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Original article

Synergistic Effects of CO_2 and LED Lighting on Accumulation of Terpenes in Roots of *Gynura bicolor*

Jin Ren[1], Shuang-sheng Guo[1,2*], Quan-yong Cheng[2], Yun-ze Shen[2], Hong-qi Feng[2]

1. Faculty of Life Sciences, Northwestern Polytechnical University, Xi'an 710072, China

2. National Key Laboratory of Human Factors Engineering, China Astronaut Research and Training Center, Beijing 100094, China

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ABSTRACT

Objective To investigate the essential oil profile and terpenes accumulation in the roots of *Gynura bicolor* (Asteracese) treated by CO_2 and LED lighting. **Methods** *G. bicolor* herbs were treated by CO_2 at the levels of 450 (control) and 1200 (elevated) μmol/mol and LED lighting with white light, RB20 (red/blue=8/2) and RB40 (red/blue=6/4). Headspace solid-phase micro-extraction-GC MS was employed to analyze terpenes from the essential oil of the roots. **Results** In all treated-roots, the major components of terpenes were (*E*)-β-farnesene, α-, β-caryophyllene, δ-, β-, γ-elemene, and α-pinene. Increasing CO_2 significantly decreased mono- and sesqui-terpenes in the roots under all light conditions, leading to the decreasing yields of the essential oils. Terpenes were at a higher level in RB20-treated roots than that in RB40-treated ones under both control and elevated CO_2. **Conclusion** CO_2 (450 μmol/mol) and 20 % blue LED lights are more conducive to the accumulation of terpenes in the roots than 1200 μmol/mol CO_2 and 40% blue LED lights.

Key words

Asteraceae; CO_2; *Gynura bicolor*; LED lighting; terpenes

1. Introduction

Gynura bicolor DC (Asteraceae, perennial herb) is called *Zibeicai*, *Buxuecai*, *Hongfengcai*, and so on in Chinese. As a traditional food and Chinese herbal medicine in China, it has the characteristics of eliminating stasis and activating blood circulation, antipyretic detoxicate, hemostasis, dephlogistication, and detumescence. It is mainly used to cure dysmenorrhea, profuse uterine bleeding, hemoptysis, trauma bleeding, cancer, and so on in clinical trial (Xie, 2001; Lu et al, 2004). Nowadays, this medicated dietary plant has attracted much attention in China and Japan due to its high nutritional value. Anthocyanins (Shimizu et al, 2010) and flavonoids (Lu et al, 2011) have been isolated from this plant and their bioactivities (Hayashi et al, 2002) have been investigated. In addition, *G. bicolor* has special flavor due to their essential oils including bioactive constituents such as caryophyllene and farnesene. α-, β-Caryophyllene and (*E*)-β-farnesene are at high levels in the leaves and roots. Caryophyllene is commonly used to treat bronchitis, inflammatory skin, and digestive ulcers (Lin and Lan, 1999). Caryophyllene and farnesene can serve as the flavoring or fixative agents. Thus, the essential oil from *G. bicolor* is the promising raw materials in pharmaceutical and fragrance industry.

The content in the essential oils is generally affected by environmental factors such as lighting, CO_2 concentration, and temperature (Tibaldi et al, 2011), and the essential oil profile

*** Corresponding author: Guo SS** Tel/Fax: +86-10-68746545 E-mail: guoshuangsheng@tom.com

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is influenced by genetic factors (Wang and Lincoln, 2004). Among various abiotic factors, enriched CO_2 concentration (Vurro et al, 2009; Tisserat and Vaughn, 2001; Penuelas and Estiarte, 1998) and light quality (Nishioka et al, 2008; Nishimura et al, 2009) can play the important roles in increasing or reducing the synthesis and accumulation of the essential oils. However, only a few studies have been reported upon the essential oils in the leaves and roots of *G. bicolor* (Shimizu et al, 2009, 2010; Lu et al, 2004), and it is unknown that how CO_2 and light affect the profile of the essential oil in *G. bicolor* roots.

Here elevated CO_2, as an increased available carbon source, and LED lighting were investigated in their effects on the concentration and composition of essential oils in *G. bicolor* roots. It is known that a plant treated by an increased available carbon source will be promoted in photosynthesis and biomass, and affected in the accumulation of secondary metabolites including phenols, anthocyanins, and terpenes. LED not only consumes less electricity but also meets the requirement of different monochromatic light quality and any combination of them, which has important effects on the accumulation of plants secondary metabolites through the induction of related gene. Besides, this study might give some guidance to the development and utilization of *G. bicolor*.

2. Materials and methods

2.1 Materials

G. bicolor seedlings were purchased from Beijing Academy of Agriculture and Forestry. All the LED lamps were purchased from Wuxi Fangzhou Technology Co. (Beijing, China). Pure carbon dioxide was purchased from Jinggao Gas Co., Ltd. (purity of 99.9%, Beijing).

2.2 Experimental design

The parameters of LED were blue LED (1 W, 460−463 nm), red LED (1 W, 625−630 nm), and white LED (1 W, full spectrum). Herbs were treated with RB20 (80% red light + 20% blue light) and RB40 (60% red light + 40% blue light), and white LED (WL)-treated herbs were the control. All light modules were placed in a controlled environment chamber (Guo et al, 2008), thus plants were exposed to the same environmental conditions.

