Interactive Effects of Drought Stresses and Elevated CO₂ Concentration on Photochemistry Efficiency of Cucumber Seedlings

Qing-Ming Li¹,³, Bin-Bin Liu², Yang Wu¹ and Zhi-Rong Zou¹∗

¹College of Horticulture, Northwest A&F University, Yangling 712100, China; ²State Key Laboratory of Soil Erosion and Dryland Farming of the Loess Plateau, Institute of Soil and Water Conservation, the Chinese Academy of Sciences, Yangling 712100, China; ³College of Horticultural Science and Engineering, Shandong Agricultural University, Tai’an 271018, China

Abstract

To reveal and quantify the interactive effects of drought stresses and elevated CO₂ concentration [CO₂] on photochemistry efficiency of cucumber seedlings, the portable chlorophyll meter was used to measure the chlorophyll content, and the Imaging-PAM was used to image the chlorophyll fluorescence parameters and rapid light response curves (RLC) of leaves in two adjacent greenhouses. The results showed that chlorophyll content of leaves was reduced significantly with drought stress aggravated. Minimal fluorescence (Fo) was increased while maximal quantum yield of PSII (Fv/Fm) decreased significantly by severe drought stress. The significant decrease of effective quantum yield of PSII (Y(II)) accompanied by the significant increase of quantum yield of regulated energy dissipation (Y(NPQ)) was observed under severe drought stress condition, but there was no change of quantum yield of nonregulated energy dissipation (Y(NO)). We detected that the coefficient of photochemical quenching (qP) decreased, and non-photochemical quenching (NPQ) increased significantly under severe drought stress. Furthermore, we found that maximum apparent electron transport rate (ETRmax) and saturating photosynthetically active radiation (PPFDsat) decreased significantly with drought stress aggravated. However, elevated [CO₂] significantly increased Fv/Fm, qP and PPFDsat, and decreased NPQ under all water conditions, although there were no significant effects on chlorophyll content, Fo, Y(II), Y(NPQ), Y(NO) and ETRmax. Therefore, it is concluded that CO₂-fertilized greenhouses or elevated atmospheric [CO₂] in the future could be favorable for cucumber growth and development, and beneficial to alleviate the negative effects of drought stresses to a certain extent.

Key words: chlorophyll fluorescence; cucumber seedlings; drought stresses; elevated CO₂ concentration; photochemistry efficiency.


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et al. 1997; Centritto et al. 2004). Most of previous studies about elevated \([\text{CO}_2]\) effect on photosynthesis have focused on carbon assimilation and metabolism (Drake et al. 1997). However, with the changes of acclimation in the photosynthetic machinery, consequent upon growth in elevated \([\text{CO}_2]\) may reduce the benefits under water deficits, when elevated \([\text{CO}_2]\) is applied for several days or weeks and sometimes even a suppression of photosynthesis occurs (Moore et al. 1999; Sawada et al. 2001). Although there are many studies on photosynthetic responses to \([\text{CO}_2]\) enrichment as well as to drought stress (Huber et al. 1984; Valle et al. 1985; Campbell et al. 1990; Ryle et al. 1992; Baker and Allen 1993; Drake et al. 1997; Vu et al. 1998), there is limited quantitative understanding of the effects of interactions between \([\text{CO}_2]\) and water deficiency (Chaves and Pereira 1992; Samarakoon and Gifford 1995; Tschaplinski et al. 1995), and much less is known about the interactive effects of these environmental variables on the fundamental processes of leaf photosynthesis (Widodo et al. 2003).

In recent years, chlorophyll \(a\) fluorescence imaging system has become ubiquitous in plant ecophysiology studies (Maxwell and Johnson 2000). As the measurement is non-destructive, rapid and convenient, chlorophyll fluorescence method has many advantages in the quantification of stress effects on photosynthesis (Krause and Weis 1991). Based on pulse amplitude modulation (PAM) and the saturation pulse method (Schreiber et al. 1986), chlorophyll fluorescence yield provides quantitative information not only on steady-state photosynthesis, but also on various mechanisms of protection against stress-induced damage by excess radiation (Govindjee 1995; Demmig-Adams and Adams 1996a; Kramer and Crofts 1996; Meng et al. 2001).

