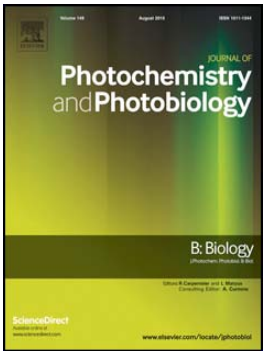

Synergistic effects of ultraviolet-B and methyl jasmonate on tanshinone biosynthesis in *Salvia miltiorrhiza* hairy roots

Cong Hui Wang, Li Ping Zheng, Hao Tian, Jian Wen Wang

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Corresponding author:

Dr. Jian Wen WANG

College of Pharmaceutical Sciences

Soochow University

Suzhou, 215123, China

E-mail address: jwwang@suda.edu.cn, bcjwwang@gmail.com

Tel.: +86 512 69561430

Fax: +86 512 65882089

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Synergistic effects of ultraviolet-B and methyl jasmonate on tanshinone biosynthesis in *Salvia miltiorrhiza* hairy roots

Cong Hui Wang [a], Li Ping Zheng [b], Hao Tian [a,c], Jian Wen Wang [a,*]

[a] *College of Pharmaceutical Sciences, Soochow University, Suzhou 215123, China*

[b] *Department of Horticulture, Soochow University, Suzhou 215123, China*

[c] *Institute of Medicinal Plants, Yunnan Academy of Agricultural Sciences, Kunming 650205, China*

ABSTRACT

Tanshinones are major bioactive diterpenoids of *Salvia miltiorrhiza* roots used for the treatment of cardiocerebral diseases. To develop effective elicitation and bioprocess strategies for the enhanced production of tanshinones, ultraviolet-B (UV-B) irradiation and methyl jasmonate (MeJA) elicitation were applied alone or in combination respectively in *S. miltiorrhiza* hairy root cultures. Our results showed 40-min UV-B irradiation at 40 μW/cm^2 stimulated tanshinone production without any suppression of root growth, suggesting a new effective elicitor to *S. miltiorrhiza* hairy root cultures for tanshinone production. Moreover, the combined treatment of UV-B irradiation and MeJA exhibited synergistic effects on the expression levels of *3-hydroxy-3-methylglutaryl-CoA reductase* (*SmHMGR*) and *geranylgeranyl diphosphate synthase* (*SmGGPPS*) genes in the tanshinone biosynthetic pathway. When hairy roots of 18-day-old cultures were exposed to the combined elicitation for 9 d, the maximum production of tanshinone reached to 28.21 mg/L, a 4.9-fold increase over the control. The combined elicitation of UV-B and MeJA was firstly used to

* Corresponding author : Tel.: +86 512 69561430; fax: +86 512 65882089.

Email address: jwwang@suda.edu.cn, bcjwwang@gmail.com (J.W. Wang).

stimulate the production of biologically important secondary metabolites in hairy root cultures.

Keywords: *Salvia miltiorrhiza*; Hairy roots; Tanshinones; UV-B; MeJA; Elicitation

1. Introduction

Salvia miltiorrhiza Bunge (Lamiaceae herb), also named Danshen, is a famous traditional herb medicine in China. It has been widely used to produce a number of traditional Chinese medicines for clinical application for cardiovascular diseases [1]. There are mainly two types of bioactive components in *S. miltiorrhiza*, the hydrosoluble phenolic acid and the liposoluble tanshinone [2]. Tanshinones as a group of abietane type diterpenes, including tanshinone I, IIA and cryptotanshinone, present important biological activities including anti-platelet aggregating [3], anti-inflammation [4], anti-oxidant and anti-tumor properties [1].

Hairy root cultures of *S. miltiorrhiza* have been established as a potential production system for tanshinones [5]. To stimulate tanshinone production, biotic and abiotic elicitations were employed in the hairy roots. Yan *et al.* reported that yeast extracts (YE) could stimulate tanshinone production (3.8-fold of the control) in a dose-dependent manner in *S. miltiorrhiza* hairy root cultures [6]. Under the treatment of silver ion Ag^{+} (30 μM), the total tanshinone content of hairy roots was enhanced by twofold [7]. Methyl jasmonate (MeJA) as an elicitor increased the tanshinone accumulation after 3 days, about 5.8 times that of the control (0.16 mg/g dry weight, DW). And the expressions of most investigated genes were also up-regulated by MeJA [8, 9].

Ultraviolet-B (UV-B) radiation (wavelength range 280 to 315 nm) has been applied as an

effective elicitor to promote the biosynthesis of important bioactive compounds such as catharanthine in *Catharanthus roseus* cell suspension cultures [10], glycyrrhizin in hydroponic and pot system [11] and taxol in *Taxus chinensis* var. mairei [12]. The phenolics content and antioxidant enzymes in *S. miltiorrhiza* leaf were increased drastically after 6 h of UV-B radiation (4.4 μW/ cm^2) [13]. However, there are no reports on eliciting tanshinone production under UV-B radiation.

The secondary metabolite production in plants could be enhanced or potentiated by incorporation of signal molecules such as jasmonate (JA) relatives with the elicitor treatment. Zhao *et al.* demonstrated the enhancement of tanshinone production by MeJA in cell cultures of *S. miltiorrhiza* [14]. The application of MeJA combined with cyclodextrin (CD) in grapevine cell culture could be responsible for the observed synergistic effect on resveratrol production and the expression levels of genes involved in the stilbene pathway [15]. The combination of MeJA and red light treatment in *Vitis vinifera* cell suspensions further increased the endogenous accumulation of anthocyanins [16]. In *S. miltiorrhiza* hairy root cultures, pre-incubation of the cultures with MeJA for 24 h or longer before YE treatment was essential to potentiate the induced tanshinone production by YE elicitation [17]. However, there has been so far neither report concerning UV-B effects on *S. miltiorrhiza* hairy roots, nor regarding its synergistic effect with MeJA. Therefore, as a follow-up to our previous characterization of UV-B elicitation [18, 19] and regulation on tanshinone biosynthesis in *S. miltiorrhiza* hairy roots [20], we therefore wish to evaluate combined elicitation of UV-B and MeJA. In this study, *S. miltiorrhiza* hairy roots were treated with MeJA and UV-B, alone or in combination, to investigate the influence and/or induction effect exerted by the concurrent elicitation stimulus on the production of tanshinone. The expression pattern of tanshinone biosynthetic genes during the elicitation was also investigated.