The experiment was conducted in the controlled chamber (Guo et al, 2008), which was located in the environmental control and life support laboratory in China Astronaut Research and Training Center (Beijing). *G. bicolor* seedlings were grown in plastic pots of 15 cm (height) × 17 cm (top diameter) × 12 cm (bottom diameter). The pots were filled with porous ceramic particles (particle size 0.5−2 mm, 57.2% of porosity, bulk density of 1.22 g/cm^3, density of 2.85 g/cm^3) and were irrigated with fresh nutrient solution (Crowndaisy Chrysanthemum Herb Garden Trail Formula of Japan, Conductivity 2−2.5 ms/cm, pH 6.3−6.4) every 2 d to ensure the abundant nutrient and water supply. Seedlings were subjected to different lighting conditions with photosynthetic active radiation (PAR) intensity at (250 ± 5) μmol/(m^2·s^1) (at 20 cm over plant canopy and adjusted as the plants grew) in a photoperiod of 16 h/8 h (light/dark) cycle. The high CO_2 treatment (1200 μmol/mol) was conducted on day 7 after light treatments. Pure CO_2 was supplied from a high concentration CO_2 cylinder and injected through a pressure regulator into the closed chamber. Online Infrared CO_2 Analysis Instrument (GXH-3011, Institutes of Huayun Analyses Instrument, Beijing) was used to measure the CO_2 concentration. The CO_2 concentration at 450 μmol/mol served as control. The air relative humidity and temperature inside the chamber were maintained at (60 ± 5) % and 24 °C/ (19 ± 1) °C (light/dark) respectively throughout the experiment (cultivation cycle: 30 d). Ventilation velocity in the chamber was about 0.8 m/s. All these environmental parameters were controlled using integrated control, monitoring, and data management system software (LabView, USA). Each experiment was conducted under identical environmental conditions and plant cultural manners except the CO_2 concentration and light quality.

In all treatments, fresh roots of seedlings were harvested on day 30, flash frozen in liquid nitrogen, and then freeze-dried with a freeze-dryer for 48 h to avoid biochemical changes due to relative enzymes activity. The freeze-dried samples were sealed in plastic bags and stored at −20 °C prior to analysis.

2.3 Extraction of essential oils

Solid-phase micro-extraction (SPME) analysis over the use of headspace trapping with solid adsorbents is rapid and simple (Li et al, 2006), so it was adopted to analyze the essential oils from *G. bicolor* roots. Solvent extraction was employed to quantitative analysis of main volatiles.

HS-SPME protocol was as follows: The lyophilized roots (1 g × triplicate) were immersed in 11.5 mL fresh physiological saline, ground thoroughly and swiftly with 20 g NaCl, then immediately placed into 50 mL triangular flask, and sealed with dispense parafilm. Then, the polydimethylsiloxane (PDMS) fiber (50/30 μm DVB/CAR/ PDMS, Supelco, USA) after conditioning was exposed to the headspace of triangular flask and essential oils were extracted for 40 min at 45 °C in water bath. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC system to desorption for 15 min.

Solvent extraction protocol was as follows: Lyophilized plant roots (1 g × triplicate) were extracted with 50 mL freshly distilled diethyl ether for 30 min at room temperature for three times after crushed to pieces. The organic layer was separated and dried over anhydrous sodium sulfate. In order to quantify the volatile constituents yield, 1-octanol was added as internal standard (IS). The extract was concentrated carefully using a rotary evaporator in vacuo to 1 mL. An aliquot of this concentrate was taken for GC-MS analysis to determine the volatile compounds.

2.4 GC-MS analysis

Blank analyses were carried out after conditioning the PDMS fiber at the recommended temperature of manufacturer so as to characterize possible contaminants from the fiber or from the chromatographic system. The GC-MS analysis was performed on a Gas Chromatograph Agilent 7890A interfaced with an Agilent 5975C Mass Spectrometer with electron impact ionization (70 eV). An Agilent DB−5MS capillary column (30 m × 250 μm, 0.5 μm) was used.

Analytical conditions were as follows: For temperature programming of HS-SPME, the oven was maintained at 50 °C for 1 min and then ramped at 2 °C/min to 180 °C, held isothermal for 4 min at 180 °C. The total run time was approximately 70 min. For temperature programming for solvent extraction, the temperature of column was maintained at 50 °C for 1 min and then gradient at 3 °C/min to 250 °C, held isothermal for 20 min at 250 °C, injected volume was 1 μL, and total run time was approximately 70 min. The injector temperature was held at 230 °C. The carrier gas was helium with a flow rate of 1 mL/min. Constant pressure was at 5.34×10^4 Pa. MS conditions were as follows: capillary direct interface temperature, 230 °C; quadrupole temperature, 150 °C. Scan time and mass range were 1 s and 40−500 *m/z*, respectively.

The relative percentage of the constituents identified was obtained by mean values of GC (FID) peak area, and semi-quantitative data were calculated using the IS method, FID response factors were calculated theoretically with effective carbon number of IS and analytic composition, which was based on the positive relationship between FID response factors and the effective carbon number of compounds.

2.5 Compound identification

The linear retention indices (LRIs) of detected compounds were calculated using *n*-alkanes (C_6–C_{30}) as reference substance. The components in essential oils were identified by comparing their retention indices (RIs) and mass spectra on the DB-5MS columns with those in literatures, commercial databases (NIST08.L), and other published mass spectra.

2.6 Statistical analysis

The data were analyzed by a two-way analysis of variance (ANOVA) in the SAS 9.2 statistical program (SAS Inc., USA), and mean separation test between treatments was performed using Duncan's multiple range tests at $P < 0.05$ as statistically significant.