We hypothesized that elevated \([\text{CO}_2]\) could increase photochemistry efficiency under drought stresses and be beneficial to cucumber seedling in drought environment, thus alleviate or offset the negative consequences of global environmental changes. Therefore, this study was conducted to reveal and quantify the effects of drought stresses and elevated \([\text{CO}_2]\) on photochemistry efficiency of cucumber seedlings by using chlorophyll a fluorescence imaging system (Imaging-PAM), and farther, to provide theory bases and technological parameters for cucumber culture under \([\text{CO}_2]\)-fertilized greenhouse or changed climatic conditions in the future.

**Results**

**The effects of drought stresses and elevated \([\text{CO}_2]\) on chlorophyll content of cucumber leaves**

For cucumber seedlings grown in both ambient and elevated \([\text{CO}_2]\), leaf chlorophyll content of seedlings decreased progressively and significantly \((P < 0.001)\) under drought stresses. Chlorophyll content of seedling leaves not subjected to drought stress was 15% and 16% higher than that of severe drought stressed seedlings in ambient and elevated \([\text{CO}_2]\), respectively. Elevated \([\text{CO}_2]\) decreased chlorophyll content slightly but not significantly, and there was no interaction between water treatments and \([\text{CO}_2]\) (Figure 1; Table 1).

**Complementary changes in \(Y(\text{II})\), \(Y(\text{NPQ})\) and \(Y(\text{NO})\) with drought stresses and elevated \([\text{CO}_2]\)**

Based on the original work of Kramer et al. (2004), the imaging-PAM chlorophyll fluorometer allows the assessment of excitation energy flux at PSII in three fundamentally different pathways, namely photochemical utilization, regulated heat dissipation (a loss process serving for protection) and nonregulated heat dissipation (a loss process due to PSII inactivity). These three fluxes are described by the quantum yields \(Y(\text{II})\), \(Y(\text{NPQ})\) and \(Y(\text{NO})\), respectively, and add up to unity (Bonfig et al. 2006). In this study, \(Y(\text{II})\) and \(Y(\text{NPQ})\) were affected significantly \((P < 0.001)\) when seedlings were subjected to severe drought stress (Table 1 and Figure 2). Compared with control, severe drought stress decreased \(Y(\text{II})\) by 12% and 13%, and increased \(Y(\text{NPQ})\) by 25% and 28% in ambient and elevated \([\text{CO}_2]\), respectively (Figure 3). However, there was no considerable change of \(Y(\text{NO})\) in both \([\text{CO}_2]\). With respect to the effect of \([\text{CO}_2]\) on the three quantum yield parameters, no significant difference was observed. Also, there was no interaction between water treatments and \([\text{CO}_2]\).

![Figure 1](image.png)

**Figure 1.** Effects of drought stresses and elevated \([\text{CO}_2]\) on chlorophyll content of cucumber leaves.

Bars represent least squared mean values ± SD \((n = 15)\) for treatments. Different lowercase letters were statistically different at the \(P < 0.05\) level. C, M and S represent control, mild drought stress and severe drought stress, respectively.
Table 1. Analysis of variance of drought stress and \([\text{CO}_2]\) and their interaction for chlorophyll content and chlorophyll fluorescence parameters of cucumber seedlings

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chl content</th>
<th>(Y(\text{II}))</th>
<th>(Y(\text{NPQ}))</th>
<th>(Y(\text{NO}))</th>
<th>(F_o)</th>
<th>(F_v/F_m)</th>
<th>(q_P)</th>
<th>NPQ</th>
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<tr>
<td>Water treatments</td>
<td>ns</td>
<td>ns</td>
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<td>ns</td>
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<tr>
<td>([\text{CO}_2])</td>
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<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
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</tr>
<tr>
<td>([\text{CO}_2] \times \text{Water treatments})</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
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ns, not significant; \(^* P < 0.05\); \(^* * P < 0.01\); \(^* * * P < 0.001\).

The effects of drought stresses and elevated \([\text{CO}_2]\) on chlorophyll fluorescence imaging of cucumber leaves and quantitative analyses

From Figures 3, 4 and Table 1, we can see that the maximal quantum yield of PSII \((F_v/F_m)\) decreased slightly under mild drought stress, but had a significant decrease \((P < 0.001)\) under severe drought stress. It was decreased by 28% and 23% than control in ambient and elevated \([\text{CO}_2]\), respectively. Compared with ambient \([\text{CO}_2]\), elevated \([\text{CO}_2]\) increased \(F_v/F_m\) by 11%, 12% and 19% under control, mild and severe drought stress conditions, respectively, and the differences were significant \((P < 0.001)\). At the same time, interaction between water treatments and \([\text{CO}_2]\) was significant \((P < 0.001)\).

