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Interactive effects of CO_2 enrichment and brassinosteroid on CO_2 assimilation and photosynthetic electron transport in *Cucumis sativus*

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ABSTRACT

CO_2 enrichment and brassinosteroids (BR) both have positive impacts on photosynthesis and plant growth. To examine the interactive effect of CO_2 enrichment and BR on photosynthesis and plant growth, CO_2 assimilation, chlorophyll fluorescence quenching, carbohydrate metabolism, photosynthetic gene transcript and enzyme activity were analyzed in leaves of young plants of cucumber (*Cucumis sativus* L.) in response to a doubling of growth CO_2 level, foliar BR application alone or in combination. Both CO_2 elevation and application of BR increased shoot biomass, leaf area, CO_2 assimilation, total soluble sugar and starch contents, transcript for photosynthetic gene and activity for enzymes involved in Benson–Calvin cycle but a combination of the two treatments resulted in a more significant effect. Although an elevation of CO_2 level had little effects on quantum efficiency of PSII (Φ_{PSII}), it significantly increased the electron flux for photosynthetic carbon reduction [J_e(PCR)] but decreased electron flux for photorespiratory carbon oxidation [J_e(PCO)]. In contrast, BR treatment increased Φ_{PSII} and this increase in Φ_{PSII} was associated with increased J_e(PCR) and J_e(PCO). Furthermore, a combined treatment of CO_2 elevation and BR resulted in an additive effect on PSII electron flux. However, alternative electron flux was almost unaltered after CO_2 enrichment and BR treatment. Thus, short term CO_2 elevation did not induce a down-regulation of photosynthesis and there was an additive effect between BR and CO_2 on the enhancement of CO_2 assimilation in leaves of young cucumber plants.

1. Introduction

The concentrations of atmospheric CO_2 are predicted to double the current levels of 380 μmol mol^{-1} by the end of this century (Watson et al., 1990). If other growth conditions are optimal, the photosynthetic rate of C_3 plants is limited by the supply of CO_2 (Kimball, 1983; Kimball et al., 2002; Long et al., 2004). Accordingly, crop CO_2 enrichment has been a powerful practice in greenhouse industry for improving produce quality and increasing crop yield. Total dry matter production increases in many species of plants are correlated with increased CO_2 levels and are most pronounced during the early stages of vegetative growth and development (Chu et al., 1992). However, research effort has not kept pace with commercial development. Whilst a great deal of data is available on photosynthesis covering many varieties of plants, the role played by CO_2 is perhaps the least understood.

Elevated CO_2 levels may increase plant productivity due to enhanced CO_2 fixation, suppressed photorespiration and/or suppressed dark respiration (Bunce, 1990; Drake et al., 1997; Amthor, 2001). In plants with a C_3 photosynthetic pathway, the enzyme Rubisco catalyses the initial carboxylation and oxygenation reactions of ribulose-1,5-bisphosphate (RuBP) (Bowes, 1993). Rubisco is not CO_2-saturated at current atmospheric CO_2 levels in C_3 plants, and an increase in atmospheric CO_2 concentration will decrease photorespiration and increase photosynthesis since the balance of carboxylation and oxygenation depends on the CO_2 and O_2 ratio at the Rubisco site (Bowes, 1993; Drake et al., 1997). However, prolonged exposure to elevated atmospheric CO_2 will lead to a decreased photosynthetic capacity in many plant species (Sage et al., 1989). This acclimation response to elevated CO_2 is often accompanied by an increase in soluble carbohydrate pools and a decrease in Rubisco protein content, activity, and activation state (Bowes, 1993; Drake et al., 1997; Pfannschmidt, 2003). However, there are significant differences in the response of mature leaves and developing leaves to CO_2 elevation (Pearson and Brooks, 1995).

Abbreviations: BR, brassinosteroids; F_v'/F_m', efficiency of excitation capture by open PSII center; J_a, alternative electron flux; J_e(PCO), electron flux for the photorespiratory carbon oxidation; J_e(PCR), electron flux for the photosynthetic carbon reduction; J_e(PSII), total electron flux in PSII; P_n, net CO_2 assimilation rate; PPFD, photosynthetic photo flux density; Φ_{PSII}, quantum efficiency of PSII; q_P, photochemical quenching coefficient; RuBP, ribulose-1,5-bisphosphate.