3. Results and discussion

3.1 Effects of CO_2 and light quality on monoterpenes

Essential oils of *G. bicolor* roots were analyzed by HS-SPME-GC-MS for the first time in this study. Six monoterpenes were identified from the roots of *G. bicolor*, and α-pinene and β-myrcene were the major monoterpenes (Table 1).

In general, CO_2 concentration and light quality had the significant effects on the percentages of monoterpenes in total essential oils (Table 1). At the control CO_2, the combination of blue and red lights apparently reduced the proportion of total monoterpenes in essential oils compared to WL light treatment. RB40 promoted the proportion of total monoterpenes in essential oils as compared with RB20 light treatment. In addition, the percentage of circular monoterpene hydrocarbons such as α-, β-pinene and α-, β-phellandrene was always higher in RB40 than that in RB20 treatment, and the proportion of acyclic monoterpenes like β-myrcene and β-ocimene was higher in RB20 than that in RB40 treatment. It indicated that more blue lights could promote the synthesis of circular monoterpene hydrocarbons, and more red lights were benefit to the accumulation of acyclic monoterpene hydrocarbons. Moreover, under elevated CO_2, β-ocimene was not detected in all the light treatments, and β-myrcene was identified only in RB20 treatment, whereas they were found in all light treatments at control CO_2. This may reveal that plants grown under the elevated CO_2 were prone to reduce the accumulation of acyclic monoterpene hydrocarbons.

Table 1 Monoterpenes identified from *G. bicolor* roots under different CO_2 concentration and light qualities ($\bar{x} \pm s$, $n = 3$)

No	Compounds	RI	450 μmol·mol^{-1} CO_2 (control)			1200 μmol·mol^{-1} CO_2		
			WL / %	RB20 / %	RB40 / %	WL / %	RB20 / %	RB40 / %
1	1*R*-α-pinene	928 (930)	3.43 ± 0.37^{aA}	1.31 ± 0.29^{bC}	2.48 ± 0.17^{aB}	3.46 ± 0.41^{aB}	4.28 ± 0.62^{aA}	2.48 ± 0.20^{aC}
2	β-phellandrene	968	0.49 ± 0.10^{aA}	0.14 ± 0.02^{bB}	0.44 ± 0.03^{aA}	0.39 ± 0.06^{bB}	0.45 ± 0.01^{aA}	0.27 ± 0.02^{bC}
3	(1S)-β-pinene	973 (976)	0.59 ± 0.05^{aB}	0.20 ± 0.01^{bC}	0.82 ± 0.12^{aA}	0.59 ± 0.08^{aA}	0.55 ± 0.03^{aA}	0.36 ± 0.01^{bB}
4	β-myrcene	984 (987)	3.45 ± 0.91	1.53 ± 0.13	1.77 ± 0.34	−	3.03 ± 0.43	−
5	α-phellandrene	1003 (1006)	0.26 ± 0.06	0.14 ± 0.01	0.24 ± 0.01	−	0.21 ± 0.01	−
6	(*E*)-β-ocimene	1041 (1044)	0.61 ± 0.05	0.39 ± 0.07	0.12 ± 0.00	−	−	−
	total monoterpenes		8.83 ± 1.08^{aA}	3.61 ± 0.97^{bC}	5.87 ± 1.21^{aB}	4.44 ± 0.78^{bB}	8.52 ± 1.34^{aA}	3.11 ± 0.62^{bB}

RI = retention indices are determined on DB-5MS column using *n*-alkanes (C_5−C_{30}) as reference compounds.

Presence of a compound is shown as its GC-FID peak area percentage (%).

Same capital letter, within a row means under the same concentration of CO_2, with no significantly different at $P < 0.05$;

Means with same small letter, within a row under the same light quality, are not significantly different at $P < 0.05$; same as below

3.2 Effects of CO_2 and light quality on sesquiterpenes

Thirty components of sesquiterpenes were identified from the roots of *G. bicolor*, in which (*E*)-β-farnesene, α-, β-caryophyllene, and δ-, β-, γ-elemene were the major compounds (Table 2). As CO_2 increased, the total sesquiterpenes proportion was rapidly decreased (Table 2) in the essential oils, and the composition of sesquiterpene hydrocarbons was changed, some of sesquiterpenes existed at control CO_2 (such as δ-cadinene, epizonarene, 3,7(11)-selinadiene and so on) were disappeared in the essential oils.

At the same CO_2 concentration, the proportion of total sesquiterpenes did not varied by light treatments, however, individual components of sesquiterpenes were changed. For example, the contents of δ-elemene, α-, β-caryophyllene, γ-elemene, and (*E*)-β-farnesene were higher in WL treatment at control CO_2 and RB20 treatment at elevated CO_2, respectively. The content of major sesquiterpenes was always decreased in RB40 treatment compared with RB20 in both control and elevated CO_2 (Table 3).

It was worth to note that non-circular sesquiterpenes of (*E*)-β-farnesene were the main sesquiterpenes in all light treatments with 15%−20% equivalent to 270−370 mg/kg DW at control CO_2 concentration, whereas α-caryophyllene became the major sesquiterpene in all light treatments at elevated CO_2 concentration although its content was only 46−52 mg/kg DW. Compared to control CO_2, (*E*)-β-farnesene was rapidly decreased by 77%−82% under the elevated CO_2 level, and decreased faster than other major compounds such as β-caryophyllene (12%−49%), α-caryophyllene (8%−23.5%), and δ-elemene (4%−37%). It might indicate that the plants were used to reduce the accumulation of acyclic sesquiterpenes at the enriched CO_2 condition.

