RESEARCH PAPER

A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*

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Abstract

The effects of 24-epibrassinolide (EBR) spray application on gas-exchange, chlorophyll fluorescence characteristics, Rubisco activity, and carbohydrate metabolism were investigated in cucumber (*Cucumis sativus* L. cv. Jinchun No. 3) plants grown in a greenhouse. EBR significantly increased the light-saturated net CO₂ assimilation rate (*A*<sub>sat</sub>) from 3 h to 7 d after spraying, with 0.1 mg l⁻¹ EBR proving most effective. Increased *A*<sub>sat</sub> in EBR-treated leaves was accompanied by increases in the maximum carboxylation rate of Rubisco (*V<sub>c,max</sub>*) and in the maximum rate of RuBP regeneration (*J<sub>max</sub>*). EBR-treated leaves also had a higher quantum yield of PSII electron transport (*f<sub>PSII</sub>*) than the controls, which was mainly due to a significant increase in the photochemical quenching (*q<sub>P</sub>*) with no change in the efficiency of energy capture by open PSII reaction centres (*F<sub>v</sub>/F<sub>m</sub>*) . EBR did not influence photorespiration. In addition, significant increases in the initial activity of Rubisco and in the sucrose, soluble sugars, and starch contents were observed followed by substantial increases in sucrose phosphate synthase (SPS), sucrose synthase (SS), and acid invertase (AI) activities after EBR treatment. It was concluded that EBR increases the capacity of CO₂ assimilation in the Calvin cycle, which was mainly attributed to an increase in the initial activity of Rubisco.

Key words: Carbohydrate metabolism, CO₂ assimilation, *Cucumis sativus*, Photosystem II, phytohormones, Rubisco activity.

Introduction

Since the isolation and identification of brassinolide from the pollen of rape as the first bioactive steroid with high plant growth-promoting activities, brassinosteroids (BRs) have received increasing attention as a new class of phytohormone (Davis, 1995; Kauschmann *et al*., 1996; Clouse and Sasse, 1998; Khripach *et al*., 1999). Studies have shown that BRs are essential for plant growth and development, and are actively involved in many physiological processes (Clouse, 1996; Steber and McCourt, 2001). BRs have pleotropic effects and can induce a broad spectrum of cellular responses including stem elongation, pollen tube growth, leaf bending and epinasty, root inhibition, induction of ethylene biosynthesis, proton-pump activation, xylem differentiation, and regulation of gene expression (Li *et al*., 1996; Sasse, 1997; Clouse and Sasse, 1998; Dhaubhadel *et al*., 1999; Li and Chory, 1999; Hu *et al*., 2000; Arteca and Arteca, 2001; Müssig *et al*., 2002). Use of BRs has also been investigated in agricultural production. Several studies have demonstrated that BRs influence plant growth, seed germination, nitrogen fixation, senescence, and leaf abscission, and enhanced tolerance against cold stress, salt stress, and diseases.

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Abbreviations, AI, acid invertase; *A*<sub>sat</sub>, light-saturated net CO₂ assimilation; BRs, brassinosteroids; EBR, 24-epibrassinolide; *F<sub>v</sub>/F<sub>m</sub>* , the maximal photochemical efficiency of PSII; *F<sub>o</sub>/F<sub>m</sub>* , the efficiency of excitation energy capture by open PSII reaction centres; *J<sub>max</sub>* , maximum potential rate of electron transport contributed to RuBP regeneration; *l*, stomatal limitation; *q<sub>PSII</sub>* , relative quantum efficiency of PSII photochemistry; *q<sub>P</sub>* , photochemical quenching; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose-1,5-bisphosphate; SPS, sucrose phosphate synthase; SS, sucrose synthase; *V<sub>c,max</sub>* , maximum carboxylation rate of Rubisco.
As a consequence, extensive research has been undertaken to develop BRs as plant growth regulators for agricultural production (Ikekawa and Zhao, 1991; Sasse, 1997). Although improved plant growth has been shown in field trials, the physiological basis of these effects is poorly understood. Previous studies found that a BR-deficient *Arabidopsis* mutant had an impaired carbohydrate metabolism and reduced biomass (Schütler et al., 2002), and application of BRs can increase sugar content in plants such as wheat and groundnut (Vardhini and Rao, 1998). However, these results do not necessarily mean that BRs have a direct role in the regulation of photosynthesis since gas exchange was usually measured in plants after several days or weeks of treatment (Braun and Wild, 1984). To date the mechanistic basis of BR-induced stimulation of CO₂ assimilation in leaves is not known.