Minimal fluorescence \((F_o)\) was not influenced by mild drought stress, but it was increased significantly \((P < 0.01)\) under severe drought stress and 10% and 12% higher than that of control in ambient and elevated \([\text{CO}_2]\), respectively (images not shown).

However, \([\text{CO}_2]\) had no significant effect on \(F_o\), and there was no interaction between water treatments and \([\text{CO}_2]\).

Although no significant difference was observed between control and mild drought stress, a significant decrease of coefficient of photochemical quenching \((q_P)\) under severe drought stress was detected, and 18% and 20% lower than that of control in ambient and elevated \([\text{CO}_2]\), respectively. Compared with ambient \([\text{CO}_2]\), elevated \([\text{CO}_2]\) significantly \((P < 0.001)\) increased \(q_P\) by 8%, 7% and 5% under control, mild and severe drought stress conditions, respectively. Also the interaction between water treatments and \([\text{CO}_2]\) was significant \((P < 0.01)\).

With respect to \(NPQ\), it was elevated by severe drought stress significantly \((P < 0.001)\), and 44% and 65% higher than control in ambient and elevated \([\text{CO}_2]\), respectively. Moreover, elevated \([\text{CO}_2]\) reduced \(NPQ\) significantly \((P < 0.05)\), irrespective of whether or not the seedlings were subjected to drought stresses. In elevated \([\text{CO}_2]\), \(NPQ\) decreased by 21%, 19% and 10% than ambient \([\text{CO}_2]\) under control, mild and severe drought stress conditions, respectively. But there was no interaction between water treatments and \([\text{CO}_2]\).

The effects of drought stresses and elevated \([\text{CO}_2]\) on rapid light response curves (RLC) of cucumber leaves

The \(ETR\) versus \(PPFD\) data were fitted and the two cardinal points (maximum apparent electron transport rate, \(ETR_{\text{max}}\) and saturating photosynthetically active radiation, \(PPFD_{\text{sat}}\)) of light-response curves were calculated. The high values of \(R^2\) supported the approach of curve fitting (Figure 5). \(ETR_{\text{max}}\) declined significantly with drought stress aggravation in both \([\text{CO}_2]\). Compared with control, mild and severe drought stresses reduced \(ETR_{\text{max}}\) by 13% and 23% in elevated \([\text{CO}_2]\), 12% and 21% in ambient \([\text{CO}_2]\), respectively. At the same time, under mild and severe drought stresses, \(PPFD_{\text{sat}}\) decreased by 7% and 19% in elevated \([\text{CO}_2]\), 8% and 24% in ambient \([\text{CO}_2]\), respectively. With respect to the effect of \([\text{CO}_2]\), \(ETR_{\text{max}}\) of control seedling was increased significantly by elevated \([\text{CO}_2]\), and 8% higher than that of in ambient \([\text{CO}_2]\). For drought stressed seedlings, there were no significant effects of \([\text{CO}_2]\) on \(ETR_{\text{max}}\). In addition, elevated \([\text{CO}_2]\) increased \(PPFD_{\text{sat}}\) by 7%, 8% and 15% under control, mild and severe drought stressed, respectively.
Discussion

In general, drought stress limits photosynthesis through stomatal closure or through metabolic impairment (Cornic 2000; Lawlor 2002). However, plants have developed several strategies (such as xanthophylls cycle, D1 repair cycle, photorespiration and etc.) to minimize the harmful effects of excessive energy under stress conditions (Ort and Baker 2002). Light energy absorbed by chlorophyll molecules in a leaf can be used to drive photosynthesis, dissipated as heat or re-emitted as chlorophyll fluorescence, which occur in competition. Hence, through measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be obtained (Lázár 1999).