Based on the previous studies (Yoshikuni et al, 2006; Steele et al, 1998; Degenhardt et al, 2009; Davis and Croteau, 2000) and our results, we proposed the biosynthetic pathways of the major sesquiterpene hydrocarbons in the roots (Figure 1). (*E*)-β-Farnesene, which is present in large quantities in control CO_2-treated plants, is considered to be generated by the directed deprotonation of *cis*-farnesyl cation. In contrast, α- and β-caryophyllene, which are contained in a large quantity in elevated CO_2-treated plants,

Table 2 Sesquiterpenes identified from *G. bicolor* roots under different CO_2 concentration and light qualities ($\bar{x} \pm s$, $n = 3$)

No	Compounds	RI	450 μmol·mol^{-1} CO_2 (control)			1200 μmol·mol^{-1} CO_2		
			WL / %	RB20 / %	RB40 / %	WL / %	RB20 / %	RB40 / %
1	δ-elemene	1330 (1326)	3.79 ± 0.94^{aA}	3.37 ± 0.43^{aA}	2.70 ± 0.64^{aB}	2.41 ± 0.70^{bA}	2.84 ± 0.26^{bA}	2.56 ± 0.58^{aA}
2	α-copaene	1370 (1362)	0.53 ± 0.12^{aA}	0.28 ± 0.15^{aB}	0.20 ± 0.04^{aB}	0.25 ± 0.08^{bA}	0.26 ± 0.10^{aA}	0.23 ± 0.07^{aA}
3	β-elemene	1384 (1385)	2.04 ± 0.65^{aA}	1.30 ± 0.27^{aB}	1.23 ± 0.38^{aB}	0.75 ± 0.29^{bA}	0.91 ± 0.41^{aA}	0.87 ± 0.35^{aA}
4	cyperene	1397 (1398)	0.76 ± 0.24^{aA}	0.30 ± 0.14^{aB}	0.26 ± 0.09^{aB}	0.47 ± 0.16^{bA}	0.51 ± 0.10^{aA}	0.35 ± 0.09^{aB}
5	β-caryophyllene	1415 (1414)	6.30 ± 0.85^{aA}	3.60 ± 0.27^{aB}	3.32 ± 0.84^{aB}	3.16 ± 0.39^{bA}	3.03 ± 0.43^{aA}	2.91 ± 0.81^{aA}
6	γ-elemene	1424 (1425)	2.83 ± 0.54^{aA}	2.35 ± 0.67^{aA}	2.04 ± 0.14^{aB}	0.51 ± 0.36^{bA}	0.41 ± 0.23^{bA}	0.52 ± 0.31^{bA}
7	isoledene	1441	0.15 ± 0.07	0.26 ± 0.11	0.35 ± 0.08	–	–	–
8	(*E*)-β-farnesene	1450 (1447)	15.26 ± 1.23^{aC}	17.29 ± 1.45^{aB}	19.99 ± 2.08^{aA}	3.39 ± 0.28^{bA}	3.65 ± 0.96^{bA}	3.76 ± 0.67^{bA}
9	α-caryophyllene	1452 (1449)	6.78 ± 1.05^{aA}	4.96 ± 0.55^{aB}	6.00 ± 0.72^{aA}	5.24 ± 0.39^{bA}	4.58 ± 0.34^{aA}	4.83 ± 0.22^{bA}
10	calamenene	1461	0.13 ± 0.02	0.17 ± 0.01	0.13 ± 0.01	–	–	–
11	4,11-selinadiene	1468	0.77 ± 0.06	–	0.31 ± 0.03	–	–	–
12	aromadendrene	1471 (1474)	–	–	–	–	0.27 ± 0.14	0.31 ± 0.09
13	β-patchoulene	1472	1.02 ± 0.24	1.90 ± 0.54	2.20 ± 0.46	–	0.21 ± 0.07	–
14	germacrene D	1476 (1480)	0.26 ± 0.05	0.20 ± 0.01	0.13 ± 0.01	–	–	–
15	δ-selinene	1482 (1478)	1.11 ± 0.45^{aB}	1.72 ± 0.27^{aA}	0.95 ± 0.21^{aB}	0.60 ± 0.17^{bA}	0.34 ± 0.04^{bB}	0.56 ± 0.13^{bA}
16	β-selinene	1483 (1481)	–	–	–	0.41 ± 0.06	0.35 ± 0.02	0.49 ± 0.08
17	α-selinene	1484 (1484)	2.22 ± 0.73	2.26 ± 0.19	2.26 ± 0.38	–	–	–
18	α-gurjunene	1497	0.93 ± 0.41		0.33 ± 0.05	0.53 ± 0.21	0.36 ± 0.10	0.53 ± 0.22
19	δ-cadinene	1512 (1513)	0.35 ± 0.02	0.32 ± 0.06	0.44 ± 0.10	–	–	–
20	epizonarene	1531	0.21 ± 0.01	0.39 ± 0.03	0.36 ± 0.05	–	–	–
21	3,7(11)-selinadiene	1535	0.15 ± 0.02	0.28 ± 0.04	0.30 ± 0.01	–	–	–
22	elixene	1552	0.22 ± 0.04	0.27 ± 0.04	0.41 ± 0.05	–	–	–
23	β-maaliene	1556	–	1.18 ± 0.16	0.71 ± 0.37	–	–	–
24	1,7,7-trimethyl-2-vinylbi-cyclo[2.2.1]hept-2-ene	1582	–	0.22 ± 0.05	0.13 ± 0.01	–	–	–
25	α-elemene	1620	0.27 ± 0.04	0.76 ± 0.14	0.32 ± 0.01	0.55 ± 0.02	–	–
26	di-epi-α-cedrene-(I)	1649	0.77 ± 0.02^{bB}	1.38 ± 0.17^{aA}	0.62 ± 0.13^{bC}	1.01 ± 0.28^{aA}	0.61 ± 0.21^{bB}	0.87 ± 0.13^{aA}
27	γ-gurjunene	1652	–	0.18 ± 0.01	–	–	0.32 ± 0.01	–
28	γ-selinene	1659	0.47 ± 0.04	0.95 ± 0.29	–	0.82 ± 0.20	–	–
29	dehydro-aromadendrene	1681	0.61 ± 0.07^{aB}	1.06 ± 0.31^{aA}	0.49 ± 0.17^{aB}	0.35 ± 0.09^{bA}	0.22 ± 0.04^{bA}	0.31 ± 0.02^{bA}
30	(*R*)-cuparene	1712	0.19 ± 0.05	0.27 ± 0.01	–	–	–	–
	total sesquiterpenes		48.13 ± 2.35^{aA}	47.24 ± 1.87^{aA}	46.18 ± 1.98^{aA}	20.4 ± 1.24^{bA}	18.93 ± 1.51^{bA}	19.14 ± 1.06^{bA}

