Effects of UV-B Radiation on Terpenoid Synthesis and Related Gene Expression in Shennongxiangju Chrysanthemum (Postprint)

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Abstract

This study utilized Shennongxiang chrysanthemum as experimental material and subjected it to UV-B radiation at an intensity of 400 W/cm² for durations of 0, 0.5, 1, 2, and 4 h to investigate the effects of UV-B radiation on terpenoid synthesis and the expression of related genes in Shennongxiang chrysanthemum. The results demonstrated that relatively short-term UV-B radiation significantly promoted the expression of genes associated with terpenoid synthesis. Compared with the control, treatments of 0.5, 1, 2, and 4 h all enhanced the expression levels of related genes to varying extents. Specifically, under the 2 h treatment, the relative expression levels of HMGR, DXR, TPS, and GPS genes reached their maximum values. Under the 4 h treatment, the relative expression levels of FPS and DXS genes reached their maximum values, with the FPS gene showing the most significant change at 69-fold compared to the control. In the MVA pathway, the contents of dehydroaromadendrene and cadinene exhibited a continuous upward trend within 4 h, consistent with the expression pattern of the FPS gene. 1-caryophyllene showed the same trend as HMGR, characterized by an initial increase followed by a decrease. In the MEP synthesis pathway, the contents of -thujone, thujone, and -thujone displayed expression trends identical to those of DXR, GPS, and TPS genes. Eucalyptol continuously increased over the 4 h UV-B radiation period, consistent with the changes observed in the DXS gene. These findings suggest that UV-B radiation influences the synthesis of terpenoids in Shennongxiang chrysanthemum by modulating the expression levels of specific key genes in their respective pathways.

Full Text

Preamble

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Effects of UV-B Radiation on Biosynthesis of Terpenoids and Expression of Related Genes in *Dendranthema indicum* var. *aromaticum*

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Abstract

This study investigated the effects of UV-B radiation on terpenoid biosynthesis and related gene expression in *Dendranthema indicum* var. *aromaticum* using UV-B irradiation at 400 $w \cdot cm^{2}$ for durations of 0, 0.5, 1, 2, and 4 h. The results demonstrated that short-term UV-B radiation significantly promoted the expression of genes related to terpenoid synthesis. Compared with the control, treatments of 0.5, 1, 2, and 4 h all enhanced gene expression to varying degrees. The relative expression levels of HMGR, DXR, TPS, and GPS genes peaked at 2 h, while FPS and DXS reached their maximum expression at 4 h. Notably, FPS showed the most dramatic change, with expression 69-fold higher than the control at 4 h. In the MVA pathway, the contents of calamenene and cadinene correlated with the sustained increase in FPS expression over 4 h, while 1-caryophyllene mirrored HMGR' s pattern of initial increase followed by decrease. In the MEP pathway, the contents of -thujone, thujone, and -thujone showed the same trend as DXR, GPS, and TPS expression, whereas cajuputole increased continuously over 4 h, consistent with DXS expression. These findings indicate that UV-B radiation affects terpenoid biosynthesis in *Dendranthema indicum* var. *aromaticum* by modulating the expression of key genes in each pathway.

Keywords: *Dendranthema indicum* var. *aromaticum*, UV-B radiation, terpenoids, gene expression

Introduction

Dendranthema indicum var. *aromaticum*, a member of the Asteraceae family, is endemic to the Shennongjia region of Hubei Province (Liu & Zhang, 1983) and possesses medicinal properties including heat-clearing, detoxification, antibacterial, anti-inflammatory, liver-calming, vision-improving, wind-dispersing, and blood pressure-lowering effects (Liu et al., 1986). The entire plant emits a distinctive fragrance, making it an important aromatic ornamental species within the genus. This natural aroma is rare among Asteraceae plants and offers broad prospects for landscape applications while holding significant value for developing new strongly-scented chrysanthemum cultivars. Previous research by our group has revealed that both leaves and petals of *D. indicum* var. *aromaticum* are densely covered with glandular trichomes, and the plant' s aroma is closely associated with terpenoid compounds secreted by these trichomes (He et al.,

2015). Genes involved in terpenoid synthesis can effectively regulate both the types and quantities of terpenoid compounds in plants.

Terpenoids are compounds composed of varying numbers of isoprene (C H) units and their derivatives. Two primary biosynthetic pathways have been identified: the mevalonate pathway (MVA) and the methylerythritol-4-phosphate pathway (MEP) (Dudareva et al., 2005). The enzymes involved in plant terpenoid biosynthesis are highly diverse, with enzyme genes determining not only the types of terpenoid products synthesized in subsequent reactions but also regulating their accumulation levels. Well-studied enzymes include HMGR and FPS in the MVA pathway (Chappell, 1995; Ji et al., 2007), and DXS, DXR, and GPS in the MEP pathway (Malhotra et al., 2014; Zhang et al., 2013). TPS operates downstream of both pathways, catalyzing substrates to form monoterpenes, sesquiterpenes, or diterpenes (Martin et al., 2012). These key enzyme genes catalyze the formation of various precursors and intermediates in terpenoid synthesis (Tang et al., 2014), occupying important branch points in the pathways or downstream positions in product formation. Modulating the relative expression of these key enzyme genes through biological approaches could potentially enhance terpenoid production.

