



Growth, morphological and photosynthetic characteristics, antioxidant capacity, biomass yield and water use efficiency of *Gynura bicolor* DC exposed to super-elevated CO₂

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ARTICLE INFO

Article history:

Received 15 July 2014

Received in revised form

16 April 2015

Accepted 4 May 2015

Available online 13 May 2015

Keywords:

Antioxidant system

Bioregenerative life support system (BLSS)

Carbon dioxide

G. bicolor

Photosynthesis

ABSTRACT

As a consequence of the increasing importance of leaf vegetables in Bioregenerative life support system (BLSS), there is an interest in enhancing both the productivity and quality of *Gynura bicolor* DC (*G. bicolor*). The effects of super-elevated carbon dioxide concentration (3000 ppm) on *G. bicolor* in different periods were investigated to reveal plausible underlying mechanisms for the growth, photosynthetic characteristics, antioxidant capacity and biomass yield. Experimental results showed that not only the photosynthetic rate but also the antioxidant capacity were increased in short-term super-elevated CO₂ concentration. However, under long-term super-elevated CO₂ concentrations, photosynthetic rate and pigment contents of *G. bicolor* significantly decreased due to photo inhibition. Elevated CO₂ can be able to dwarf the *G. bicolor* plants. The leaves in high CO₂ concentration were curled, which may affect the photosynthetic efficiency. At 3000 ppm level, leaf senescence accelerated but biomass yield had no significant difference. Furthermore, elevated CO₂ controlled transpiration rate and then enhanced water use efficiency. Superoxide dismutase (SOD) activity, peroxide activity (POD) and total antioxidant capacity (T-AOC) in 3000 ppm condition were significantly lower than that in 400 ppm condition. On the contrary, catalase (CAT) activity, glutathione (GSH) content and hydrogen peroxide (H₂O₂) were significantly enhanced under high CO₂ concentration.

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1. Introduction

The global atmospheric carbon dioxide concentration ([CO₂]), presently at about 385 μmol mol⁻¹ CO₂ mol⁻¹

air, may surpass 700 μmol mol⁻¹ before the end of this century [1]. A rise in atmospheric [CO₂] and other greenhouse-effect gases is expected to cause changes in global climate, including increases in air temperatures and shifts in regional scale rainfall patterns, which could result in decreased soil water availability in some areas of the world [2,3]. Growing plants under elevated CO₂ may neutralize or mitigate the negative effects of the stressful environmental conditions on production, maintaining or even improving the antioxidant system [4,5].

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Establishment of Bioregenerative life support system (BLSS) is an ultimate way to provide food, oxygen and water for astronauts carrying out long-term manned spaceflight and deep space exploration [6,7]. Plants will be an integral part in BLSS for long-term, human space exploration [8,9]. CO₂ enrichment is the sole source of carbon for plants, which will act as carbon fertilizer, resulting in far reaching consequences for plant production generally, grain yield and quality specifically [10,11]. More importantly, the CO₂ levels for advanced life support systems may be at super elevated levels [12] and CO₂ may be used as a pressurizing gas for growing plants on Moon or Mars. It is very important to select plants which are resistant to high CO₂ concentration. The effects of carbon dioxide on plant growth have been widely studied, especially in conjunction with increasing concerns about rising CO₂ concentrations in the Earth's atmosphere [12,13]. In general, these studies have shown that increases in CO₂ (e.g., a doubling from 350 to 700 $\mu\text{mol mol}^{-1}$) increase photosynthetic rates for C₃ species and decrease water use per unit area of vegetation for C₃ and C₄ species [14,15]. The present atmosphere [CO₂] limits the growth of C₃ crop plants, which show responses to elevated [CO₂] via reduced photorespiration and enhanced photosynthetic rates, thereby increasing their growth and yield.