With drought aggravated, significant decreased chlorophyll content and increased $F_o$ were observed in this study. We supposed that an increase of $F_o$ is probably related to a decrease in chlorophyll content under drought stress conditions, because less chlorophyll may cause a lower rate of re-absorption of the emitted fluorescence light, and thus result in an increased total fluorescence emission (Havaux 1993; Barber 1998). Generally, $F_o$ and $F_{v'/F_m}$ were widely used as reliable diagnostic
indicators of photoinhibition (He et al. 1996; Valladares and Pearcy 1997). In this study, the significant increase of Fo and the significant decrease of Fv/Fm were observed under severe drought stress, which indicate the occurrence of photoinhibitory damage in response to water stress (Epron et al. 1992), and/or the reversible inactivation or downregulation of PSII (Tezara and Lawlor 1995; Tourneux and Peltier 1995; Tezara et al. 2002). However, some researchers reported that Fv/Fm was unaffected by water deficit, which means there is absence of photoinhibition or no loss in the yield of PSII photochemistry, and confirms the resistance of the photosynthetic machinery to water deficit (Tezara and Lawlor 1995; Tourneux and Peltier 1995; Chaves et al. 2002; Cornic and Fresneau 2002). The discrepancies among these results could be possibly due to different plant materials and measuring conditions.

With respect to the effects of elevated [CO$_2$], decrease in leaf chlorophyll had been reported in *Phaseolus vulgaris* L., pea and soybean (Radoglou and Jarvis 1992; Xu et al. 1994). In addition, Vu et al. (2001) have observed chlorophyll content and chloroplast density/volume increased for mature leaves of rice grown at high [CO$_2$]. But in this study, there were no significant effects of elevated [CO$_2$] on chlorophyll content and Fo, which agreed with the study on young leaves of 3-week-old soybean plants in doubled [CO$_2$] (Sicher et al. 1995). More important, we observed that Fv/Fm increased significantly ($P < 0.001$) in elevated [CO$_2$], and there was significant ($P < 0.001$) interactive effect between water treatments and [CO$_2$]. Moreover, the positive effect of elevated [CO$_2$] on Fv/Fm was more remarkable under severe drought stress than control. From this point, elevated [CO$_2$] could be beneficial for cucumber seedlings to tolerate and/or adapt drought stress more effectively by increasing maximal quantum yield of PSII.

$Y(II)$ is directly related to the rate at which CO$_2$ is assimilated by the leaf (Genty et al. 1989, 1990; Harbinson et al. 1990; Krall and Edwards 1990, 1991; Cornic and Briantais 1991; Edwards and Baker 1993; Rolfe and Scholes 1995; Siebke et al. 2001). The significant decrease of $Y(II)$ was detected under severe drought stress in this study, which indicated that photosynthetic CO$_2$ assimilation was inhibited severely. At the same time, significant increased $Y(NPQ)$ accompanying
Figure 5. Effects of drought stresses and elevated [CO$_2$] on rapid light response curves (RLC) of the relative photosynthetic electron transport rate (ETR) of cucumber leaves.

Data points with same symbols are means of four AOIs, which were fitted against a single exponential function detailed in Materials and Methods (lines, $R^2$ = regression coefficient). The numbers at horizontal long dash give means ± SD ($n$ = 12) of ETR$_{\text{max}}$, and different lowercase letters were statistically different at the $P < 0.05$ level. The numbers at the right side of the vertical short dash give PPFD$_{\text{sat}}$, i.e. PAR at 0.9 ETR$_{\text{max}}$, and different lowercase letters were statistically different at the $P < 0.05$ level. C, M and S represent control, mild drought stress and severe drought stress, respectively. E and A represent elevated and ambient [CO$_2$], respectively.