2. Materials and methods

2.1. Hairy root culture

S. miltiorrhiza hairy roots have been established by the infection of leaf discs with *Agrobacterium rhizogenes* R1601 in our laboratory [21]. The hairy roots were maintained on hormone-free Murashige & Skoog (MS) medium [22] containing 8 g/L agar and 30 g/L sucrose and grown at 25°C in the dark. For experiments in flasks, hairy roots were incubated in 50 ml hormone-free MS medium containing 3% (w/v) sucrose in 330 ml flasks (cap diameter 55 mm, bottom diameter 70 mm, height 110 mm) on an orbital shaker (120 rpm) at 25°C [7]. Hairy roots were harvested and dried at 40°C to a constant weight (yielding the dry weight, DW).

2.2. UV-B irradiation and MeJA treatments

The treatment of UV-B irradiation or MeJA were applied to the hairy root cultures (hairy root density of 3.75 g/L at day 0) on day 18, 2-3 days before the stationary phase based on a previous study [17]. UV-B irradiation was artificially provided by UV-B fluorescent lamps (30 W, λ_{max} 315 nm, Philips, the Netherlands) using fixtures mounted 45 cm above the culture jars. The plastic cap of the culture jar was taken off during the treatment of UV-B radiation. The spectrum of the UV-B lamp was given in our previous report [19]. Thick cellulose diacetate film (0.125 mm, Shanghai Plastic Company, China) was wrapped on the lamps to filter out the UV-C (< 280 nm) radiation.

The intensity of UV-B irradiation at the top of the suspension cultures was 40 μW/cm^2, measured with an ultra violet intensity meter (Spectra Physics, USA). In addition, hairy roots were also supplemented simultaneously with 100 μM MeJA (Sigma) according to Liang *et al.* [23], in combination or not with UV-B irradiation. The equal volume of distilled water added to the hairy root culture was used as the control treatment. All treatments (control, UV-B irradiation, 100 μM MeJA, and 100 μM MeJA plus UV-B irradiation) were performed in three independent experiments with 4 bottles hairy root culture each, and the results were represented by the mean ± standard deviation (S.D.).

2.3. Extraction and assay of tanshinone

The dried hairy roots were ground and extracted by methanol/dichloromethane (3:1, v/v). Tanshinone content was analyzed by high performance liquid chromatography (HPLC) with UV detection at 275 nm, using a 250 mm × 4.6 mm Elite SinoChrom ODS-BP C18 column, and a mobile phase consisting of acetonitrile : water at 55 : 45 (v/v) [17]. The content of cryptotanshinone, tanshinone I and tanshinone IIA was quantified with a genuine standard (Sigma).

2.4. RNA isolation and quantitative real-time PCR analysis

Total RNA extracted by RNAprep pure Plant Kit (Tiangen, China) was reversely transcribed using AMV reserve transcriptase (Fermentas, Canada) to generate cDNA. Primers used for quantitative real-time polymerase chain reaction (qRT-PCR) were listed in Table 1. The quantitative

real-time PCR was performed according to manufacturer's instruction (Bio-Rad, USA) under the following condition: 94°C for 3 min, and then 40 cycles of 94°C for 1 min, 30 s at 56°C and 72°C for 30 s.

2.5. Statistical analysis

Data analyses were carried out using Microsoft Excel and expressed as mean ± S.D. Student's *t*-test was applied for comparison of the means of two groups. $P < 0.05$ is considered statistically significant.

3. Results

3.1. The effects of UV-B on growth and tanshinones of hairy roots

S. miltiorrhiza hairy roots were subjected to UV-B irradiation (40 μW/cm^2) for 0-60 min (Fig. 1). The UV-B exposure time had no significant influence on biomass of the hairy roots. However, the content of all three tanshinones was increased by UV-B irradiation. Among the three tanshinones, cryptotanshinone was most responsive to UV-B irradiation and reached maximum (0.13 mg/g DW), 3.4-fold than that of control after 40 min of UV-B irradiation, while the content of total tanshinone reached the peak (0.38 mg/g DW), 1.8-fold than that of control. To best characterize tanshinone accumulation after UV-B exposure, 40-min radiation was chosen for subsequent study.

3.2. The effects of combined elicitation with MeJA

In the time course assay, 18-day-old hairy roots were subjected to different treatments: 40 min UV-B irradiation, 100 μM MeJA or the combined treatment (MeJA + UV-B) (Fig. 2). Compared with the control, hairy root subjected to UV-B irradiation displayed a slight increase of biomass at first, and then had no significant change until up to day 9 with significant decrease. Unlike UV-B irradiation, exogenous MeJA showed suppression on biomass of hairy roots. Hairy root grown in the presence of MeJA plus UV-B irradiation, similar to MeJA-elicited, inhibited significantly the growth of hairy roots when compared to the control or UV-B irradiation (Fig. 2).

