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Changes in volatile constituents and phenols from *Gynura bicolor* DC grown in elevated CO_2 and LED lighting

Jin Ren [a], Shuang-Sheng Guo [b,a,*], Xiu-Lan Xin [c], Liang Chen [c]

[a] Faculty of Life Sciences, Northwestern Polytechnical University, Xi'an 710072, China
[b] National Key Laboratory of Human Factors Engineering, China Astronaut Research and Training Center, Beijing 100094, China
[c] College of Bioengineering, Beijing Polytechnic, Beijing 100029, China

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ABSTRACT

Volatile constituents and phenols of *Gynura bicolor* DC grown under three different CO_2 levels of ambient (450 μmol mol^{-1}) and elevated (1200, 2000 μmol mol^{-1}) and three LED light conditions of WL (white light), RB20 (red/blue = 8/2) and RB40 (red/blue = 6/4) were investigated to improve efficient production of bioactive compounds in this vegetables under controlled environment. Results showed that increased CO_2 caused a significant decrease in volatile terpenoids from *G. bicolor* DC leaves as compared with ambient CO_2 at any light treatments. Plants grown under ambient CO_2 with RB20 LED lighting had the highest yield of volatile terpenoids. In addition, CO_2 elevation from 450 to 1200 improved the production of phenols (including anthocyanins, flavonoids and phenolics), while increasing CO_2 from 1200 to 2000 appeared to negate such promotion. Furthermore, phenols contents were promoted by RB20 as compared with WL light treatment, whereas those were not further improved when increasing blue lights up to 40% (RB40). Our results indicated that the better choices for *G. bicolor* DC to produce more volatile terpenoids and phenols under controlled environment were at ambient and elevated CO_2 levels with RB20 LED lighting condition, respectively.

1. Introduction

Gynura bicolor DC. (*G. bicolor*), belonging to Asteraceae family, is commercially cultivated as a traditional vegetable in south China, like Guangdong, Fujian, Hainan, Jiangsu, Taiwan, where it is also called 'Guanyinxian', 'Hongfencai', 'Zibeicai' and 'Xuepicai' and so on. Its leaves have a reddish-purple color and a good flavor, associated with the presence of anthocyanins and volatile compounds (Shimizu et al., 2009, 2010, 2011; Lu et al., 2004; Lu et al., 2011; Chen et al., 2012). Recent studies also revealed that *G. bicolor* had high anti-oxidative activity (Maeda, 2006), the apoptosis-inducing effect on cancer cells (Hayashi et al., 2002) and other bioactivities (Hsieha et al., 2013; Wu et al., 2013) in which phenols and volatile constituents were considered to be the main biologically active compounds. Hence, a method for increasing the concentrations of phenols and volatile components in the leaves while simultaneously promoting growth is essential for efficient production of plants. Growing plants under controlled environment could be an alternative, which allows the precise control of optimized environmental conditions with maximum biomass and bioactive compounds production, and ensures the plants free from biotic and abiotic contaminations with consistent biochemical profiles (Zobayed et al., 2005).

Volatiles and phenols are considered to be the main bioactive compounds of interest in this plant, and they have physiological implications on plant biological behavior, their quantity and quality were affected by environmental factors (Bianco, 2010). Any change in the levels of these bioactive compounds caused by varying the CO_2 environment and lighting condition would have important commercial and ecological significance (Reitz et al., 1997; Vaughn and Spencer, 1993). However, to our best knowledge, no data have been collected regarding *G. bicolor* volatile compounds and phenols changes under elevated CO_2 condition with artificial LED lighting. So, in this paper the authors investigated for the first time some biochemical responses of this plant when subjected to different CO_2 levels and LED light qualities so as to determine the efficient production conditions under controlled environments.

* Corresponding author at: China Astronaut Research and Training Center, National Key Laboratory of Human Factors Engineering, Beijing 100094, China. Tel.: +86 010 6874 6545; fax: +86 010 6874 6545.
E-mail addresses: guoshuangsheng@tom.com, xuyuchenxi@hotmail.com (S.-S. Guo).

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2. Materials and methods

2.1. Plant materials and growth conditions

Lighting system: All the LED lamps were purchased from Wuxi Fangzhou Technology Co. (Beijing, China) and the parameters of LEDs were blue LED (1 W, 460–463 nm), red LED (1 W, 625–630 nm) and white LED (full spectrum, 1 W). Plants were treated with RB20 (80% red lights + 20% blue lights) and RB40 (60% red lights + 40% blue lights), and white LED (WL)-treated plants were the control. All light modules were placed inside a controlled environment chamber (length 8 m × diameter 2.65 m) with non-reflective panel between treatments to prevent light contamination (Guo et al., 2008). The total photosynthetic active radiation (PAR) intensity of WL, RB20 and RB40 were 249.37 ± 2.24, 250.70 ± 1.22, 251.03 ± 1.92 μmol m^{-2} s^{-1} at the height of plant canopy (below the lighting about 20 cm), respectively. The percentages of blue light in RB20 and RB40 over the total PAR were about 9.52% and 21.89%.

