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Ozone effects on high light-induced photoinhibition in *Phaseolus vulgaris*

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ABSTRACT

In this study the response to photoinhibition of photosynthesis and subsequent recovery was examined in plants of *Phaseolus vulgaris* L. cultivar 'Pinto' exposed to charcoal-filtered air or to ozone (O_3) at 150 nL L^{-1} either for 3 h, or for 5 h. The responses were analysed using chlorophyll fluorescence imaging and by conventional fluorometry. Compared to control plants maintained in charcoal-filtered air, in plants exposed for 3 h to O_3 and then subjected to high light treatment, the results show an increased tolerance to photoinhibition. Plants exposed to the same O_3 concentration but for the longer 5-h period, were not tolerant to the photoinhibition treatment and, instead showed visible symptoms of damage (chlorosis and necrosis) clearly attributable to the longer O_3 exposure. Here the detrimental effects of O_3 aggravated the effects of the high light photoinhibitory treatment. The leaves exposed to the shorter O_3 treatment (150 nL L^{-1} for 3 h) developed an ability to counteract the negative effects of a high light exposure probably because the O_3 had activated an antioxidant system able to protect the photosynthetic machinery.

1. Introduction

The light energy absorbed by chlorophyll in the membranes of the thylakoids can be utilised photochemically to drive electron transport, or to be dissipated non-photochemically or be re-emitted radiatively as fluorescence. These three processes are competitive with one another, so measurements of any one of them (e.g. fluorescence) can also provide information on the other two. Chloroplast represents a sensitive target [1] for many biotic and abiotic stresses to which plants are often subjected and, consequently the photosynthetic process can be directly affected. Because chlorophyll fluorescence measurement is non-invasive and extremely efficient [2] is widely used to study changes in the photosynthetic process and, especially, changes in PSII photochemistry. In particular, the F_v/F_m ratio is a useful measurement that typically ranges between 0.80 and 0.83 [3] and its decrease under stress conditions is indicative of the photoinhibition of photosynthesis [4], namely a decrease in photosynthetic activity induced by light exposure. Photoinhibition is often reversible (dynamic photoinhibition) and does not involve permanent damage to the photosynthetic system. However, severe photoinhibition over a long period can degrade the photosynthetic components (chronic photoinhibition or photodamage) as a result of the formation of highly reactive free oxygen radicals. The protein D1, a core protein of PSII is especially vulnerable [5].

It is widely reported that many abiotic [6–10] and biotic [11–13] stresses induce photoinhibition of PSII photochemistry because the stresses influence the degree of susceptibility to photoinhibition and thus exacerbate the adverse effects [14–16]. Air pollution causes many negative-going biochemical and physiological effects in plants and photosynthesis appears to be a principal target of one of them, O_3 [17–19]. It is reported [20,21] that plants subjected to this pollutant are more susceptible to photoinhibition.

Imaging chlorophyll fluorescence represents a non-invasive and quantitative tool by which it is possible to determine localized changes in the photosynthetic process and this permits detection of the early stages of many different types of stress [7,4,8,12,13,22]. This is particularly the case when leaves are characterised by a significant surface heterogeneity of chlorophyll fluorescence emission. The analysis of chlorophyll fluorescence during photosynthetic induction and under different illuminations permits the determination of numerous parameters such as the maximum efficiency of PSII, F_v/F_m, the proportion of absorbed light which is utilised for photosynthetic electron transport, Φ_{PSII} or the coefficient q_{NP}, which estimates the amount of light energy dissipated non-photochemically as heat [23].

To help clarify the mechanisms of tolerance and survival ability after O_3 exposure, the objective of this study was to determine if leaves subjected to two acute doses of O_3 become more susceptible to photoinhibition induced by high light intensity.

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2. Materials and methods

2.1. Plant material

Seeds of *Phaseolus vulgaris* (L.) cultivar 'Pinto' were germinated in washed vermiculite under controlled environment conditions with a temperature regime of 23/18 °C (T) (day/night) and an 8-h photoperiod at a light intensity of about 200 μmol m^{-2} s^{-1}. Approximately 5 days after germination, seedlings were transplanted into pots containing a steam-sterilized soil:peat:perlite mixture (1:1:1 by volume) and placed under controlled environment conditions with a temperature regime of 25/20 °C (day/night), a relative humidity (RH) of 80/55% (day/night) and a 14-h photoperiod at a photosynthetic flux density (PFD) of 400 μmol m^{-2} s^{-1}.

When the primary leaves were completely expanded, 30 uniform bean plants were randomly divided into three similar groups of 10 plants each. One group was placed in a controlled environment chamber ($T = 25 \pm 3$ °C and RH 85 ± 4%) under an irradiance of 400 μmol m^{-2} s^{-1} PFD and ventilated with charcoal-filtered air. The remaining two groups were exposed to the same controlled environment conditions with respect to T, RH and light but were exposed to 150 nL L^{-1} O_3 for either 3 h or for 5 h.

2.2. Ozone treatment

Ozone was generated with a Fisher (Mod. 500, Meckenheim, Germany) ozone generator. The O_3 concentration inside the chamber was continuously monitored with a Monitor-Labs Analyser (Mod. 8810, Englewood, USA) connected to a PC. Details of O_3 treatment are reported more fully in Guidi et al. [24].