Accumulation of tanshinones showed notable diversity according to the elicitor type within 9 days. All three treatments significantly induced the accumulation of tanshinone (Fig. 3). After UV-B treatment, total tanshinone content in *S. miltiorrhiza* hairy roots reached the peak value on day 3, about 0.61 mg/g DW, which was 1.5-fold of that of control (Fig. 4A). The response of tanshinone I content to the elicitation demonstrated similar pattern with cryptotanshinone. The contents of cryptotanshinone and tanshinone I reached the top on day 3, which were 0.22 mg/g DW (2-fold) and 0.27 mg/g DW (1.5-fold), respectively. Then they gradually decreased to control level. Therefore, the results show that UV-B irradiation had positive effects on tanshinone accumulation. In MeJA treatment, the maximum total tanshinone content was recorded on day 7, reached 1.46 mg/g DW, which was approximately 3.2-fold higher than that of control. The cryptotanshinone content was significantly enhanced on day 5, reached highest (0.65 mg/g DW) on day 7, 6.2-fold of that of control. The tanshinone I content gradually increased along with treatment time, reached

0.46 mg/g DW (3.4 folds) on day 9 while the tanshinone IIA content reached its highest (0.36 mg/g DW) on day 7. There was synergistic effect between UV-B irradiation and MeJA treatment within tanshinone accumulation. In combined treatment group (MeJA+UV-B), total tanshinone content reached its highest (2.26 mg/g DW), which was nearly 6.7-fold of that of control. The cryptotanshinone and tanshinone I content gradually increased along with the combined treatment, reached a higher level on day 9, 0.97 mg/g DW (9.9 folds) and 0.93 mg/g DW (6.1 folds), respectively. As to tanshinone IIA, the response pattern displayed some differences. Tanshinone IIA content reached the top on day 7, which was 0.46 mg/g DW (4.8 folds) (Fig. 4D).

3.3. Effects of elicitation on total tanshinone production

The growth situation of *S. miltiorrhiza* hairy roots was observed after 5 days (Fig. 5A). Compared with the control, the color of hairy roots treated with different elicitors became redder. In addition, the reddening degree of hairy root was in conformity with the production of tanshinones (data not shown). Total tanshinone production refers to the volumetric production of total tanshinones (the sum of cryptotanshinone, tanshinone I and tanshinone IIA). Total tanshinone production in hairy root cultures of *S. miltiorrhiza* during the treatment was all found to be raised (Fig. 5B). During UV-B irradiation period, the production of total tanshinone in *S. miltiorrhiza* hairy roots reached 6.88 mg/L on day 3, about 1.5-fold of that of control, and then gradually decreased to control level. In MeJA and UV-B+MeJA group, total tanshinone production had similar varying tendency and reached the top on day 9, which were 17.93 mg/L (3.7 folds) and 28.21 mg/L (5.9 folds), respectively. All three treatments significantly induced the production of

tanshinones, and MeJA+UV-B treatment was more effective than UV-B or MeJA alone. When hairy roots of 18-day-old cultures were exposed to the 40 min UV-B irradiation (40 μW/cm^2) combined with 100 μM MeJA for 9 days, the maximum production of tanshinone reached to 28.21 mg/L, a 4.9-fold increase over the control.

3.4. The changes of transcript profiles during the elicitation

The expression pattern of key genes in tanshinone biosynthetic pathway was measured after 3, 7 days by quantitative real-time PCR (Fig. 6). The expression of *3-hydroxy-3-methylglutaryl-CoA reductase* (*SmHMGR*), *1-deoxy-D-xylulose-5-phosphate reductoisomerase* (*SmDXR*), *1-deoxy-D-xylulose-5-phosphate synthase* (*SmDXS1*), *2-deoxy-D-xylulose-5-phosphate synthase* (*SmDXS2*), *geranylgeranyl diphosphate synthase* (*SmGGPPS*), *copalyl diphosphate synthase* (*SmCPS*) and *kaurene synthase-like* (*SmKSL*) increased significantly on day 3 (Fig. 6A). In detail, during UV-B irradiation period, the expression of these genes increased to more than 3 times of the untreated control, and in particular the expression of *SmDXS2* and *SmCPS* reached 6.2 and 7.3 times, respectively. In MeJA treatment group, the expression of *SmGGPPS*, *SmCPS* and *SmKSL* improved obviously, and reached 17.3, 13.6 and 18.0 times of the untreated control severally. In combined treatment group (MeJA+UV-B, Fig. 6A), the expression of *SmHMGR*, *SmGGPPS*, *SmCPS* and *SmKSL* reached 10.0, 9.6, 16.2 and 20.5 times of control, but the expression of other tested genes was only slightly induced.

After 7 days of UV-B irradiation, the expression of tested genes except *SmGGPPS* was

induced steadily, and *SmHMGR* was enhanced the most with 3.9 times of the untreated control. In MeJA group, the expression of *SmGGPPS* increased 8.7 times and *SmKSL* increased 15.4 times of the untreated group. The expression of *SmHMGR* and *SmGGPPS* increased significantly during combined treatment (MeJA+UV-B, Fig. 6B), and reached very high level (7.2 and 12.4 times of the untreated control).

4. Discussion

As elicitation was an effective process strategy for stimulating tanshinone biosynthesis, many biotic elicitor (carbohydrate fractions of microbial cells, salicylic acid, JA, or MeJA) and abiotic stressors (osmotic stress and heavy metal salts) have been used in *S. miltiorrhiza* hairy root cultures [20]. 30 μM Ag^{+} was supplied to the cultures at 2-3 days before the stationary growth phase of hairy roots and the total tanshinone content was increased by twofold over the control, while the root growth was suppressed to nearly 30% lower [7]. Sorbitol at 50-70 g/L raised the osmolality of the culture medium to 410-500 mmol/kg, 1.5-2.0 times of that in the control and the tanshinone content of roots increased by 4.5-fold. Sorbitol as an osmoticum still inhibited the root biomass [24]. In the present study, we report for the first time UV-B irradiation has an effect on the accumulation of tanshinone in *S. miltiorrhiza* hairy root cultures. The exposure of *S. miltiorrhiza* hairy root cultures to 40 μW/cm^2 UV-B irradiation (40 min) significantly increased the tanshinone levels (on average 77.2% higher) (Fig. 1). Cryptotanshinone content varies most significantly and increased 238.2% higher than that in control. Moreover, the hairy roots showed strong tolerance to the UV-B irradiation, retaining a stable and even higher biomass than that of the control. Our results suggested

that UV-B irradiation was a new effective elicitor to *S. miltiorrhiza* hairy root cultures for tanshinone production.