Terpenoid synthesis in plants is influenced not only by developmental stages but also by biotic factors such as insects and pathogens, as well as abiotic factors including light, temperature, and humidity (Liang et al., 2017). Among photic ecological factors, UV-B radiation is particularly significant, as it can directly or indirectly affect plant morphology, biomass, photosynthesis, physiological metabolism, proteins, and nucleic acids, thereby influencing plant growth and development (Pu et al., 2017). Exposure to UV-B radiation can alter gene expression, enzyme activity, and secondary metabolism in some plants (Searles et al., 2001).

This study employed UV-B radiation to treat *D. indicum* var. *aromaticum* introduced from the Shennongjia region of Hubei. Using GC-MS and qRT-PCR techniques, we examined terpenoid compounds and related gene expression in leaves under different radiation durations to investigate the effects of UV-B radiation on terpenoid biosynthesis and related gene expression.

Materials and Methods

1.1 Plant Materials

Dendranthema indicum var. *aromaticum* was collected from Shennongjia, Hubei (110°23 57 E, 31°28 07 N) and cultivated in the nursery of the College of Landscape Architecture, Northeast Forestry University. In May 2017, healthy and uniform stem tips were selected for cutting propagation. After rooting, the cuttings were transplanted into pots and grown in the college greenhouse for approximately 35 days. When plants developed 7–8 functional leaves, vigorous, uniform, and pest-free individuals were selected for experimentation.

1.2 Experimental Procedures

1.2.1 UV-B Radiation Treatment Uniform *D. indicum* var. *aromaticum* plants were subjected to UV-B radiation using UV-B lamps (Nanjing Huaqiang Electronics Co., Ltd.). The radiation intensity at the third functional leaf from the top was set to 400 $w \cdot cm^{2}$, as measured by a UV340B ultraviolet radiometer (Shenzhen Xinbaorui Instrument Co., Ltd.). Experimental plants were divided into five groups (five plants per group, three replicates) and exposed to UV-B radiation for 0, 0.5, 1, 2, or 4 h. At each time point, 3 g of the third functional leaf (approximately 12–15 leaves) was collected from each group, immediately frozen in liquid nitrogen, and stored at –80 °C for subsequent analysis.

1.2.2 Analysis of Terpenoid Synthesis-Related Genes Total RNA was extracted from treated leaves using the TRNzoI Universal Total RNA Extraction Kit (TIANGEN) according to the manufacturer' s instructions. cDNA was synthesized using the ReverTra Ace qPCR RT Master Mix with gDNA Remover kit and stored at -20 °C. Primers were designed using Primer Premier 5.0 software based on *D. indicum* var. *aromaticum* transcriptome data for HMGR, DXR, TPS, GPS, FPS, and DXS genes. qRT-PCR was performed using the TransStart® Top Green qPCR SuperMix with CmEF1 as the internal reference gene, with three technical replicates per sample. Primer sequences are listed in Table 1 . Relative gene expression was calculated using the 2^(-ΔΔCt) method (Livak & Schmittgen, 2001).

1.2.3 Extraction and Analysis of Leaf Terpenoids Frozen leaf samples were ground to a fine powder in a mortar. The powder was extracted three times with 100 mL dichloromethane (2 s per extraction) and filtered through a funnel containing 20 g anhydrous sodium sulfate into a round-bottom flask. The extract was concentrated at 40 °C using a rotary evaporator, transferred to a brown sample vial, adjusted to a final volume of 0.5 mL, and stored at 0 °C (Hu et al., 2012).

Terpenoid analysis was performed using a GC-MS system (Hewlett-Packard, USA). GC conditions: HP-5MS column (30 m × 0.25 mm × 0.25 m), injection volume 1 L, split ratio 5:1, temperature program: 50 °C for 30 min, then increased to 160 °C at 5 $°C \cdot min^{1}$, and finally to 270 °C at 10 $°C \cdot min^{1}$, with a solvent delay of 3.5 min. MS conditions: EI ionization, quadrupole temperature 150 °C, ion source temperature 230 °C, interface temperature 280 °C, ionization energy 70 eV, electron multiplier voltage 2100 V, scan range (m/z) 4–500, NIST08L spectral library. Terpenoids were identified using Turbo Mass 5.4.2 GC/MS software, and relative contents were calculated using peak area normalization.

1.3 Data Processing and Analysis

Data were analyzed for correlation using SPSS 17.0, and calculations and charting were performed using Excel 2003.

Results and Analysis

2.1 Effects of UV-B Radiation on Expression of Terpenoid Synthesis-Related Genes

Using CmEF1 as the internal reference, qRT-PCR was employed to detect expression levels of HMGR, DXR, TPS, GPS, FPS, and DXS during 4 h of UV-B radiation. The results revealed distinct expression patterns across treatment durations.