In a previous study, it was reported that EBR pretreatment significantly alleviated chilling injury and photoinhibition in cucumber seedlings (Yu et al., 2002b). During this experiment, a sharp increase in net photosynthetic rate was observed after EBR treatment.

The main objective of this study was to determine the mechanisms by which BRs affect the photosynthetic characteristics of cucumber seedlings grown in greenhouse conditions. Changes in photosynthesis after EBR treatment were examined through gas exchange, chlorophyll fluorescence, Rubisco activity, and carbohydrate metabolism analysis.

**Materials and methods**

**Plant material and EBR treatment**

Cucumber (*Cucumis sativus* L. cv. Jinchun No. 3) seeds were sown in a growth medium containing a mixture of soil and perlite (1:1; v:v) in 12 cm plastic pots in a greenhouse. Seven days after sowing, groups of six seedlings were transplanted into a container (40×25×15 cm) filled with half-strength Enshi nutrient solution (Yu and Matsui, 1997). The mean daily maximum and minimum air temperature was maintained at 27°C and 18°C, respectively. Three different experiments were carried out. Each treatment had 36 seedlings at the third leaf stage.

**Experiment I**: To test the concentration effects of 24-epibrassinolide (EBR, Sigma, USA) on photosynthesis, cucumber seedlings were sprayed at 14.00 h with either 0.01, 0.1, or 1.0 mg l⁻¹ EBR or distilled water at 07.00 h. Leaf area, leaf mass per area, CO₂ gas exchange, and chlorophyll fluorescence quenching, were measured at 3, 24, 72, 120, and 168 h after treatment. Leaf samples were simultaneously harvested from randomly selected control plants and EBR-treated plants, frozen immediately in liquid nitrogen and stored at −80°C until required for analysis of chlorophyll content, soluble protein content, Rubisco content, and activity assays. At intervals, plants were harvested for biomass and leaf area analysis according to Nogués et al. (2002).

**Experiment II**: To examine the effects of EBR on photorespiration and carbohydrate metabolism, cucumber plants were sprayed with 0.1 mg l⁻¹ EBR or distilled water at 07.00 h. A}_{sat} was determined under both photorespiratory (21% O₂) and non-photorespiratory (2% O₂) conditions 3, 6, 9, and 24 h after treatment. Meanwhile, leaf samples were harvested and frozen immediately in liquid nitrogen and stored at −80°C until used for the determination of the activities of sucrose synthase (SS), sucrose phosphate synthase (SPS), and acid invertase (AI) or freeze-dried for the determination of sucrose, starch, and hexose contents.

**Gas exchange and chlorophyll fluorescence measurements**

Leaf gas-exchange was measured using an infrared gas analyzer (model CIRAS-1, PP System, Hertford, UK) on the second true leaf of each seedling (Yu et al., 2002a). In all three experiments, A}_{sat} was measured by maintaining the air temperature, air relative humidity, CO₂ concentration, and photosynthetic photon flux density (PPFD) at 25°C, 80–90%, 350 μmol mol⁻¹, and 1000 μmol m⁻² s⁻¹, respectively. In experiment II, assimilation versus intercellular CO₂ concentration ([A]/[C]) curves were also measured (von Caemmerer and Farquhar, 1981). Estimation of maximum carboxylation rate of Rubisco (V_{c,max}) and maximum rates of RuBP regeneration (J_{max}) were made by fitting a maximum likelihood regression below and above the inflexion of the [A]/[C] response using the method of McMurtrie and Wang (1993). Stomatal limitation (l), the proportion of photosynthesis that is limited by stomatal conductance, was calculated according to Farquhar and Sharkey (1982).