JA or its relative MeJA has been reported as another effective elicitor for stimulating tanshinone in *S. miltiorrhiza* hairy root cultures. Under the treatment of 100 μM MeJA, tanshinone accumulation was enhanced after 3 days, reaching a maximum value 0.93 mg/g DW on day 9, about 5.8 times that of the control [8]. In our present study, tanshinone accumulation reached a maximum value 1.46 mg/g (4.2 fold of that of control) on day 7 and total tanshinone production reached the top on day 9, which was 17.93 mg/L (3.7 folds) after MeJA treatment, which was consistent with previous reports on the elicitation. However, our results showed that UV-B irradiation combined with MeJA produced additive effects on the induction of tanshinone production and reached the top on day 9, which was 28.21 mg/L, about 5.9 times of that of control (Fig. 5B). The combination of MeJA and UV-B irradiation enhanced the tanshinone production in *S. miltiorrhiza* hairy root cultures much more significantly than the two used separately. From a biotechnological point of view, the increase of productivity after the synergistic elicitor treatment is of great practical value in bioreactors where the production can be scaled up and operated efficiently [14, 25].

The plant defense responses in secondary metabolites to combined elicitation of UV-B and other stress have been reported. The combined stress of UV-B and lower temperature (17°C) could enhance anthocyanin content in apple fruit [26]. 100 μM nitroprusside could significantly enhance UV-B-induced flavonoid accumulation in *Ginkgo biloba* callus [27]. The non-protein amino acid β-aminobutyric acid (BABA) pretreatment followed by YE elicitation could strongly promote YE-induced tanshinone production in *S. miltiorrhiza* hairy root cultures [28]. Moreover, the combination of YE and sorbitol resulted in much higher tanshinone content 1.48 mg/g DW, which

was approximately 10.0-fold of that in the control and 2.0-fold of that by YE or sorbitol alone, respectively. In this study we found the synergistic effects between UV-B and MeJA on tanshinone biosynthesis. UV-B irradiation combined with MeJA produced additive effects on the induction of tanshinone production and reached the top on day 9, which was 28.21 mg/L, about 5.9 times of that of control and 1.6 times of that by MeJA (Fig. 5B). When compared to other separated or combined elicitation, the combination of UV-B with MeJA is an effective elicitor on tanshinone in *S. miltiorrhiza* hairy root cultures.

The change of elicitation conditions may modify the plant response via an extensive reprogramming of gene expression. The expression levels of key genes involved in tanshinone biosynthesis exhibited different response strength at different stages under the elicitation in our study. UV-B irradiation induced all of the tested genes at early stage (day 3), consistent with the enhancement of tanshinone production. Then, the expression of these genes except *SmGGPPS* was induced steadily and *SmHMGR* was enhanced the most on day 7. Under the treatment of MeJA, tanshinone accumulation enhanced with a peak value on day 7 (Fig. 4). Accordingly, mRNA expressions of genes including *SmHMGR*, *SmDXR*, *SmDXS1*, *SmDXS2*, *SmGGPPS*, *SmCPS* and *SmKSL* was induced more significantly by MeJA than UV-B irradiation (Fig. 6B), which was consist with the change of tanshinone production (Fig. 5B). Kai *et al.* reported that MeJA increased most of the tested genes in tanshinone biosynthetic pathway including *acetyl-CoA C-acetyltransferase* (*SmAACT*), *3-hydroxy-3-methylglutaryl-CoA synthase* (*SmHMGS*), *SmHMGR*, *SmDXR*, *SmDXS2*, *4-(cytidine 5-diphospho)-2-C-methyl-derythritol kinase* (*SmCMK*), *isopentenyl-diphosphate delta-isomerase* (*SmIPPI*), *SmGGPPS*, *SmCPS* in *S. miltiorrhiza* hairy root cultures [8, 29]. Under the combined treatment of UV-B irradiation and MeJA, the expression of all

the tested genes was induced more significantly on day 3 (Fig. 6A). After 7 days, although the expression level of other 5 genes decreased, the expression of *SmHMGR* and *SmGGPPS* kept the higher levels, which implied the possible role of those genes responding to the combined stimuli. HMGR catalyzing the conversion of HMG-CoA to mevalonate, is considered as the first key step in the mevalonate (MVA) pathway in plant isoprenoid synthesis [30]. It was reported that there was a crosstalk between MVA and 1-deoxy-*D*-xylulose phosphate (DXP) pathways for tanshinone biosynthesis in *S. miltiorrhiza* hairy roots [17, 31]. The important branch point from basic terpenoid precursor to universal diterpenoid precursor is determined by the condensation reaction catalyzed by GGPPS [32]. Our results here are indicative of the relationship between those key genes for tanshinone biosynthesis and combined elicitation. Further exploration on the synergistic effects of the combined elicitors should provide valuable insights into reputational mechanism and develop effective process strategies for tanshinone production in the hairy root cultures.

5. Conclusion

In summary, we have successfully demonstrated that 40-min UV-B irradiation at 40 μW/cm^2 could enhance the accumulation of tanshinone without any suppression of root growth, suggesting a new effective elicitor to *S. miltiorrhiza* hairy root cultures. Moreover, when hairy roots of 18-day-old cultures were exposed to the UV-B irradiation with 100 μM MeJA for 9 days, the maximum production of tanshinone reached to 28.21 mg/L, a 4.9-fold increase over the control. *SmHMGR* gene involved in MVA pathway and the "late" genes including *SmGGPPS*, *SmCPS* and *SmKSL*, all showed increases at transcription level, coinciding with the induction of the combined

treatment of UV-B irradiation and MeJA. The combined elicitation (UV-B + MeJA) has been proved an effective strategy for substantial enhancement of tanshinone production in *S. miltiorrhiza* hairy roots.