by no significant changed Y(NO) was observed under severe drought stress both in ambient and elevated [CO$_2$]. Thus we supposed that the cucumber seedlings could alleviate damage to photosynthetic mechanism processes to a certain extent by regulating the dissipation of excessive excitation energy into harmless heat (Kramer et al. 2004), and there was no severe irreversible photodamage to PSII or loss of D$_1$ protein occurred (Demmig et al. 1987; Critchley and Russell 1994; Demmig-Adams and Adams 1996b; Kramer et al. 2004). Although there were no significant changes and no interaction between water treatments and [CO$_2$], little is known about the effect of [CO$_2$] on these parameters.
The ability to maintain the PSII quinone acceptors partially oxidized or the overall “openness” of PSII reaction centers, estimated by qP, is directly related to the rate of consumption of NADPH and ATP which are the products of photosynthetic electron transport (Rosenqvist 2001; Ort and Baker 2002). Decreases in the rate of consumption of NADPH and ATP can result in an increase in non-photochemical quenching (NPQ) in the PSII antennae. Demmig-Adams and Adams (1996b) suggested that NPQ was a good indicator for “excess light energy” which is primarily dissipated via xanthophyll cycle in leaves in the presence of a transthylakoidal ΔpH. With increasing levels of stress, the increases in NPQ can be insufficient to maintain the PSII electron acceptors partially oxidized (Ort and Baker 2002). Dietz et al. (1985) has proposed that a high level of NPQ would result from a higher thylakoid energization because of a lower energy demand in nonphotorespiratory conditions. In this study, qP declined while NPQ increased significantly under severe drought stress. So we presumed that the thylakoids could remain highly energized to dissipate excess light energy at the cost of declined photochemical quenching, so as to avoid photodamage to photosynthesis apparatus. Furthermore, we found that elevated [CO₂] increased qP while decreased NPQ significantly (P < 0.001), irrespective of water conditions, and there was a significant (P < 0.01) interactive effect on qP between water treatments and [CO₂]. Changes in carbon assimilation in elevated [CO₂] necessitate changes in the partitioning of absorbed energy between heat dissipation and photochemistry in the thylakoid membrane (Pammenter et al. 1993; Drake et al. 1997), we speculated that elevated [CO₂] might increase photosynthesis capacity and electron transport rate away from PSII to maintain or stimulate high photosynthetic process by increasing qP and decreasing NPQ. This might be the main mechanism of cucumber seedlings in response to elevated [CO₂]. Nevertheless, it was noteworthy that the extent of effects of severe drought stress on qP and NPQ were more severer in elevated [CO₂] than in ambient [CO₂] (qP, 20% and 18% lower than control, NPQ, 65% and 44% higher, respectively; Figure 4). In this sense, we supposed that the effect of elevated [CO₂] on photochemistry efficiency depend on water conditions faced by plants, and the negative effects induced by drought stress will be relatively more serious in the future at elevated [CO₂] (Wu and Wang 2000).

For the ecophysiological characterization of the photosynthetic capacity of a plant, RLC and the resulting cardinal points provide highly interesting parameters (Rascher et al. 2000). We observed that ETRmax and PPFDsat declined significantly with drought stress aggravation in both [CO₂]. This meant that drought stress decreased maximal photosynthetic electron transport potential and subsequently decreased the capacity of preventing photodamage. At the same time, elevated [CO₂] increased PPFDsat significantly, irrespective of the water conditions. That is to say, elevated [CO₂] can alleviate the drought stress-induced photoinhibitory damage by improving saturating photosynthetically active radiation.

In conclusion, chlorophyll content, Y(II), Fv/Fm, qP, ETRmax and PPFDsat declined, while Fo, Y(NPQ) and NPQ increased with drought stress aggravated. That is to say, drought stress down-regulated photosynthesis capacity or linear electron transport rate in leaves of cucumber seedlings. Elevated [CO₂] alleviated the negative effects of drought stresses to a certain extent mainly by increasing Fv/Fm, qP and PPFDsat, and decreasing NPQ. Therefore, we supposed that CO₂-fertilized greenhouses or elevated atmospheric [CO₂] in the future could be favorable for cucumber growth and development, and beneficial to alleviate the negative effects of drought stresses to a certain extent. Furthermore, the above four parameters could be used to evaluate the interactive effects of drought stresses and elevated [CO₂] on photochemistry efficiency of cucumber seedlings.

Materials and Methods

Greenhouse and environment monitor

The experiment was conducted during 10–17 June 2007 in two adjacent identical test greenhouses (3 m × 6 m cross-section area, ridge height 2.5 m) located at Northwest A&F University, Yangling (108°13′ E, 34°15′ N), China. The greenhouses were equipped with an environment control system (Auto Company, Beijing, China) to supply CO₂ from a compressed CO₂ gas cylinder controlled by solenoid valve. During the treatments, [CO₂] was monitored by Telaire 7001 Carbon Dioxide Monitor (Hoyt Electrical Instrument Works Inc., NH, USA), and CO₂ was automatically injected into the greenhouse when necessary to maintain the target concentration. In addition, a HOBO Data Loggers (MicroDAQ.com, Ltd., NH, USA) was fixed in each greenhouse to record [CO₂], air temperature and relative humidity every 30 min. And photosynthetic photon flux density (PPFD) was measured by Dual Radiation Meter (Apogee Instruments Inc., CA, USA) every two hours (Figure 6).