Chlorophyll fluorescence was measured using a system that combined a gas exchange system (PP Systems, Hitchin, UK) and a pulse-modulated fluorimeter (FMS-2, Hansatech Instruments Ltd., Norfolk, UK) (Yu et al., 2002a). The cuvette of the gas exchange system was modified to accept the fibre optic of the fluorimeter at a 60° angle without significantly interfering with PPFD distribution at the leaf surface. Minimal fluorescence (F₀) was measured under a weak pulse of modulating light over a 0.8 s period, and maximal fluorescence (F_m) was induced by a saturating pulse of light (8000 μmol m⁻² s⁻¹) applied over 0.8 s. The maximal quantum efficiency of PSII was determined as F₀/F_m, where F₀ is the difference between F₀ and F_m. An actinic light source (600 μmol m⁻² s⁻¹) was then applied to achieve steady-state photosynthesis and to obtain F_s (steady-state fluorescence yield), after which a second saturation pulse was applied for 0.7 s to obtain F_w (light-adapted maximal fluorescence). Fluorescence parameters were calculated by the FMS-2, based on the dark-adapted and light adapted fluorescence measurements. The quantum efficiency of PSII (Φ_{PSII}) and the efficiency of excitation capture by open PSII centres were calculated as (F_w−F_s)/F_m and F_s/F_w, respectively (Genty et al., 1989). Photochemical quenching (q_P) was calculated as (F_w−F_s)/(F_w−F_0) (van Kooten and Snel, 1990).

**Measurement of total chlorophyll, soluble protein, and Rubisco contents and Rubisco activity**

The total Chl content was determined by the method of Arnon (1949). The frozen leaf sample was homogenized using a chilled pestle and mortar with cooled extraction buffer containing 50 mM TRIS-HCl (pH 7.5), 1 mM EDTA, 1 mM MgCl₂, 12.5% (v/v) glycerin, 10% PVP, and 10 mM β-mercaptoethanol. The homogenate was centrifuged at 15 000 g for 15 min at 4°C. The soluble protein in the supernatant was estimated as described by Bradford...
(1976) using bovine serum albumin as a standard. The Rubisco content was determined spectrophotometrically by formamide extraction of the Coomassie Brilliant Blue R-250-stained subunit bands separated by SDS-PAGE (Makino et al., 1994) using calibration curves made with Rubisco purified from cucumber leaves.

Rubisco activity was measured spectrophotometrically by coupling 3-phosphoglyceric acid formation with NADH oxidation at 25 °C according to Lilley and Walker (1974) with some modification (Nakano et al., 2000). The total activity was assayed after the crude extract was activated in a 0.1 ml activation mixture containing 33 mM TRIS-HCl (pH 7.5) 0.67 mM EDTA, 33 mM MgCl2, 10 mM NaHCO3 for 15 min. Initial Rubisco activity measurements were carried out in a 0.1 ml reaction medium containing 5 mM HEPES-NaOH (pH 8.0) 1 mM NaHCO3, 2 mM MgCl2, 0.25 mM DTT, 0.1 mM EDTA. 1 U creatine phosphokinase, 1 U 3-phosphoglyceric phosphokinase, 1 U glyceraldehyde 3-phosphate dehydrogenase, 0.5 mM ATP, 0.015 mM NADH2, 0.5 mM phosphocreatine, 0.06 mM RuBP, and 10 μl extract. The change in absorbance at 340 nm was monitored for 90 s.

Measurements of carbohydrate content and enzyme activity of carbohydrate metabolism

Freeze-dried samples were used for the determination of carbohydrate content. Sucrose, starch, and hexose (glucose and fructose) content were determined using a modified phenol–sulphuric acid method (Buysse and Merckx, 1993). One hundred milligrams of sample was extracted overnight in 25 ml 80% ethanol (v/v) and the supernatant was analysed for hexose, sucrose, and total sugars. The residue was boiled for 3 h in 10 ml 2% HCl (v/v) and the supernatant was used for starch content.