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Table 1 Some genes in the tanshinone biosynthetic pathway and primer pairs used for qRT-PCR

Name	Sequence
HMGR-F	5’-GCAACATCGTCTCCGCCGTCTACA-3’
HMGR-R	5’-GATGGTGGCCAGCAGCCTGGAGTT-3’
DXR-F	5’-CATGCGTTTGCCTATTCTGTAC-3’
DXR-R	5’-ACTAAGAACTCCGGTCATGGTG-3’
DXS1-F	5’-AATGCGCCACCCATAAAGC-3’
DXS1-R	5’-ATCTGTTCATGCACCAGCTCTCT-3’
DXS2-F	5’-GGCGTCGTCTTGTGGAGTTA-3’
DXS2-R	5’-AGCCACGTCGTTGGTGTTAT-3’
GGPPS-F	5’-GTCGGATTCATCCACTCCACCACC-3’
GGPPS-R	5’-CTCTCTGCGTCGACACTCTG-3’
CPS-F	5’-GAGGGAGAGGTGAGGAAGGAA-3’
CPS-R	5’-AGGGAACAAAAGTTGAAAAGG-3’
KSL-F	5’-GTGTGACCCTTCTGCTAGCA-3’
KSL-R	5’-TGCATTGTCTTGGGAAGATG-3’
β-actin-F	5’-AGGAACCACCGATCCAGACA-3’
β-actin-R	5’-GGTGCCCTGAGGTCCTGTT-3’

Figure legends

Fig. 1. Hairy roots growth and tanshinone accumulation of *S. miltiorrhiza* under UV-B irradiation (40 μW/cm^2). 18-day-old hairy roots were exposed to 0, 20, 40, or 60 min UV-B irradiation and harvested at 72 h after exposure. Data are mean ± S.D. of three replicates. "**" shows significant difference ($p < 0.01$) and "***" shows significant difference ($p < 0.001$) when compared to the control.

Fig. 2. Biomass accumulation of hairy root cultures of *S. miltiorrhiza*. 18-day-old hairy roots were treated with 40 min UV-B irradiation (40 μW/cm^2), 100 μM MeJA alone or in combined with 40 min UV-B irradiation (40 μW/cm^2) and harvested at different time after treatments. Data are mean ± S.D. of three replicates. The small letters indicate the significant difference ($p < 0.05$) between the data of the different treatments: a, UV-B irradiation *versus* control; b, MeJA *versus* control; c, MeJA plus UV-B irradiation *versus* control; d, MeJA plus UV-B irradiation *versus* UV-B irradiation; e, MeJA plus UV-B irradiation *versus* MeJA.

Fig. 3. Chromatograms of tanshinones in the hairy roots after different treatments. A. control; B. 40-min UV-B irradiation (40 μW/cm^2); C. 100 μM MeJA alone; D. 100 μM MeJA in combined with 40 min UV-B irradiation (40 μW/cm^2). All samples were treated on day 18 post inoculation and harvested on day 7 after different treatments.

Fig. 4. Time course of tanshinone contents in *S. miltiorrhiza* hairy roots after different treatment. 18-day-old hairy roots were treated with 40 min UV-B irradiation (40 μW/cm^2), 100 μM MeJA alone or in combined with 40 min UV-B irradiation (40 μW/cm^2) and harvested at different time after treatments. Data are mean ± S.D. of three replicates. The small letters indicate the significant difference ($p < 0.05$) between the data of the different treatments: a, UV-B irradiation *versus* control;

b, MeJA *versus* control; c, MeJA plus UV-B irradiation *versus* control; d, MeJA plus UV-B irradiation *versus* UV-B irradiation; e, MeJA plus UV-B irradiation *versus* MeJA.

Fig. 5. Tanshinone production of *S. miltiorrhiza* hairy roots. (A) Hairy root cultures observed on day 5 after the treatments. (B) Total tanshinone production in hairy root cultures of *S. miltiorrhiza*. 18-day-old hairy roots were treated with 40 min UV-B irradiation (40 μW/cm^2), 100 μM MeJA alone or in combined with 40 min UV-B irradiation (40 μW/cm^2), and harvested at different time after treatments. Data are mean ± S.D. of three replicates. The small letters indicate the significant difference ($p < 0.05$) between the data of the different treatments: a, UV-B irradiation *versus* control; b, MeJA *versus* control; c, MeJA plus UV-B irradiation *versus* control; d, MeJA plus UV-B irradiation *versus* UV-B irradiation; e, MeJA plus UV-B irradiation *versus* MeJA.

Fig. 6. The expression levels of tanshinone biosynthesis genes. 18-day-old hairy roots were treated with 40 min UV-B irradiation (40 μW/cm^2), 100 μM MeJA alone or in combined with 40 min UV-B irradiation (40 μW/cm^2) and harvested on day 3 (A) or day 7 (B) after treatments. Data are mean ± S.D. of three replicates. The small letters indicate the significant difference ($p < 0.05$) between the data of the different treatments: a, UV-B irradiation *versus* control; b, MeJA *versus* control; c, MeJA plus UV-B irradiation *versus* control; d, MeJA plus UV-B irradiation *versus* UV-B irradiation; e, MeJA plus UV-B irradiation *versus* MeJA.

Fig. 1.