G. bicolor seedlings (the Beijing Academy of Agriculture and Forestry) were grown in round shaped 15 cm (height) × 17 cm (top diameter) × 12 cm (bottom diameter) plastic pots. 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^{-1}, PH6.3–6.4) every two days to ensure the abundant nutrient and water supply. Each pot had one seedling and the pots were placed randomly in growth modules. Actual net planting area was about 1.1 m (length) × 0.7 m (width) × 3 (light treatments) in our experiment, 24 seedlings were planted in each light treatment, this results in a planting density of 31 plants m^{-2}. Seedlings were initially maintained under low lighting for 3 days to acclimate the new circumstances after being transplanted in the plots, then subjected to different lighting treatments with PAR at 250 ± 5 μmol m^{-2} s^{-1} (adjusted as the plants grew) in a photoperiod of 16 h/8 h light/dark cycle. Within the light module, the pots were randomly shifted every two days to avoid any positional effects. The high CO_2 treatments of 1200 (1.2k) and 2000 (2k) μmol mol^{-1} were conducted on the seventh day after initiating light treatments. Pure carbon dioxide (99.9% purity; Jinggao gas co.ltd, Beijing) was supplied from a high concentration carbon dioxide cylinder and injected through a pressure regulator into the closed chamber. Online infrared CO_2 analysis instrument (GXH-3011, the institutes of Huayun analysis instrument, Beijing) was used to measure the CO_2 concentration. Ambient CO_2 (450 μmol mol^{-1}) concentration served as the CO_2 control. Air Relative Humidity inside the chamber and the temperature were maintained at 60% ± 5% and 24/19 °C ± 1 °C (light/dark), respectively, throughout the experiment (cultivation cycle: 30 days). Wind velocity in the chamber was about 0.8 m s^{-1}. 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 CO_2 concentration and light quality.

For all treatments, seedlings were harvested on the 30th day. Fresh leaves of plants were flash frozen in liquid nitrogen and then freeze-dried with a freeze-dryer for 48 hr to avoid biochemical changes due to relative enzymes activity. The freeze-dried samples were sealed in plastic bags and stored at −20 °C until analysis.

2.2. Analysis and determination of volatile constituents

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 volatile constituents from *G. bicolor* DC leaves. Solvent extraction was employed to carry out quantitative analysis.

2.2.1. Headspace solid-phase micro-extraction (HS-SPME)

HS-SPME protocol was as follows. The lyophilized leaves (1 g × triplicate) were immersed in freshly physiological saline, grind thoroughly and swiftly with NaCl, then immediately placed into 50 mL triangular flask, 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 volatile constituents 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.

2.2.2. Gas chromatography–mass spectrometry analysis (GC–MS)

Blank analysis was run after conditioning the PDMS fiber at the manufacturer's recommended temperature 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 film thickness) was used to separate different constituents.

Analytical conditions were as follows. The oven was maintained at 50 °C for 1 min and then ramped at 2 °C min^{-1} to 180 °C, held isothermal (4 min) at 180 °C, total run time was approximately 70 min; injector temperatures were held at 230 °C. The carrier gas was helium with a flow rate of 1 mL min^{-1}, constant pressure, 7.7421 psi. 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 from *G. bicolor* leaves was obtained by mean values of GC (FID) peak area.

2.2.3. Solvent extraction and GC–MS analysis

Solvent extraction protocol was as follows. Lyophilized leaf samples (1 g × triplicate) were extracted with distilled dichloromethane for 60 min at 80 °C in a circulating water bath after crushed to pieces. The greenish organic layer was separated, dried over anhydrous sodium sulfate. In order to quantify the volatile constituents yield, 1-octanol was added as internal standard. The extract was concentrated carefully using a rotary evaporator in vacuo to ca. 1 mL. An aliquot of this concentrate was taken for GC–MS analysis to determine volatile compounds.

GC–MS analysis for solvent extraction were similar to that for HS-SPME except for the temperature programming, in which the oven was maintained at 50 °C for 1 min and then ramped at 3 °C min^{-1} to 250 °C, held isothermal (20 min) at 250 °C, injected volume was 1 μL and total run time was approximately 87 min. Semi-quantitative data was calculated using the internal standard method, FID response factors were calculated theoretically with effective carbon number of internal standard and analytic composition, which was based on the positive relationship between FID response factors and effective carbon number of compounds. Actual response factors of α,β-caryophyllene (commercially available, ChromaDex) were measured using purified compounds according to Shimizu et al. (2009).

2.2.4. Compounds identification

The linear retention indices (LRIs) of detected compounds were calculated using *n*-alkanes (C_6–C_{30}) as the reference materials. Volatile components were identified by comparing their RIs on the DB-5MS columns with those of literatures or with those of authentic compounds available in our laboratory. Further identification