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Several studies showed that the mature leaves were more sensitive to the photosynthetic acclimation induced by elevated CO_2 (Gesch et al., 1998; Sakai et al., 2006). To avoid photosynthetic acclimation and reduced growth vigour at the late growth stage, farmer prefer to introduce CO_2 enrichment at the early growth stage instead of the whole growth stage in the commercial production. Until now, experiments on climate change under controlled environmental conditions have focused primarily on temperate plant species with a prolonged growth period (Sage et al., 1989; Gesch et al., 1998; Vu et al., 1999, 2001). Furthermore, there are close interactions between elevated CO_2 and other environmental factors such as temperature, nutrients, water availability and ozone in the atmosphere (Prasad et al., 2003; Mateos-Naranjo et al., 2010). In addition, CO_2 elevation had a significant effect on the levels of indole-3-acetic acid (IAA), gibberellins (GAs), cytokinins (CKs) and abscisic acid (ABA), and ethylene in the leaves or in the xylem sap (Yong et al., 2000; Li et al., 2002; Seneweera et al., 2003; Teng et al., 2006; Wang et al., 2009; Tao et al., 2010). However, a few studies to date have been conducted to examine the interactive effects of CO_2 elevation and exogenous plant growth substances on the physiology of greenhouse crops such as tomatoes and cucumber although CO_2 enrichment is widely used in their production.

Brassinosteroids (BRs) are a family of over 40 naturally occurring plant steroid hormones that are ubiquitously distributed in the plant kingdom (Clouse and Sasse, 1998; Bishop and Koncz, 2002; Krishna, 2003; Montoya et al., 2005). BRs play prominent roles in various physiological processes including the induction of a broad spectrum of cellular responses, such as stem elongation, pollen tube growth, xylem differentiation, leaf epinasty, root inhibition, induction of ethylene biosynthesis, proton pump activation, regulation of gene expression and photosynthesis, and adaptive responses to environmental stress (Clouse and Sasse, 1998; Dhaubhadel et al., 1999; Khripach et al., 2000; Krishna, 2003; Yu et al., 2004). As potent plant growth regulators, BRs are now widely used to enhance plant growth and yield of important agricultural crops (Khripach et al., 2000; Divi and Krishna, 2009). We have previously shown that application of exogenous BR increases photosynthetic CO_2 assimilation in cucumber plants, which may provide an important mechanism for increased growth and yield in BR-treated plants (Yu et al., 2004). Recently, we found that BR increased whilst brassinazole (Brz), a specific inhibitor of BR biosynthesis, decreased the maximum Rubisco carboxylation rates ($V_{c,max}$), total and, to a greater extent, initial Rubisco activity and the Rubisco activation state (Xia et al., 2009a). Furthermore, BR upregulated whilst Brz downregulated the expressions of *rbc*L, *rbc*S and other Calvin–Benson cycle genes. In this regard, there is an intriguing possibility that BR and CO_2 enrichment could have additive effects on photosynthesis by increasing RuBP carboxylation capacity by Rubisco and depressing photorespiration.

Cucumber is widely cultivated in greenhouse in the world. Due to its effectiveness in improving growth vigor and productivity, CO_2 enrichment has been widely adopted in its commercial production around the world especially at the early plant growth stage. In the following study, we grew cucumber plants under two atmospheric CO_2 levels with or without BR application to test the hypothesis that BR and CO_2 enrichment could have additive effects on the increase in biomass accumulation and CO_2 assimilation. Furthermore, carbohydrate accumulation, Calvin–Benson cycle gene transcripts and enzyme activity were analyzed to determine whether elevated CO_2-induced photosynthetic acclimation occurs in young developing leaves and if so whether BR could alleviate elevated CO_2-induced photosynthetic acclimation by modifying photosynthetic gene transcript and enzyme activity.