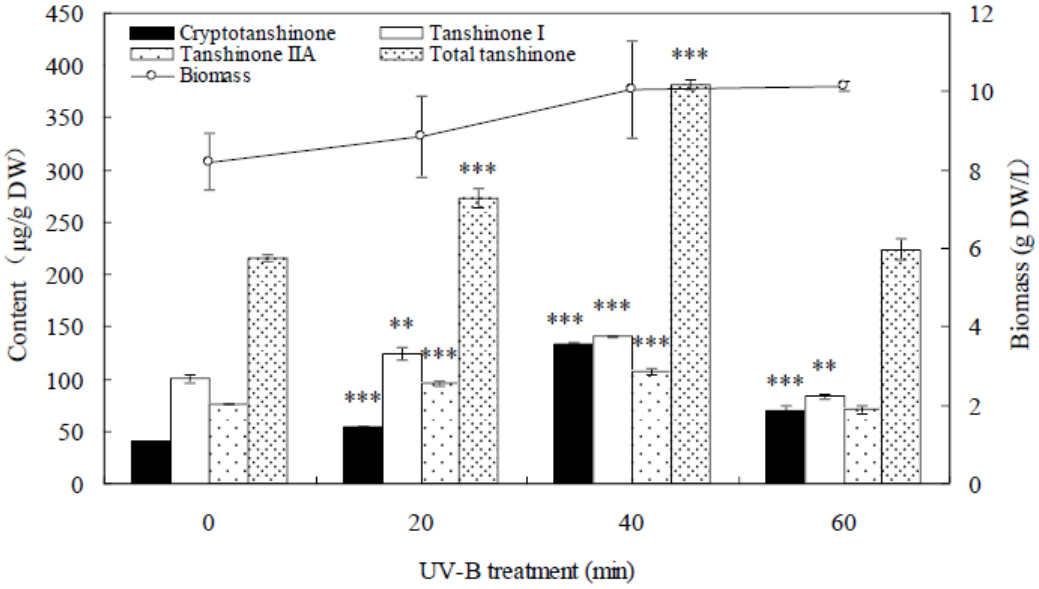


Fig. 2.

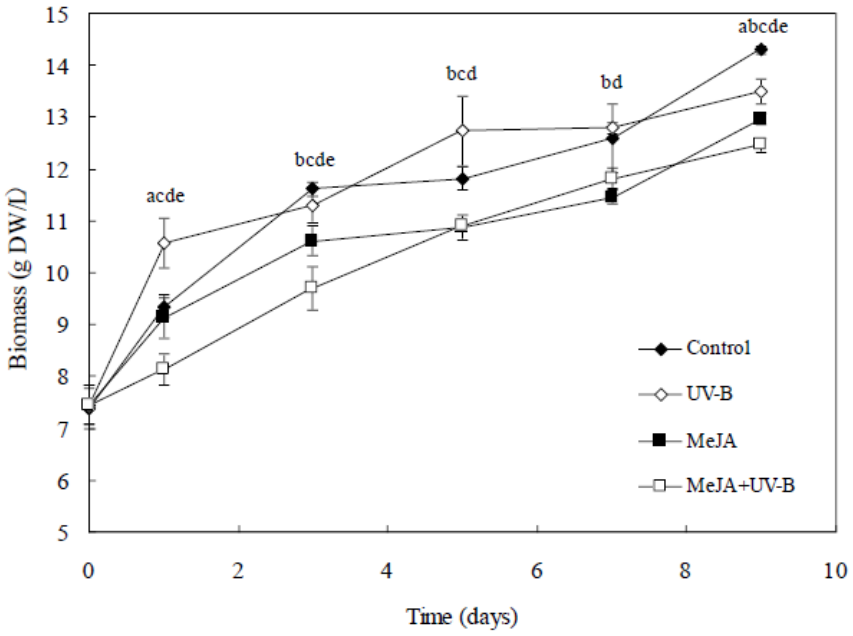


Fig. 3.

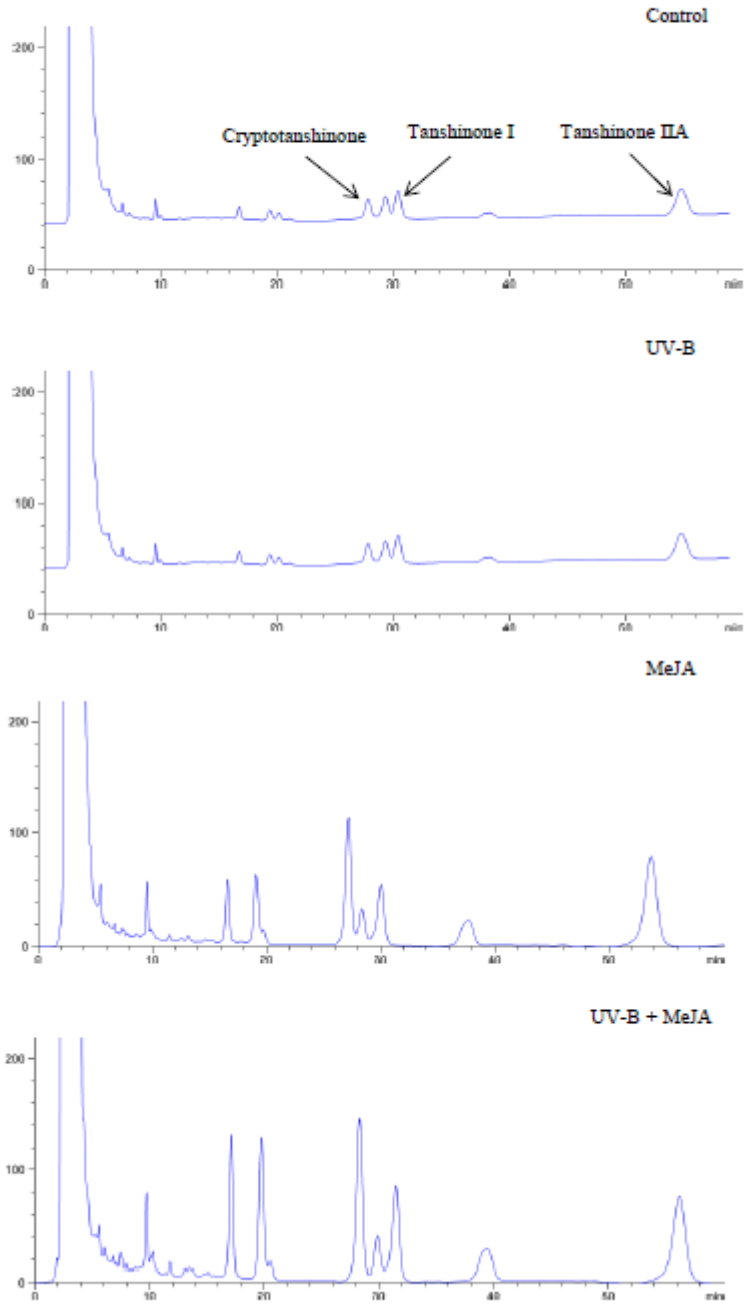


Fig. 4.