Table 3 Quantitative changes of main terpene components in *G. bicolor* roots under different CO_2 concentration and light qualities ($\bar{x} \pm s$, $n = 3$)

Compounds	450 μmol·mol^{-1} CO_2 (control)			1200 μmol·mol^{-1} CO_2		
	WL / (mg·kg^{-1})	RB20 / (mg·kg^{-1})	RB40 / (mg·kg^{-1})	WL / (mg·kg^{-1})	RB20 / (mg·kg^{-1})	RB40 / (mg·kg^{-1})
1*R*-α-pinene	85.34 ± 3.90^{aA}	26.31 ± 2.10^{bC}	33.84 ± 2.70^{aB}	31.13 ± 1.80^{bB}	49.30 ± 3.70^{aA}	23.88 ± 1.50^{bC}
β-myrcene	85.94 ± 2.75	30.16 ± 1.44	24.10 ± 1.21	–	34.20 ± 2.77	–
δ-elemene	93.39 ± 4.17^{aA}	67.25 ± 3.24^{aB}	36.47 ± 2.48^{aC}	21.50 ± 1.34^{bB}	32.35 ± 2.07^{bA}	24.41 ± 1.39^{bB}
β-elemene	50.20 ± 3.58^{aA}	26.00 ± 1.77^{aB}	16.63 ± 0.54^{aC}	6.66 ± 1.16^{bC}	10.34 ± 0.36^{bA}	8.32 ± 0.24^{bB}
β-caryophyllene	156.34 ± 6.42^{aA}	72.30 ± 5.17^{aB}	45.20 ± 2.19^{aC}	28.31 ± 1.33^{bB}	34.83 ± 0.89^{bA}	27.96 ± 1.35^{bB}
γ-elemene	69.82 ± 4.25^{aA}	46.95 ± 3.55^{aB}	27.56 ± 1.56^{aC}	4.53 ± 0.57^{bB}	4.73 ± 0.37^{bAB}	4.97 ± 0.52^{bA}
(*E*)-β-farnesene	373.57 ± 8.94^{aA}	342.71 ± 5.38^{aB}	268.10 ± 9.12^{aC}	30.04 ± 1.72^{bC}	41.39 ± 1.42^{bA}	35.68 ± 0.97^{bB}
α-caryophyllene	167.17 ± 6.31^{aA}	99.06 ± 3.79^{aB}	81.02 ± 2.56^{aC}	46.66 ± 2.92^{bB}	52.21 ± 1.68^{bA}	46.10 ± 0.69^{bB}