Plant material and growth condition

Seeds of cucumber (Cucumis sativus L. cv. Jinyou No.1) were surface-sterilized in 1% (v/v) hypochlorite solution for 15 min. After rinsing in distilled water, seeds were imibed in water for about 6–8 h and germinated for about 48 h in darkness at 26–28 °C, and then sown in plastic pots placed in one of greenhouse. The pots (diameter 10 cm, height 10 cm, one seed per pot) contain 3:1:1 (v/v) mixture of peat, perlite and vermiculite. When seedlings were grown to two leaves, identical seedlings were selected and transplanted in darkened plastic containers (length 35 cm, breadth 28 cm, and height
Figure 6. The dynamics of air temperature, relative humidity, [CO$_2$], and photosynthetic photon flux density (PPFD) in the greenhouses during treatments.

The data were logged during 10–17 June 2007. Average temperature were 27.8 °C and 27.5 °C in elevated- and ambient CO$_2$-greenhouse, respectively; average RH 47% and 45%; average [CO$_2$] 380 μmol/mol and 760 μmol/mol; average PPFD 749 μmol·m$^{-2}$·s$^{-1}$ and 746 μmol·m$^{-2}$·s$^{-1}$. 
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13 cm, 8 plants per container). Nine containers were placed randomly in each greenhouse and grown hydroponically with full strength Yamazaki cucumber nutrient solution, containing 0.5 mM NH₄H₂PO₄, 2.0 mM Ca(NO₃)₂·4H₂O, 3.2 mM KNO₃, and 1.0 mM MgSO₄·7H₂O, the trace elements were full strength. The electrical conductivity (EC) and pH of the nutrient solutions were maintained at 2.2–2.5 ms/cm and 6.8–7.0, respectively. The nutrient solutions were aerated every two hours by air pumps, supplemented to original volumes every day, and refreshed every six days.

Experiment design

The experiment consisted of two [CO₂] treatments (ambient [CO₂], 380 ± 10 μmol/mol and elevated [CO₂], 760 ± 20 μmol/mol, designated A and E; Figure 6 [CO₂]) and three water treatments for each [CO₂]. The water treatments were simulated by PEG 6 000: nutrient solution, nutrient solution + 5% PEG 6 000 and nutrient solution + 10% PEG 6 000, designated C, M and S, respectively.

When the third leaf of cucumber fully expanded, one greenhouse was supplied CO₂ and maintained 760 ± 20 μmol/mol, the other was maintained ambient. At the same time, PEG 6 000 was dissolved in the nutrient solutions to simulate rhizosphere drought stresses according to the design. In each greenhouse, water treatments were designed completely randomly.

Chlorophyll content measurement, chlorophyll fluorescence kinetics and rapid light curve imaging

After seven days’ treatment, the portable chlorophyll meter (SPAD-502, Minolta, Japan) was used to measure chlorophyll content (Netto et al. 2005). Three seedlings were selected randomly for each treatment to measure chlorophyll content of the third leaf, and five readings was obtained for each third leaf, avoiding main veins during measurements.

After measurement was made, a MINI-version of the Imaging-PAM (Heinz Walz GmbH, Effeltrich, Germany) was used to image chlorophyll fluorescence kinetics parameters on the adaxial side of the same leaves according to Schreiber et al. (1986). Y(II) was calculated as the quotient (Fm’ − F)/Fm’ (Genty et al. 1989). qP was calculated according to Schreiber et al. (1986): qP = (Fm’ − F)/(Fm’ − Fo’), with Fo’ being estimated by the equation Fo’ = Fo(Fv/Fm + Fo/Fm’) (Oxborough and Baker 1997). NPQ was calculated according to Bilger and Björkman (1990): NPQ = (Fm − Fm’)/Fm’. Y(NPQ) and Y(NO) were calculated according to Kramer et al. (2004).

Rapid light curves (RLC) were recorded after each chlorophyll fluorescence kinetics measurement. To determine the cardinal points of light-response curves, using SigmaPlot version 10.0 (SPSS Science, Chicago, IL, USA), the ETR versus PPFD data were fitted using a single exponential function: f(x) = a (1 − e−bx). From the results of the equation, cardinal points were determined with a = ETRmax, and PPFDsat was reached at 0.9 ETRmax (Rascher et al. 2000). ETR was calculated by the equation ETR = 0.5 × Y(II) × PAR × 0.84 μmol·m⁻²·s⁻¹ (Schreiber et al. 1994).

The data for the chlorophyll content are means of 15 measurement values, and all chlorophyll fluorescence parameters values are means of 12 replications. For the overall means comparison, Duncan’s post hoc test was used to denote the significant differences at P < 0.05 for the interaction of all factors.

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