Gynura bicolor DC (*G. bicolor*), which originates in the tropical area of East Asia, is one of perennial vegetables grown for its edible young immature leaves and stems. It contains relatively high nutritional value of vitamin, ferrite, manganese and flavonoids with higher antioxidant activity and serve as one of the candidate plants in the life support system [5]. Recent studies have shown that a diet rich in antioxidants can mitigate the harmful effects of the stressful environmental conditions during long-term space flight [4,16]. Therefore, planting the vegetables rich in antioxidants in the space environment cannot only increase the dietary diversity of the astronauts, but also reduce the risk of space radiation to the astronauts [16]. The responses of different species to high CO₂ were significantly different. Hence, it is necessary to understand the mechanisms underlying the different effects of high CO₂ concentration on the yield and antioxidant capability of *G. bicolor*. Moreover, as a consequence of the growing global demand for food, and the increasing awareness of the importance of fruits and vegetables in the human diet, plant physiologists, agriculturists, and plant breeders have demonstrated increasing interest not only in enhancing crop productivity but also in improving the nutritional and health-promoting qualities of commonly consumed fruits and vegetables [17].

In this study, we evaluated the response of growth, morphological and photosynthetic characteristics, antioxidant capacity, biomass yield and water use efficiency of *G. bicolor* plants under different CO₂ concentrations (400 and 3000 ppm) in the closed environment growth chambers. The results provide some basic information on the optimal cultivation pattern for stable, good quality, high yields of leaf vegetables in BLSS.

2. Materials and methods

2.1. Cultivation conditions

G. bicolor DC plants (16 seedlings per treatment) were initially maintained under low lighting for 3 days to acclimate the new growth circumstances after being transplanted in the pots, then subjected to different CO₂ treatments. Mixtures of red plus white LEDs (R+W, R: W=1: 1) were used [9]. For all treatments lighting was continuous (24/0 h light/dark). Photosynthetic photon flux density (PPFD) levels were measured at the top of plant canopy with a quantum sensor (Li-250 A, Li-Cor, USA). PPFD was about 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for all the treatments. Air temperature and relative humidity were maintained in growth chambers at $21 \pm 1.3^\circ\text{C}$ and $70 \pm 3.4\%$, respectively. The modified Hoagland nutrient solution (Hoagland and Arnon, 1950) included: $[\text{NH}_4^+]$ 9.0 mg/L; $[\text{Ca}^{2+}]$ 80.1 mg/L; $[\text{K}^+]$ 117.2 mg/L; $[\text{Mg}^{2+}]$ 24.05 mg/L; $[\text{NO}_3^-]$ 310.4 mg/L; $[\text{H}_2\text{PO}_4^-]$ 48.5 mg/L; $[\text{SO}_4^{2-}]$ 96.2 mg/L and the pH was 5.6–5.8.

One chamber was set to maintain the CO₂ concentration at 400 ppm (ambient conditions) and the other at 3000 ppm (super-elevated CO₂ conditions). CO₂ monitoring was done with non-dispersive infrared CO₂ sensors (T6615, USA). Pure carbon dioxide (99.9% purity; Haipu 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 CO₂ analysis instrument (CICS) [18] was used to measure the CO₂ concentration. Ambient CO₂ concentration served as the control.

2.2. Morphological and physiological analyses

Plants were harvested at 15 days after planting (DAP), wherein 6 plants were selected randomly from each treatment to conduct morphological measurements. Plant height (PH) and root height (RH) was measured from plant base to tip of the main stem with straight scale. The fresh weight ($\text{FW}=m_1$, g plant⁻¹) of plants were determined, and then put it in the drying oven (85 °C) for 48 h. The dried weight was expressed as m_2 . Biomass of the fresh and dry weight were measured with an electronic balance. Water content (WC) was calculated according to the following equation:

$$\text{WC} = \frac{m_1 - m_2}{m_1} \times 100\% \quad (1)$$

2.3. Photosynthetic characteristics analyses

2.3.1. Chlorophyll and carotenoid contents

Chlorophyll was extracted from the leaves of 6 plants at a similar position within each treatment randomly when plants were harvested at 15 days. The minced sample with 0.1 g (fresh weight, FW) each, were placed in a mortar, and a little of quartz sand and calcium carbonate powder, and 2 to 3 ml of 95% ethanol were added, and then homogenized.

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