2. Materials and methods

2.1. Plant materials and treatments

Seeds of cucumber (*Cucumis sativus* L. cv. Jinchun No. 3) were sown directly in a growth medium containing a mixture of peat, vermiculite and perlite (6:3:1, v:v:v) in plastic trays. Average day/night temperatures were 26/17 °C in a greenhouse with natural sunlight as light source. When the first true leaf was fully expanded, seedlings were transplanted into plastic pots (15 cm diameter and 15 cm deep, one seedling per pot) and watered daily with half-strength Enshi nutrient solution (Yu and Matsui, 1997). After 3 weeks after sowing, the seedlings at the 3-leaf stage were set into Conviron E15 (Conviron, Manitoba, Canada) controlled environment growth chambers and allowed to acclimate for 2 days under 12-h photoperiod (8 am–8 pm), temperature of 25/18 °C (day/night), photosynthetic photo flux density (PPFD) of 400 μmol m^{-2} s^{-1} above canopy and CO_2 of 380 μmol mol^{-1}. Then, the seedlings were set into 4 growth chambers with four treatments: control (ambient CO_2 of 380 μmol mol^{-1}); BR (ambient CO_2 of 380 μmol mol^{-1} + 0.1 μM 24-epibrassinolide); elevated CO_2 (CO_2 of 760 μmol mol^{-1}); and elevated CO_2 + BR (CO_2 of 760 μmol mol^{-1} + 0.1 μM BR). The aqueous BR solution was made with a very low level of ethanol (0.01, v/v) and sprayed onto the leaves at day 1 and day 5. After 7 days, plants were harvested for biomass and leaf area analysis according to Xia et al. (2009a). Meanwhile, a subset of samples were taken, frozen immediately in liquid nitrogen and stored at −80 °C before biochemical and molecular analysis. Each treatment had 8 plants with four replicates.

2.2. Leaf gas exchange and chlorophyll fluorescence analysis

Leaf gas exchange measurements were coupled with measurements of chlorophyll fluorescence using an open gas exchange system (LI-6400; LI-COR, Inc., Lincoln, NE, USA) with an integrated fluorescence chamber head (LI-6400-40 leaf chamber fluorometer; LI-COR, Inc.) on the 4th leaves. For all cases unless otherwise stated, gas exchange and chlorophyll fluorescence parameters were measured under the growth conditions at 25 °C, 80% relative humidity, 1–1.3 kPa leaf-to-air vapor pressure deficit, 380 or 760 μmol mol^{-1} CO_2 and 400 μmol m^{-2} s^{-1} incident PPFD, respectively. Chlorophyll fluorescence parameters were calculated on the basis of the light-adapted fluorescence measurements as described by Zhou et al. (2004) and Ogweno et al. (2008). Quantum efficiency of PSII (Φ_{PSII}), efficiency of excitation capture by open PSII center (F'_v/F'_m), and photochemical quenching coefficient (q_P) were calculated as $(F'_m - F_s)/F'_m$, $(F'_m - F'_o)/F'_m$ and $(F'_m - F_s)/(F'_m - F'_o)$, respectively (Genty et al., 1989; van Kooten and Snel, 1990).

2.3. Estimation of the rate of alternative electron flow

The rate of electron transport through PSII [J_e(PSII)] was measured as described by Harley et al. (1992). The rate of oxygenation by Rubisco (V_o) was estimated following von Caemmerer and Farquhar (1981) and the rate of carboxylation by Rubisco (V_c) was estimated as described by Miyake and Yokota (2000). Under atmospheric conditions, the electron fluxes in the two cycles can be expressed as $J_e(PCR) = 4 \times V_c$ and $J_e(PCO) = 4 \times V_o$, respectively (Krall and Edwards, 1992). An alternative flux, J_a, caused by electrons that are not used by the PCR and/or PCO cycles in the total electron flux driven by PSII, can be estimated from $J_e(PSII) - J_e(PCR + PCO)$ (Miyake and Yokota, 2000; Zhou et al., 2004).