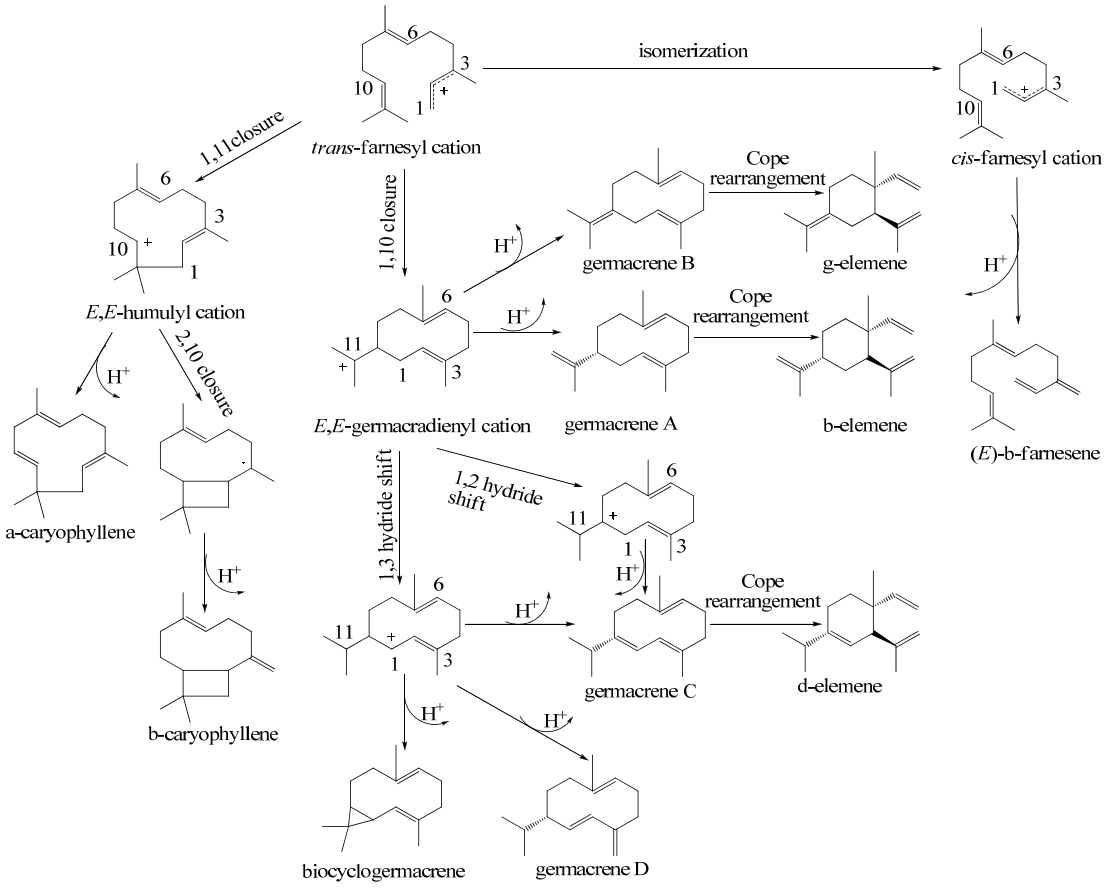

Figure 1 Proposed biosynthetic pathways for sesquiterpene hydrocarbons in volatile oils from *G. bicolor* roots

are produced by the cyclization of *trans*-farnesyl cation from C-1 to C-11. Therefore, we proposed that deprotonation occured prior to cyclization of the *trans*- or *cis*-farnesyl cation at control CO_2 condition instead in elevated CO_2 condition, which suggested that the growing environments could significantly affect the production of sesquiterpene hydrocarbons.

Germacrenes A, B, C, and bicyclogermacrene were not found in this study, whereas the corresponding elemane derivatives (β-, γ-, and δ-elemene) had a great proportion of the total essential oils in the roots of *G. bicolor* in all treatments, which did not support Shimizu et al (2011). Since germacrane sesquiterpenoids were generally accompanied by their corresponding elemane cope rearrangement products in gas chromatographic analyses of essential oils. Cope rearrangement of germacrenes to elemene was facile at high temperature, some concern was considered about whether one or the other may be an artifact due to the high temperature encountered during the hydrodistillation or the gas chromatographic analysis (Setzer, 2008). However, germacrenes A, B, C, and bicyclogermacrene were also important intermediates in the biosynthesis of other sesquiterpenoids (Colby et al, 1998; de Kraker et al, 1998), which might be why germacrenes were not detected in all treatments.

The resulting carbocation undergoes a range of

cyclizations, secondary cyclization, hydride shifts, and deprotonateion to a neutral intermediate, some of which are preceeded by isomerization, re-protonation, and cope rearrangements to other products. The numbering of carbon atoms of intermediates and products refers to that for *trans*-farnesyl cation.

Recent reports showed that β-caryophyllene had several biological activities such as antibiotic (Sabulal et al, 2006), anti-inflammatory (Fernandes et al, 2007), anticarcinogenic (Legault and Pichette, 2007; Park et al, 2011; Amiel et al, 2012), anti-oxidative (Calleja et al, 2012), and local anaesthetic activities (Ghelardini et al, 2001). Gertsch et al (2008) demonstrated that β-caryophyllene was a dietary cannabinoid, because it acted as a selective nonpsychoactive cannabinoid receptor type 2 (CB2) receptors agonist in foodstuff.

Differed from other classical cannabinoids, it can protect brain cells from ischemia injury without any psychoactive side effects (Choi et al, 2013). Given the abundance of CB2 receptors in distinct disease tissues, the therapeutic potential of β-caryophyllene is broad (Bento et al, 2011; Horvath et al, 2012; Klauke et al, 2014; Gertsch et al, 2008). (*E*)-β-Farnesene, an acyclic sesquiterpene, is different from caryophyllene. Turkez et al, (2014) reported their potentiating neuroprotective effects on hydrogen peroxide-induced neurotoxicity. Elemene was also largely isolated along with (*E*)-β-farnesene and α-, β-caryophyllene from roots. Nowadays, β-elemene has been intensively studied in cancer diseases (Adio, 2009; Liu et al, 2011; Wang et al, 2005; Chen et al, 2011; Sun et al, 2009; Li et al, 2009; Wang et al, 2011; Chen et al, 2012). Therefore, *G. bicolor* roots could be a useful raw material due to these bioactive compounds. Furthermore, it was important to improve the accumulation of them in the roots of *G. bicolor* by controlling the environmental factors.

3.3 Effects of CO_2 and light quality on total essential oils

The major components of the essential oils in the roots from all treatments were terpenes and aldehydes (> 80% of the total essential oils) (Figure 2A). Essential oils in the roots contained large quantities of aldehydes including hexanal, (*E*)-2-hexenal, (*E*)-2-nonenal, and (*E*,*E*)-2,4-nonadienal, and they also contained alcohols (such as eucalyptol and linalool), esters (methyl salicylate and 1-octen-3-yl-acetate) and other volatile compounds (butylated hydroxytoluene, 2-methoxy-3-(1-methylethyl)-pyrazine, and 2-methoxy-3-(1-methyl propyl)-pyrazine). Shimizu et al (2010) reported that methoxypyrazines (2-methoxy-3-(1-methylethyl)-pyrazine and 2-methoxy-3-(1-methylpropyl)-pyrazine) were identified as aroma-impact compounds in the roots. The data about aldehydes, alcohols, esters, and other volatile compounds are shown in Table 4.