Sucrose phosphate synthase (SPS) and sucrose synthase (SS) were extracted at 0–4 °C according to Lowell et al. (1989). SPS activity was assayed at 37 °C by the method of Hubbard et al. (1989). The reaction mixtures (70 μl) contained 50 mM HEPES-NaOH (pH 7.5), 15 mM MgCl2, 1 mM EDTA, 5 mM NaF, 6 mM UDP-Glucose, 4 mM Fru 6-P, 20 mM Glc 6-P, and 20 μl crude enzyme. Reaction mixtures were incubated for 30 min at 37 °C and incubation was terminated with 70 μl of 5 M NaOH. Tubes were placed in boiling water for 10 min to destroy any unreacted fructose or fructose 6-P. After cooling, 1 ml of a mixture of 0.14% anthrone in 13.8 M H2SO4 was added and incubated at 40 °C in a water bath for 20 min. Colour development of cooled solutions was measured at 620 nm and the SPS activity was calculated. SS was assayed in both the synthetic (SS-s) and cleavage (SS-c) directions with the method of Lowell et al. (1989). Reaction mixtures (70 μl) for SS synthetic directions contained 80 mM HEPES (pH 8.5), 5 mM KCN, 5 mM NaF, 100 mM fructose, 15 mM UDPG, and 20 μl desalted extract. Other assays used identical conditions to those for SPS. The mixtures (490 μl) used for sucrose cleavage contained 80 mM MES (pH 5.5), 5 mM NaF, 100 mM sucrose, and 5 mM UDP. Reactions proceeded for 30 min at 30 °C and were terminated by the addition of 490 μl DNS reagent. Tubes were heated in boiling water for 5 min. After cooling, colour development was measured at 520 nm. Acid invertase (AI) was extracted as described by Miron and Schaffer (1991). Activity was assayed in a reaction mixture that consisted of 4% sucrose, 50 mM sodium acetate buffer (pH 4.5), and an aliquot of enzyme solution in a total volume of 1 ml. The reaction mixture was incubated at 30 °C for 15 min. Reducing groups formed in the reaction mixture were measured (Endo et al., 1990).

Statistical methods

Statistical treatments of the data were performed by ANOVA and using the Student’s t-test for comparison of means.

Results

Effects of EBR on gas exchange and chlorophyll fluorescence parameters

In order to study whether EBR can directly stimulate photosynthesis in cucumber leaves, the net photosynthetic rates at saturated PPFD (A sat) and at growing light intensity (A) were measured with increasing concentrations of EBR (Fig. 1). In this experiment, A was measured at 16.00 h, 26 h after treatment. Interestingly, EBR showed a concentration-dependent effect on A sat, which was increased by 61% following treatment with 0.01 mg l−1 EBR. A sat reached its maximum value at 0.1 mg l−1 EBR, which corresponded to 210% of the control. No increase of A sat was observed at 1 mg l−1 EBR. For control plants, A at growing light intensity (600 μmol m−2 s−1) was not significantly different from A sat (measured at 1000 μmol m−2 s−1) since the saturated point of PPFD for CO2 assimilation rate was about 600 μmol m−2 s−1 (Yu et al., 2002b). Similarly, A measured at growing light intensity was significantly increased by EBR treatment (0.01 mg l−1 and 0.1 mg l−1). Consequently, EBR at 0.1 mg l−1 EBR was used in Experiments II and III.

The time-course of gas exchange parameters showed that the effects of EBR on photosynthesis lasted approximately 7 d and the stimulatory effects were most significant from 24 h to 72 h (Fig. 2A). EBR also significantly increased stomatal conductance and transpiration throughout the experiment especially up to 72 h, but had no significant effects on intracellular CO2 concentrations (data not shown). The response of net photosynthetic rate to the intercellular CO2 concentration was examined and analyses of A/Ci curves allowed the determination of the maximum carboxylation rate of Rubisco (Vc,max), maximum potential rate of electron

![Fig. 1. Effects of 24-epibrassinolide (EBR) at different concentrations on the light-saturated rate of CO2 assimilation at 1000 μmol m−2 s−1 (A max) (filled circles) and CO2 assimilation rate (open circles) at growing light intensity (600 μmol m−2 s−1, Experiment I). Measurements were carried out at 16.00 h, 26 h after EBR treatment. Leaf temperature was maintained at 25±0.5 °C with 360 μmol mol−1 CO2. Data are shown as the means of four replicates and the standard errors are shown when larger than the symbols.](https://academic.oup.com/jxb/article-abstract/55/399/1135/524955)
transport contributed to ribulose-1,5-bisphosphate (RuBP) regeneration \( (J_{\text{max}}) \), and stomatal limitation \( (l) \). EBR treatment significantly increased \( V_{c,\text{max}} \) and \( J_{\text{max}} \) from 3 h to 72 h. One day after EBR treatment, for example, \( V_{c,\text{max}} \) and \( J_{\text{max}} \) increased by 33.2% and 23.4%, respectively. EBR treatment, however, had no significant effects on \( l \) (Fig. 2D).