2.3. Photoinhibitory treatments and recovery

Photoinhibition of photosynthesis was induced in a room at ambient temperature (about 25 °C). Susceptibility to photoinhibition was quantified by monitoring changes in F_v/F_m as a function of exposure to high light of about 1000 μmol m^{-2} s^{-1} at the leaf surface provided by a metal halide lamp (250 W; Powerstar HQI-TS, Osram GmbH, Germany). A heat filter containing 3 cm of water was placed between the lamp and leaf to ensure that temperature remained roughly constant. Plants were allowed to recover at room temperature for 48 h in the dark.

2.4. Chlorophyll fluorescence imaging

Imaging was performed using an IMAGING-PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany). The Universal Sample Holder IMAG-USH was mounted on the Stand IMAG-S. This structure provides a convenient means for mounting the CCD-Camera and the LED-Ring-Array. The LED-Ring-Array consisted of blue light-emitting diodes (LEDs) directed at a fixed angle and distance onto the object area. The LEDs provided the pulse-modulated excitation light, actinic illumination and saturating light pulses. The instrument uses a charge-coupled device (CCD) camera (IMAGE-K, Allied Vision Technologies) to capture Chl fluorescence images as a function of time and light sources and irradiances. The CCD camera was protected from stray excitation light by a long-pass filter (Schott, RG 645; Mainz, Germany) and from long-wavelength radiation ($\lambda < 780$ nm) by a short-pass filter (Balzers, Calflex-X, Bingen, Germany). Details of the measurements are reported in [18]. The current fluorescence yield (F_t) was measured continuously and the F_0 images were recorded in a quasi-dark state. The maximum fluorescence yield F_m was determined with a saturating pulse of 8000 μmol m^{-2} s^{-1} PFD for 1–2 s duration. The images of F_0 and F_m were subtracted and divided $[(F_m - F_0)/F_m]$ to generate an image of the maximum quantum efficiency of PSII photochemistry F_v/F_m. The current fluorescence yield F_t and the maximum light-adapted fluorescence (F'_m) were determined in the presence of an actinic illumination of 400 μmol m^{-2} s^{-1}, then Φ_{PSII} was computed as the quotient $(F'_m - F_t)/F'_m$ [25]. Images of the fluorescence parameters were displayed by means of a false-colour code ranging from black (0.00–0.040) via red, yellow, green and blue to purple (1.00).

2.5. Fluorometer determination

Measurements were carried out with a PAM-2000 Chlorophyll Fluorometer (Walz) on leaves similar to those utilised for the imaging technique. Further details of this instrumentation have been reported previously [26]. The measured area of the leaves was dark-adapted for 40 min before each measurement and then F_0 was measured using a modulated beam of 25 Hz. A saturating flash (8000 μmol m^{-2} s^{-1} PFD) of light for 800 ms was used to determine maximal fluorescence F_m. Fluorescence induction was started with actinic light (about 400 μmol m^{-2} s^{-1}) and superimposed with 800 ms saturating pulses (10.000 mol m^{-2} s^{-1} PFD) at 20-s intervals to determine maximal fluorescence in the light-adapted state (F'_m). A steady-state level of fluorescence F_s was achieved ($F_t - F'_0$), approximately 20-min after switching to the next higher light level. Minimal fluorescence in the light-adapted state (F'_0) was determined immediately after turning off the actinic source in the presence of a far-red (>710 nm) background for 10 s to ensure maximal oxidation of PSII electron acceptors. The maximum PSII quantum efficiency of PSII photochemistry $[F_v/F_m = (F_m - F_0)/F_m]$ and non-photochemical quenching $[q_{NP}; 1 - (F'_v/F_v)]$ were calculated according to [27]. The effective PSII quantum yield (Φ_{PSII}) was determined as $(F'_m - F_s)/F'_m$ [25] while the values of the quantum efficiency of open PSII reaction centres under the given light conditions ($\Phi_{exc.}$) were calculated as $F'_v/F'_m = (F'_m - F'_0)/F'_m$. The coefficient of photochemical quenching, q_L, is a measurement of the fraction of open PSII reaction centres based on the lake model of PSII antenna pigment organisation. This was defined by Kramer et al. [28] as $q_P \times F'_0/F_s$. The apparent rates of photosynthetic electron transport (ETR) were estimated as: ETR = $(\Phi_{PSII}) \times 0.5 \times$ PFD $\times 0.8$, where 0.5 accounts for the excitation of both PSII and PSI and 0.8 represents the average value for leaf absorbance.

2.6. Statistical analysis

Each experiment was performed at least twice with 30 plants for each. Means and standard deviations were calculated from pooled data of three replicates for each experiment. Data were subjected to two-way analysis of variance (ANOVA). When the significance of the interaction was significant, the least significant difference (LSD) was calculated for $P = 0.05$.

3. Results

Susceptibility to photoinhibition in bean leaves was determined as changes in the F_v/F_m ratio and the images of the ratio are reported in Fig. 1. Initial values of F_v/F_m were 0.796, 0.784 and 0.741 for plants maintained in charcoal-filtered air, or treated with a single exposure to O_3 for 3 h, or for 5 h, respectively. The results indicate that treatment with O_3 for 5 h induced a slight photoinhibition. The exposure of control plants (charcoal-filtered air for 5 h) at a light intensity of 1000 μmol m^{-2} s^{-1} resulted in a significant reduction in F_v/F_m ($P < 0.01$) (Fig. 1b), while plants treated with O_3 for 3 h showed an increased tolerance to photoinhibition with less reduction in F_v/F_m (Fig. 1f). Plants treated with O_3 for 5 h and then exposed to high light showed a reduction in F_v/F_m ratio values similar to those recorded in control