The expression levels of HMGR, DXR, TPS, and GPS showed an initial increase followed by a decrease with prolonged UV-B exposure (Figures 1 [Figure 1: see original paper]–4 [Figure 4: see original paper]). These genes exhibited steady increases during the first 0–1 h, reached maximum expression at 2 h with significant differences compared to 0 h, and declined markedly by 4 h.

In contrast, FPS and DXS expression showed continuous increases throughout the treatment period (Figures 5 [Figure 5: see original paper]–6 [Figure 6: see original paper]). FPS expression rose sharply within 0.5 h, changed minimally by 2 h, and peaked at 4 h with a significant difference from the control, showing an overall upward trend. DXS expression changed little within 0.5 h, increased rapidly at 1 and 2 h, and reached maximum expression at 4 h, also demonstrating a significant difference from the control. The most pronounced change was observed in FPS, whose expression at 4 h was 69 times that of the control.

2.2 Effects of UV-B Radiation on Terpenoid Synthesis in *D. indicum* var. *aromaticum*

The MVA pathway participates in the synthesis of sesquiterpenes, triterpenes, and polyterpenes, while the MEP pathway is involved in monoterpene, diterpene, and tetraterpene biosynthesis. This study focused on sesquiterpenes in the MVA pathway (-cubebene, 1-caryophyllene, calamenene, and cadinene) and monoterpenes in the MEP pathway (cajuputole, camphor, -thujone, thujone, and -thujone). Based on the gene expression analysis, we selected the 2 h and 4 h time points, which showed significant changes, for detection of terpenoid metabolites.

As shown in Table 2 , relative contents of MVA pathway terpenoids exhibited varying trends with prolonged UV-B radiation. -Cubebene showed a pattern of initial decrease followed by increase (17.04%, 15.37%, and 18.05% at 0, 2, and 4 h, respectively). 1-Caryophyllene increased significantly then decreased (2.61%, 28.36%, and 21.32% at 0, 2, and 4 h). Calamenene and cadinene showed continuous increases, with calamenene rising from 4.51% to 7.97% and cadinene from 0.7% to 3.16% over 4 h.

In the MEP pathway, cajuputole content increased continuously (4.76%, 5.93%, and 10.63% at 0, 2, and 4 h), while camphor content decreased continuously (6.04%, 4.1%, and 2.1%). The relative contents of -thujone, thujone, and -thujone showed initial increase followed by decrease, with all except -thujone

remaining higher at 4 h than at 0 h. Specifically, -thujone was 1.9%, 2.63%, and 1.31%; thujone was 0.68%, 1.01%, and 0.97%; and -thujone was 6.34%, 10.36%, and 8.36% at 0, 2, and 4 h, respectively.

Discussion

UV-B radiation affects plant genetic material, including gene expression (Hartmann et al., 1998; Safrany et al., 2008). Dolzhenko et al. (2010) found that UV-B radiation altered terpenoid biosynthesis gene expression in peppermint (*Mentha piperita* L.). Similarly, this study demonstrated that UV-B radiation affected the expression of HMGR, DXR, TPS, GPS, FPS, and DXS in *D. indicum* var. *aromaticum*, with different radiation durations producing varying effects (Qi et al., 2014). In the MVA pathway, HMGR expression increased then decreased within 4 h, while FPS showed continuous increase. In the MEP pathway, DXR, GPS, and TPS increased then decreased, whereas DXS showed continuous increase. These results suggest that UV-B radiation exerts differential effects on genes within the same pathway.

Plant secondary metabolite synthesis is primarily determined by the activity of biosynthetic enzymes and gene expression levels (Broun, 2004). This study found that in the MVA pathway, the contents of calamenene and cadinene correlated with FPS expression trends, while 1-caryophyllene matched HMGR patterns. In the MEP pathway, -thujone, thujone, and -thujone contents paralleled DXR, GPS, and TPS expression, and cajuputole content aligned with DXS expression. These findings indicate that UV-B radiation influences terpenoid synthesis in *D. indicum* var. *aromaticum* by modulating key gene expression in each pathway. Altering the expression of key enzymes in the MVA and MEP pathways can affect downstream metabolite synthesis (Enfissi et al., 2005; Morris et al., 2006).

This study demonstrates that UV-B radiation affects terpenoid synthesis-related gene expression in *D. indicum* var. *aromaticum*, with short-term radiation significantly promoting expression of all studied enzyme genes. Expression levels remained above control levels throughout the 4 h period, though the degree of promotion varied among different enzymes. Most terpenoid compounds showed trends consistent with key genes in their respective pathways, with synthesis levels exceeding controls under short-term UV-B radiation. However, -cubebene in the MVA pathway showed a decrease-then-increase pattern 不同于 HMGR and FPS trends, and camphor content in the MEP pathway decreased continuously, contrasting with DXR, GPS, TPS, and DXS patterns. These results suggest that terpenoid synthesis involves more complex regulatory mechanisms beyond key gene control, warranting further investigation.

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Note: Figure translations are in progress. See original paper for figures.