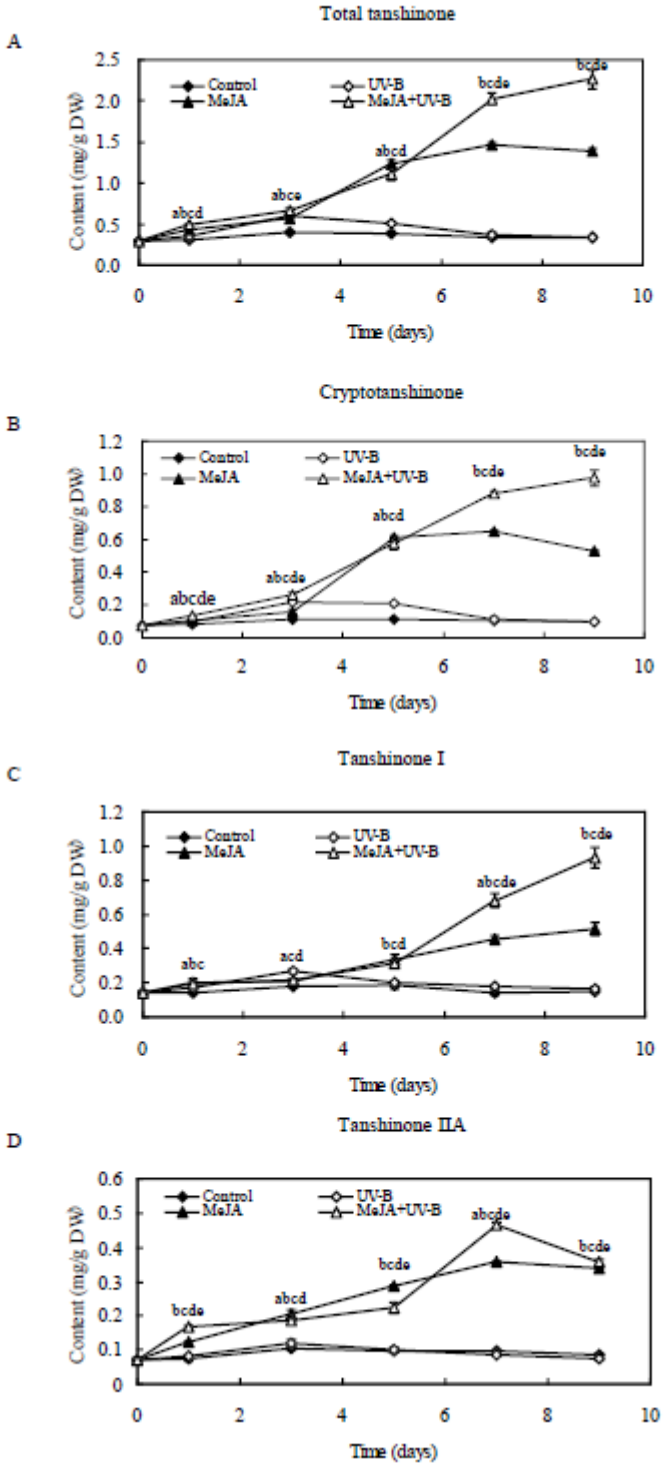
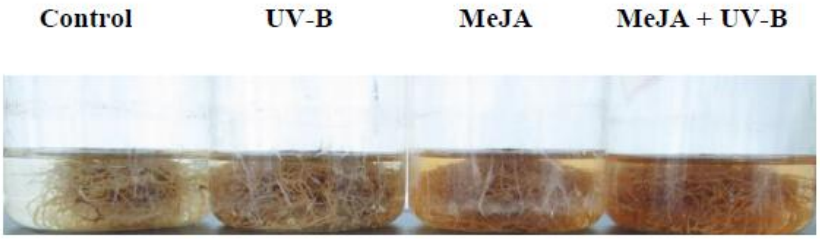


Fig. 5.