Quantum yield of PSII \( (\Phi_{\text{PSII}}) \) for control leaves remained almost constant throughout the experiment \( (0.32-0.38, \text{Fig. 3A}) \). Compared with control leaves, EBR-treated leaves exhibited significantly higher \( \Phi_{\text{PSII}} \) values after treatment, reaching 0.45 by 24 h post-treatment. This increase in \( \Phi_{\text{PSII}} \) was mainly due to a significant increase in photochemical quenching \( (q_p, \text{Fig. 3B}) \), with no change in the efficiency of energy capture by open PSII reaction centres \( (F_v/F_m) \) (Fig. 3C).
Maximal quantum efficiency of PSII \( (F_v/F_m) \) remained at c. 0.83 for both control and EBR-treated leaves (data not shown).

Effects of EBR on Rubisco content, Rubisco activity, leaf mass, leaf area, and chlorophyll content

EBR treatment had little effect upon Rubisco content or upon the total Rubisco activity of the leaves, however, EBR significantly increased the initial Rubisco activity 3 h after EBR treatment (Fig. 4). Interestingly, the magnitude of this increase was similar to that of both \( A_{\text{sat}} \) and \( V_{c,\text{max}} \) (Fig. 2A, B).

The effects of EBR treatment on leaf mass per area, leaf area index, total chlorophyll content, and soluble protein content are shown in Table 1. EBR treatment had little effect upon leaf mass per area, total chlorophyll content or soluble protein content (Table 1), but significantly increased leaf area, which was increased by 54.9% at the end of the experiment.

Effects of EBR on photorespiration and carbohydrate metabolism

The possibility that EBR-induced increases in \( A_{\text{sat}} \) were associated with changes in photorespiration or limitations in inorganic phosphate supply to the chloroplasts was also examined by changing the atmospheric O\(_2\) content from 21% to 2% (Experiment III; Fig. 5). For both untreated and EBR-treated leaves, \( A_{\text{sat}} \) showed typical diurnal changes, with lowest \( A_{\text{sat}} \) at 16.00 h (9 h after EBR treatment). EBR increased \( A_{\text{sat}} \) by between 20.3% and 57.4% under photorespiratory conditions and by between 15.8% and 55.0% under non-photorespiratory condition 3 h and 24 h after treatment, respectively. Significant increases (between 25.5% and 44.5%) were observed in \( A_{\text{sat}} \) measured at low O\(_2\) partial pressure in all measurements regardless of the treatments. There were, however, no significant differences in the percentage increase of \( A_{\text{sat}} \) or the percentage decrease of \( \Phi_{\text{PSII}} \) upon transition from a 21% O\(_2\) to a 2% O\(_2\) atmosphere between the control and EBR treatment during the experiment (Fig. 5).

EBR treatment had no effect on hexoses or sucrose content of the leaves, but significantly increased the total soluble sugars and starch content 3 h after EBR treatment (Fig. 5). Furthermore, after 6 h, the sucrose, soluble sugar, and starch content in EBR-treated leaves increased by 32.6%, 37.2%, and 81.8%, respectively. However, no difference in starch content between the two treatments was observed 9 h after treatment.

Increases in carbohydrate metabolite content were accompanied by increases in sucrose phosphate synthase (SPS) activity, acid invertase activity (AI), and activity of sucrose synthase (SS)-synthetic direction in EBR-treated leaves, which increased by 113.9%, 192.5%, and 23.2%, respectively, 6 h after treatment (Fig. 7). SS-cleavage direction was initially slightly inhibited 6 h after treatment.

