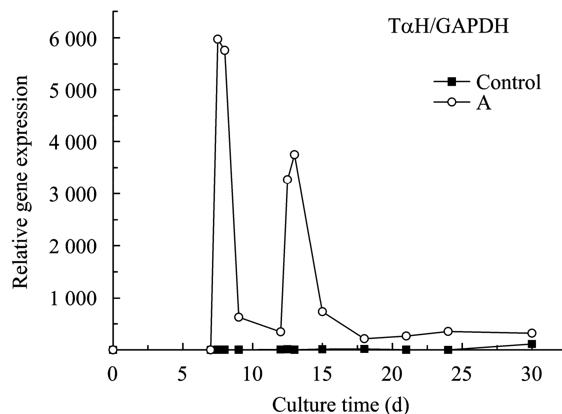

Fig. 7 TαH mRNA expression of *T. chinensis* cell suspension cultures under control and double DHPJA elicitation with sucrose feeding (A).

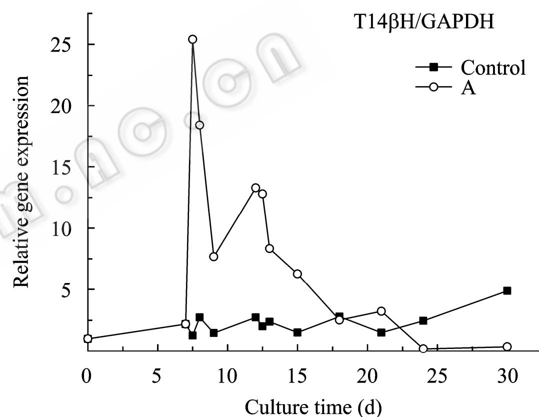

Fig. 8 T14βH mRNA expression of *T. chinensis* cell suspension cultures under control and double DHPJA elicitation with sucrose feeding (A).

Tc yield changes. Elicitation could improve expression of six genes. While along with culture course, high expression of the genes weakened. Elicitation for the second time would promote high expression of the genes again.

When *T. chinensis* cell culture was elicited with DHPJA, there is a preference towards one side of the taxane biosynthetic pathway branch—13α-hydroxylation which coincides with the research of Ezekiel's[3]. While 13α-hydroxylated paclitaxel did not accumulate in our cell culture, this is because on one hand, TαH expression level is too low in the uninduced cell line. On the other hand, paclitaxel could only be produced after multiple steps behind 13α-hydroxylation. While 14α-hydroxylated Tc accumulated to a greater extent when cell cultures were treated with double elicitation

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and sucrose feeding because Tc yield was relatively high in the uninduced cell line. Our research work suggest that future efforts to enhance paclitaxel accumulation in Taxus cell suspension cultures should first focus on improving the *TαH* expression more greatly.

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REFERENCES

[1] Baloglu E, Kingston DGI. The taxane diterpenoids. J Nat Prod, 1999, 62(10): 1448−1472.

[2] Itokawa H. Taxoids occuring in the genus Taxus//Itokawa H, Lee KH, eds. Taxus-The Genus Taxus. London, UK: Taylor & Francis, 2003: 35−78.

[3] Nims E, Dubois CP, Roberts SC, et al. Expression profiling of genes involved in paclitaxel biosynthesis for targeted metabolic engineering. Metab Eng, 2006, 8: 385−394.

[4] Kikuchi Y,Yatagai M. The commercial cultivation of Taxus species and production of taxoids//Itokawa H, Lee KH, eds. London, UK: Taxus-The Genus Taxus. Taylor & Francis, 2003:151−178.

[5] Takeya K. Plant tissue culture of taxoids//Itokawa H, Lee KH, eds. Taxus-The Genus Taxus. London, UK: Taylor & Francis, 2003: 134−150.

[6] Zhao ZJ, Xu YF, Qian ZG, et al. Novel fluoro- and hydroxyl-containing jasmonate derivatives as highly efficient elicitors in suspension cultures of *Taxus chinensis*. Bioorg Med Chem Lett, 2004, 14(18): 4755−4758.