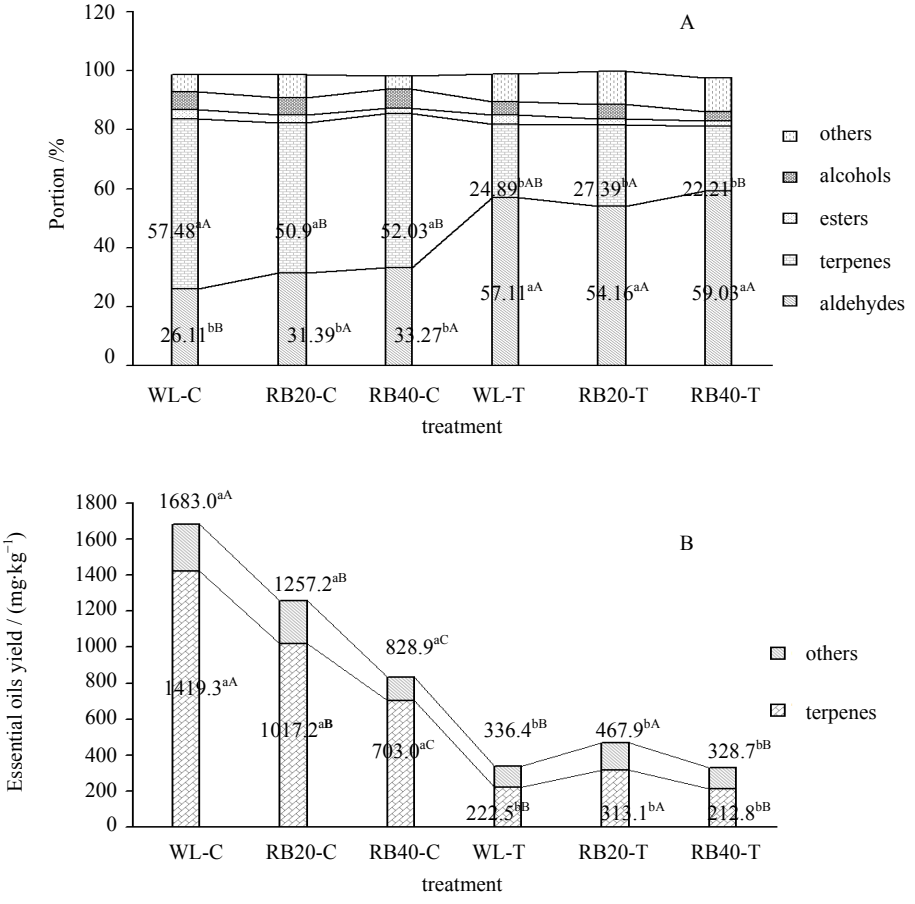

Figure 2 Proportion of volatile compound classes (A) in essential oils and total contents of essential oils (B) from *G. bicolor* roots under different CO_2 levels and light qualities (n = 3)

Table 4 Composition of other volatile oils identified in HS-SPME obtained from *G. bicolor* roots under different CO_2 concentration and light qualities ($\bar{x} \pm s$, $n = 3$)

No	Compounds	RI	450 μmol·mol^{-1} CO_2 (control)			1200 μmol·mol^{-1} CO_2		
			WL / %	RB20 / %	RB40 / %	WL / %	RB20 / %	RB40 / %
1	hexanal	797	12.21 ± 0.57^{bC}	18.13 ± 0.27^{bB}	23.37 ± 0.93^{bA}	36.59 ± 1.35^{aA}	33.30 ± 0.83^{aB}	35.34 ± 0.74^{aA}
2	(*E*)-2-hexenal	846	5.70 ± 0.42^{bB}	5.52 ± 0.31^{bB}	6.61 ± 0.28^{bA}	8.89 ± 0.41^{aA}	8.33 ± 0.38^{aA}	8.30 ± 0.44^{aA}
3	1-hexanol	860	0.90 ± 0.23	−	−	1.73 ± 0.26	2.21 ± 0.42	−
4	2-ethyl-1-hexanol	1024	1.76 ± 0.33	2.14 ± 0.24	2.00 ± 0.38	−	−	−
5	eucalyptol	1028	0.92 ± 0.15^{aC}	1.45 ± 0.17^{aB}	2.44 ± 0.34^{aA}	0.89 ± 0.27^{aA}	0.93 ± 0.12^{bA}	0.99 ± 0.20^{bA}
6	(*E*)-2-octenal	1054	0.70 ± 0.10	−	−	1.22 ± 0.11	1.23 ± 0.14	1.41 ± 0.10
7	2-methoxy-3-(1-methyleth yl)-pyrazine	1083 (1087[d])	1.50 ± 0.24^{bB}	1.93 ± 0.15^{aA}	1.81 ± 0.19^{bA}	1.80 ± 0.23^{aB}	1.84 ± 0.10^{aB}	2.22 ± 0.16^{aA}
8	linalool	1096 (1098)	1.58 ± 0.12^{bB}	2.10 ± 0.15^{aA}	1.60 ± 0.09^{bB}	1.84 ± 0.12^{aB}	1.90 ± 0.08^{aB}	2.21 ± 0.10^{aA}
9	1-octen-3-yl-acetate	1103 (1106)	2.69 ± 0.24	2.25 ± 0.30	1.50 ± 0.20	0.90 ± 0.15	−	−
10	(*E*)-2-nonenal	1157	3.80 ± 0.26^{aA}	3.43 ± 0.10^{bB}	1.35 ± 0.13^{bC}	4.10 ± 0.31^{aB}	4.41 ± 0.29^{aB}	6.53 ± 0.35^{aA}
11	2-methoxy-3-(1-methylpropyl)-pyrazine	1161 (1164)	1.82 ± 0.13^{aB}	2.11 ± 0.23^{aA}	1.75 ± 0.10^{aB}	1.60 ± 0.15^{aB}	1.51 ± 0.20^{bB}	1.82 ± 0.05^{aA}
12	methyl salicylate	1188	0.73 ± 0.07	0.41 ± 0.03	−	0.22 ± 0.10	0.33 ± 0.05	0.60 ± 0.08
13	(*E*,*E*)-2,4-nonadienal	1212	1.92 ± 0.14^{bA}	1.80 ± 0.22^{bA}	0.91 ± 0.18^{bB}	2.20 ± 0.11^{aB}	2.26 ± 0.15^{aB}	2.94 ± 0.25^{aA}
14	butylated hydroxytoluene	1496	1.84 ± 0.26^{bB}	3.61 ± 0.35^{bA}	1.02 ± 0.12^{bC}	6.11 ± 0.40^{aA}	6.53 ± 0.35^{aA}	6.43 ± 0.32^{aA}
	other volatiles		38.22 ± 1.53^{bB}	44.81 ± 1.62^{bA}	44.90 ± 1.23^{bA}	67.89 ± 1.80^{aA}	66.11 ± 2.09^{aA}	69.54 ± 1.90^{aA}