Discussion

Until now, there has been little evidence to show that BRs are directly involved in the regulation of photosynthesis. In this paper, EBR significantly stimulated photosynthesis, showing the greatest effects at 0.1 mg l\(^{-1}\) (Fig. 1). This enhanced effect appeared as soon as 3 h after EBR treatment (Figs 2, 4). Braun and Wild (1984) showed that continuous application of BRs led to an increased CO\(_2\) fixation rate, however, the observed increase in photosynthetic rate was measured on the sixth day after treatment. It is worth noting that the relatively low rates of \( A_{\text{sat}} \) in Fig. 1 were likely due to the diurnal change of CO\(_2\) assimilation (in Experiment I plants were measured at
Consequently, both factors might be responsible for the by a significant change in conductance and transpiration rate were not accompanied or both. In this study, EBR increases in stomatal or non-stomatal (namely mesophyll) factors or both. In this study, EBR increases in stomatal conductance and transpiration rate were not accompanied by a significant change in \( c_i \) (data not shown). Consequently, both factors might be responsible for the increased photosynthetic rate caused by EBR treatment. There are several possible candidates for the mesophyll factor affecting photosynthesis: (i) reduced photorespiration; (ii) feedback control by carbohydrate metabolism; (iii) improved efficiency of photosynthetic energy conversion; and (iv) improved efficiency of photosynthetic carbon fixation. In this study, the proportion of \( A_{\text{sat}} \) measured under photorespiration conditions compared with that measured under non-photorespiratory conditions showed no significant difference (Fig. 5), showing that EBR did not increase net photosynthetic rate by reducing photorespiration. Furthermore, if photosynthesis was limited by carbohydrate metabolism, any increase in net photosynthetic rate would be accompanied by a reduction in the carbohydrate content, together with increases in the activities of the carbohydrate metabolism enzymes after EBR treatment. Figure 6 clearly shows that an increase in \( A_{\text{sat}} \) was followed by increases in sucrose, soluble sugars, and starch content, together with significant increases in acid invertase, sucrose phosphate synthase, and sucrose synthase (Fig. 7). This observation also rules out the possibility that EBR treatment increased photosynthesis by sugar-signal-induced feedback regulation (Paul and Foyer, 2001). It is likely that increased photosynthetic \( CO_2 \) assimilation provided more carbohydrate for metabolism and export to sink. Sink strength could be stimulated due to direct effects of enhanced substrate availability, and also through the stimulation of the expression of genes encoding enzymes involved in the carbohydrate metabolism (Koch, 1996; Paul and Foyer, 2001). In agreement with this, a BR-deficient \textit{Arabidopsis} mutant has been found to have decreased starch and sucrose contents, and reduced activities of invertase as compared with the wild type (Schlüter et al., 2002). As \( CO_2 \) assimilation was not correlated with changes in the proportionate increase in \( A_{\text{sat}} \) in response to a switch from 21% to 2% \( O_2 \) (Fig. 5), \( P_i \)-limitation could not play a role in the increase in photosynthesis observed in EBR-treated leaves. \( P_i \)-limitation of \( CO_2 \) assimilation is generally observed at low temperature and high \( CO_2 \) levels (Sage, 1994).

### Table 1. Effects of EBR treatment on leaf mass per area, leaf area, total chlorophyll, and soluble protein contents in cucumber leaves (Experiment II)

EBR was applied at a concentration of 0.1 mg l\(^{-1}\) at 07.00 h. Data are shown as the means of four replicates ±SE. Two asterisks indicate a significant difference between treatments at the 1% level.

<table>
<thead>
<tr>
<th>Hours after EBR treatment</th>
<th>Leaf mass per area (FW g m(^{-2}))</th>
<th>Leaf area (cm(^2) plant(^{-1}))</th>
<th>Total Chl content ((\mu)g cm(^{-2}))</th>
<th>Soluble protein content (g m(^{-2}))</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>EBR</td>
<td>Control</td>
<td>EBR</td>
</tr>
<tr>
<td>3 h</td>
<td>144.5±9.7</td>
<td>147.4±12.7</td>
<td>142.9±11.7</td>
<td>145.2±13.8</td>
</tr>
<tr>
<td>24 h</td>
<td>150.3±2.9</td>
<td>163.4±8.5</td>
<td>155.8±20.0</td>
<td>179.0±14.7</td>
</tr>
<tr>
<td>72 h</td>
<td>156.6±10.9</td>
<td>169.4±4.7</td>
<td>233.2±19.6</td>
<td>297.9±21.0**</td>
</tr>
<tr>
<td>120 h</td>
<td>149.7±4.7</td>
<td>154.4±9.5</td>
<td>307.2±30.8</td>
<td>438.9±21.3**</td>
</tr>
<tr>
<td>168 h</td>
<td>148.4±3.7</td>
<td>149.8±7.5</td>
<td>357.5±41.9</td>
<td>553.7±28.4**</td>
</tr>
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</table>

**Fig. 5.** Changes in the light-saturated rate of \( CO_2 \) assimilation (\(A_{\text{sat}}\), A) and the quantum efficiency of PSII (\(\Phi_{\text{PSII}}\), B) measured under both photorespiratory (21% \( O_2 \)) and non-photorespiratory (2% \( O_2 \)) conditions in control and EBR-treated plants (Experiment III). (open circles) Control at 21% \( O_2 \); (filled circles) EBR at 21% \( O_2 \); (open squares) control at 2% \( O_2 \); (filled squares) EBR at 2% \( O_2 \). Leaf temperature was maintained at 25±0.5 °C with 1000 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) incident PPFD and 360 \( \mu \)mol mol\(^{-1}\) \( CO_2 \). EBR was applied at a concentration of 0.1 mg \( l^{-1}\) at 07.00 h. The shading indicates nighttime and the arrow indicates the time for EBR application, respectively. Data are shown as the means for four replicates and the standard errors are shown when larger than the symbols.