A



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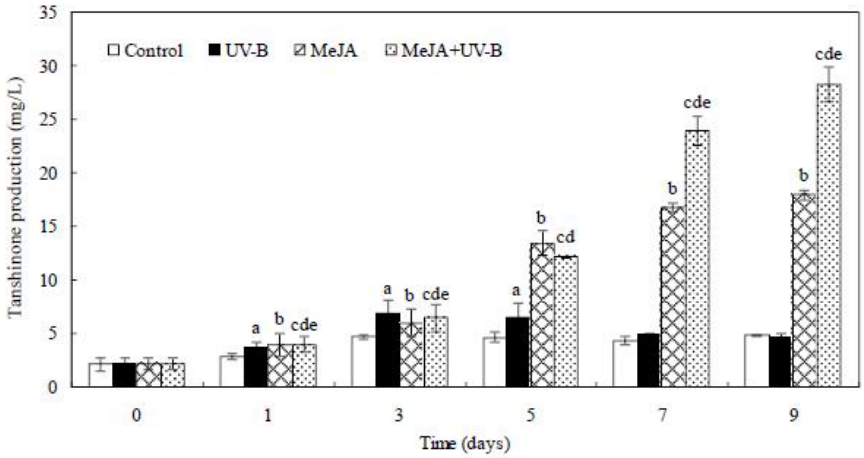
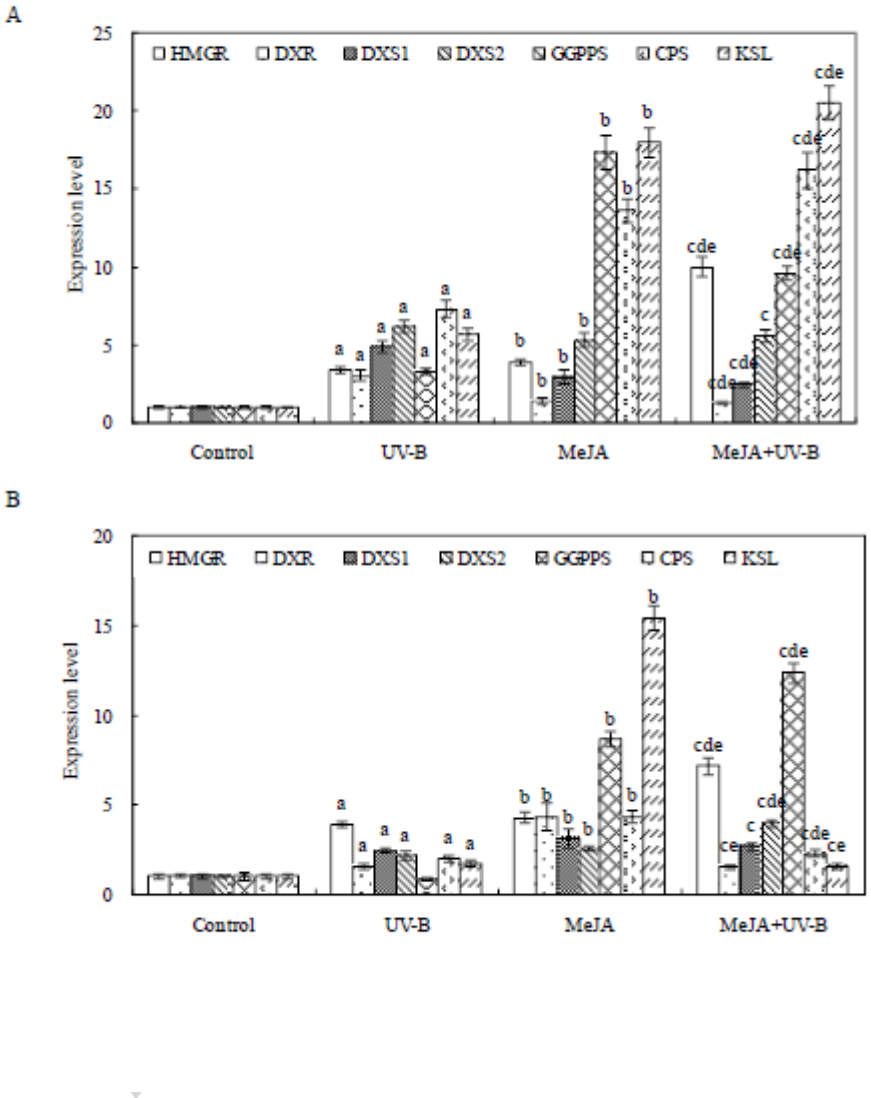
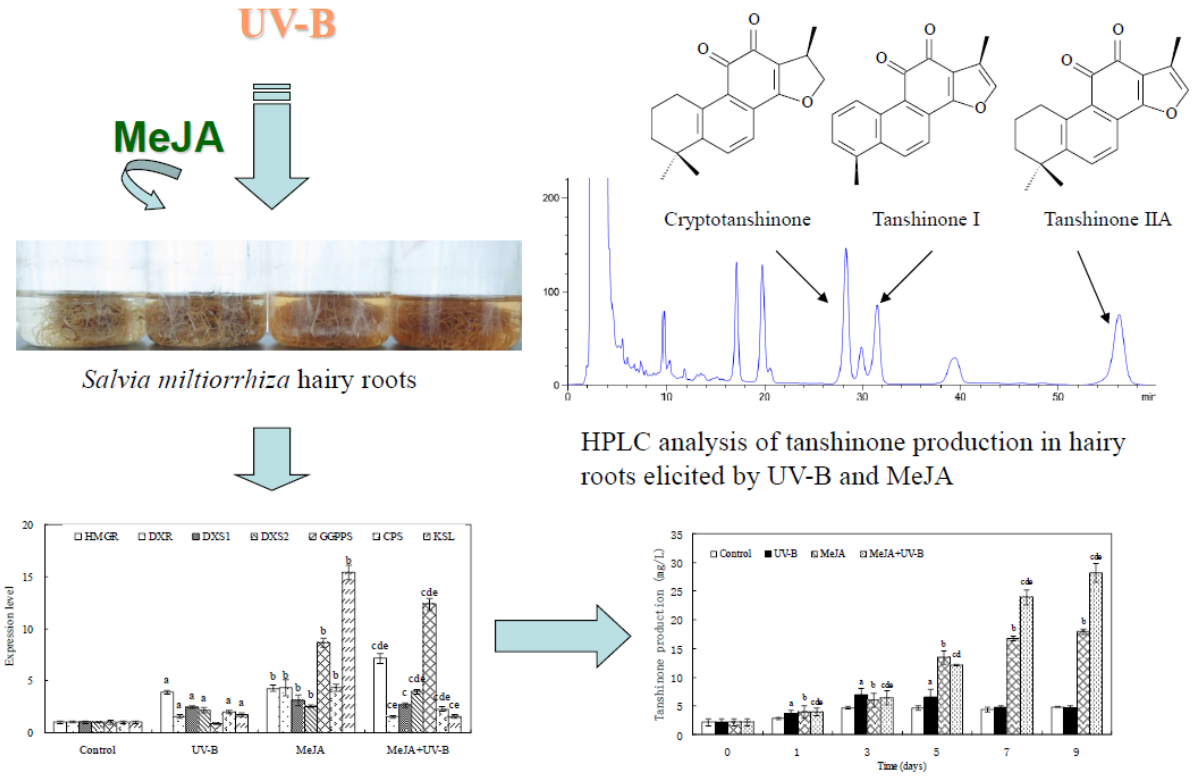


Fig. 6.





Graphical abstract

Highlights

► Tanshinones in *S. miltiorrhiza* hairy roots were stimulated by 40 μW/cm^2 UV-B.

► 100 μM MeJA increased total tanshinone content to 1.5 mg/g DW.

► The combined elicitation induced a 4.9-fold increase in tanshinone production.

► *SmHMGR* and *SmGGPPS* expression were promoted during the elicitation.