At any light treatments, aldehydes showed a significant increase in essential oils from the roots under elevated CO_2 condition, while terpenes showed a significant decrease (Figure 2A). Under the control CO_2 concentration, aldehydes in the essential oils were at a higher level under WL light condition than the combination of red and blue, whereas terpenes were at a higher level under the combination of red and blue light conditions than under WL condition. However, there were no significant differences in the proportion of aldehydes and terpenes between RB20 and RB40 treatments. At the elevated CO_2, light quality did not affect the percentage of aldehydes in essential oils, while the percentage of terpenes decreased with the reinforcement of blue light.

The increased CO_2 greatly reduced the yield of the total essential oils (Figure 2B). At the control CO_2, the rapid reduction of terpenes largely contributed to the decrement of total essential oils in the roots. Vurro et al (2009) reported that the WL-treated roots had a higher yield than the red and blue light-treated ones, which was contrast under elevated CO_2. At the same CO_2 level, the elevation of blue light from 20% to 40% reduced the amount of the total essential oils, which implied that more blue light did not increase the synthesis and accumulation of essential oils in *G. bicolor* roots. Yield variation of terpenes in different treatments was the same as that of the total essential oils.

The rapid reduction of terpenes largely contributed to the decrement of total essential oils in the roots. Vurro et al (2009) found that CO_2 enrichment promoted phenol contents in essential oils, but reduced the contents of monoterpenes and sesquiterpenes, which was in agreement with our results. They thought that it might be the consequence of increased water availability and reduced water loss via transpiration (Wullschleger et al, 1994; Mohamed et al, 2002), or related to the decrease in oxidative stress under elevated CO_2. The reduced terpenes at elevated CO_2 in this study may be explained by the fact that CO_2-enrichment seems to induce the relaxation of anti-oxidant defense system through decreasing the basic rate of the formation of reactive oxygen species (ROS) and activation of O_2, potentially resulting in less need for the synthesis of defensive anti-oxidant. Furthermore, there were studies reported that the increased CO_2 concentration down-regulated gene expression related to defense signaling lipoxygenase 7 (lox7), lipoxygenase 8 (lox8), and 1-aminocyclo-propane-1-carboxylate synthase (acc-s) (Zavala et al, 2008), suppressed the jasmonic acid signaling pathway (Guo et al, 2012), and then reduced the resistance and tolerance of plants defense against invasive insects.

Insect behavior is influenced by terpenoids (Aharoni et al, 2005), the reduction of terpenoids regarding to plant defense at the increased CO_2 could lead to increase plants susceptibility to invasive crop pests. In addition, the decreased terpenes in essential oils and the reduced activity of some anti-oxidant enzyme like SOD, POD, and GR in the leaves from *G. bicolor* (data not shown) were also demonstrated the proposed notion of the depressed defensive anti-oxidant status in elevated CO_2 level under controlled environment.

4. Conclusion

In summary, the increased CO_2 concentration not only decreases the amount of mono- and sesquiterpenes, but also changes their composition. The significance of the effects of elevated CO_2 on the plant essential oils is more apparent than that of LED lighting treatments. Elevated CO_2 decreases the accumulation of terpenes from *G. bicolor* roots under controlled environment without any stress factors like invasion of insects or UV radiation. This study will play a guiding role on the actual production of

the medicinal plants, for instance, increasing CO_2 concentration is applied in the greenhouse in order to promote plants yields, but this measure is bound to reduce the effective ingredients concentration of essential oils, eventually decrease the medicinal value of this plant. Moreover, the results in this study will be applicable to future space farming, controlling higher CO_2 concentration is inevitable measures in a closed controlled environment so as to improve plant photosynthesis to release more O_2 for astronauts breathe and achieve larger plant biomass, which also would lead to reduce the medicinal value of this plant, making poor health effects on astronauts. Therefore, this situation should be considered in planting *G. bicolor* under elevated CO_2 condition. Additionally, LED light source has received good application in the plant factory and controlled plant chamber nowadays, controlling a certain percentage of the blue lights would have a greater impact on the active ingredients in different medicinal plants.

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