16.00 h) and due to using young leaves (second leaves on the plants at the third leaf stage).

Changes in \( CO_2 \) assimilation may be attributable to either stomatal or non-stomatal (namely mesophyll) factors or both. In this study, EBR increases in stomatal conductance and transpiration rate were not accompanied by a significant change in \( c_i \) (data not shown). Consequently, both factors might be responsible for the
EBR treatment resulted in large increases in the photosynthetic capacity of leaves, which was accompanied by an increase in both $V_{c,max}$ and $J_{max}$. The increase in $A_{sat}$ (Fig. 2) was accompanied by an increase in Rubisco activity measured in vivo from $V_{c,max}$ (Fig. 2) and in vitro by biochemical assay (Fig. 4). To date, a hormone-induced increase in Rubisco activity has rarely been reported. The increase in $J_{max}$ caused by EBR treatment (Fig. 2) was accompanied by an increase in the quantum efficiency of electron flux through PSII ($\phi_{PSII}$, Fig. 3). The simultaneous increase in $V_{c,max}$ and $J_{max}$ caused by EBR treatment is attributed to (i) the increase in the initial Rubisco activity (Fig. 4) resulting in an increase in $V_{c,max}$, whilst (ii) an
increase in the activity of other Calvin-cycle enzymes may result in an increase in the rate of RuBP regeneration ($J_{\max}$) (Baker et al., 1997; Nogue& and Baker, 2000). Accordingly, the impact of EBR on the activities of other Calvin cycle enzymes requires further studies.

EBR treatment significantly increased $\Phi_{\text{PSII}}$ (Fig. 3), which is a product of the efficiency of excitation energy capture by open PSII reaction centres ($F_c/F_m$) and the proportion of ‘open’ PSII reaction centres ($q_p$) (Genty et al., 1989). An increase in $q_p$ (Fig. 3) is attributable to an increase in the rate of consumption of reductants and ATP produced by non-cyclic electron transport relative to the rate of excitation of open PSII reaction centres (Nogue& and Baker, 2000). Consequently, the increase in the quantum yield of PSII electron transport in EBR-treated leaves (Fig. 3) reflects the increased demand in the Calvin cycle for ATP and NADPH.

The mechanism by which EBR exerts an effect on Rubisco activity is not known and warrants further investigation. Khripach et al. (1999) suggested that BRs affect the biosynthesis of enzymes via an effect on gene expression and/or an effect of BRs on cell membranes. The first effect will be responsible for the slow reaction of plants to hormones, and the second one for the rapid ones. The data presented in this study, with a rapid effect of BRs on plants, may suggest a direct effect of BRs in leaves. Furthermore, the mechanism of action of BRs in leaves is a light-dependent process, which suggests a relationship between BRs and the action of phytochrome (Khripach et al., 1999). In fact, several indications point to the important role of BRs in the control of photomorphogenesis. BR-deficient mutants display de-repression of some light-induced genes in the dark such as rbcS (Szekeres et al., 1996).

The photosynthetic productivity of a plant is determined by the quantity of photosynthetically active radiation intercepted and the efficiency with which this intercepted radiation is utilized for net dry matter production. Factors associated with the EBR-induced increases in the efficiency of light utilization have been addressed above. However, EBR-induced increases in leaf area (Table 1) will be associated with an increase in the ability of plants to capture more photosynthetically active radiation and, consequently, be a major factor in determining the EBR-induced increases in the photosynthetic productivity of these plants. This is in accordance with literature stating that BRs influence cell division (and consequently leaf size), leaf anatomy, and stomatal frequency (Hu et al., 2000; Arteca and Arteca, 2001; Schlüter et al., 2002).

In conclusion, this study has demonstrated that EBR is effective in increasing photosynthesis, particularly the capacity of CO$_2$ assimilation in the Calvin cycle which were mainly attributed to an increase in the initial activity of Rubisco.

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References


Role of brassinosteroids in photosynthesis

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