[7] Hefner J, Rubenstein SM, Ketchum REB, et al. Cytochrome P450-catalyzed hydroxylation of taxa-4(5), 11(12)-diene to taxa-4(20),11(12)-dien-5a-ol: the first oxygenation step in Taxol biosynthesis. Chem Biol, 1996, 3(6): 479−489.

[8] Koepp AE, Hezari M, Zajicek J, et al. Cyclization of geranylgeranyl diphosphate to taxa-4(5), 11(12)-diene is the committed step of taxol biosynthesis in Pacific yew. J Biol Chem, 1995, 270(15): 8686−8690.

[9] Wildung MR, Croteau R. A cDNA Clone for Taxadiene synthase, the diterpene cyclase that catalyzes the committed step of Taxol biosynthesis. J Biol Chem, 1996, 271(16): 9201−9204.

[10] Wang W, Shi Q, Zhu P, et al. cDNA cloning, expression and characterization of taxadiene synthase, a diterpene cyclase from *Taxus chinensis*. Acta Bot Sin, 2002, 44(2): 181−187.

[11] Jennewein S, Long RM, Williams RM, et al. Cytochrome P450 taxadiene 5α-hydroxylase, a mechanistically unusual monooxygenase catalyzing the first oxygenation step of Taxol biosynthesis. Chem Biol, 2004, 11(3): 379−387.

[12] Ketchun RE, Rithner CD, Qiu D, et al. *Taxus* metabolomics: methyl jasmonate preferentially induces production of taxoids oxygenated at C-13 in *Taxus* x *media* cell cultures. Phytochemistry, 2003, 62: 901−909.

[13] Ketchum RE, Horiguchi T, Qiu D, et al. Administering cultured Taxus cells with early precursors reveals bifurcations in the taxoid biosynthetic pathway. Phytochemistry, 2007, 68(3): 335−341.

[14] Jennewein S, Croteau R. Taxol: biosynthesis, molecular genetics and biotechnological applications. Appl Microbiol Biotechnol, 2001, 57(1/2): 13−19.

[15] Jennewein S, Rithner CD, Williams RM, et al. Taxoid metabolism: taxoid 14β-hydroxylase is a cytochrome P450-dependent monooxygenase. Arch Biochem Biophys, 2003, 413(2): 262−270.

[16] Walker KD, Floss HG. Detection of a phenylalanine aminomutase in cell-free extracts of *Taxus brefolia* and preliminary characterization of its reaction. J Am Chem Soc, 1998, 120(21): 5333−5334.

[17] Walker KD, Klettke K, Akiyama T, et al. Cloning, Heterologous Expression and characterization of a phenylalanine aminomutase involved in Taxol biosynthesis. J Biol Chem, 2004, 279(52): 53947−53954.

[18] Walker K, Long R, Croteau R. The final acylation step in taxol biosynthesis: cloning of the taxoid C13-side chain-N-benzoyl- transferase from Taxus. Proc Natl Acad Sci USA, 2002, 99(14): 9166−9171.

[19] Walker K, Fujisaki S, Long R, et al. Molecular cloning and heterologous expression of the C-13 phenylpropanoid side chain-CoA acyltransferase that functions in Taxol biosynthesis. Proc Natl Acad Sci USA, 2002, 99(20): 12715−12720.

[20] Qian ZG, Zhao ZJ, Xu YF, et al. Novel chemically synthesized hydroxyl-containing jasmonates as powerful inducing signals for plant secondary metabolism. Biotechnol Bioeng, 2004, 86(7): 809−816.

[21] Qian ZG, Zhao ZJ, Xu YF, et al. Highly efficient strategy for enhancing taxoid production by repeated elicitation with a newly synthesized jasmonate in fed-batch cultivation of *Taxus chinensis* cells. Biotechnol Bioeng, 2005, 90(4): 516−521.

[22] Zhang CH, Mei XG, Liu L, et al. Enhanced paclitaxel production induced by the combination of elicitors in cell suspension cultures of *Taxus chinensis*. Biotechnol Lett, 2000, 22(19): 1561−1564.

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