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Research article

Effects of acute O_3 stress on PSII and PSI photochemistry of sensitive and resistant snap bean genotypes (*Phaseolus vulgaris* L.), probed by prompt chlorophyll "a" fluorescence and 820 nm modulated reflectance

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ABSTRACT

The response of PSII and PSI photochemistry to acute ozone (O_3) stress was tested in a "model plant system", namely the O_3 sensitive (S156) and O_3 resistant (R123) genotype pairs of *Phaseolus vulgaris* L., during a phenological phase of higher O_3 sensitivity (pod formation). The modulation of the photosynthetic activity during O_3 stress was analysed by measuring gas exchanges, Prompt Fluorescence (PF, JIP-test) and 820 nm Modulated Reflectance (MR), a novel techniques which specifically detects the changes in the redox state of P700 and plastocyanin. The results showed that, coherently with genotypic-specific O_3 sensitivity, the response of the two snap bean genotypes differed for the intensity and time of onset of the considered physiological changes. In fact, despite leaf injury and gas exchanges reduction appeared concurrently in both genotypes, S156 showed a PSII down regulation already after the first day of fumigation (DOF), and an enhancement of Cyclic Electron Flow of PSI after the second DOF, whereas R123 showed only slight adjustments until the third DOF, when the activity of both photosystems was down-regulated. Despite these differences, it is possible to distinguish in both genotypes an early O_3 response of the photochemical apparatus, involving PSII only, and a following response, in which PSI activity and content are also modulated. The measurement of the MR signal, performed simultaneously with the PF measurements and the JIP-test analysis, has allowed a better understanding of the role that PSI plays in the O_3 stress response of the S156/R123 model plant system.

Abbreviations: A (μmol CO_2 m^{-2} s^{-1}), net assimilation rate; ABS/RC, effective antenna size of an active reaction center (RC); Ci/Ca (dimensionless), ratio of substomatal and ambient CO_2 concentration; DI_0/RC, effective dissipation in an active RC; DOF, day of Fumigation; ET_0/RC, electron transport in an active RC; F_0, minimal fluorescence, of the dark adapted leaf, when all RCs are open; F_m, maximal fluorescence of the dark adapted leaf, when all RCs are closed; FR, far red light; g_s (mmol m^{-2} s^{-1}), stomatal conductance; MR, modulated reflectance of 820 nm light; MR_{min}, minimal MR_t/MR_0 value, a transitory steady state, with equal oxidation and re-reduction rates of P700 and PC; MR_t/MR_0, ratio between modulated 820 nm reflection intensity at time t (MR_t), and value of the 820 nm reflection of the sample at the onset of the actinic illumination (between 0.3 and 1 ms, MR_0); PF*, prompt fluorescence transient of a sample exposed to the second light pulse of the measuring protocol, after 1 s of darkness from the first pulse; PF, prompt fluorescence transient of a dark-adapted sample; PI_{ABS}, Performance Index (potential) for energy conservation from photons absorbed by PSII to the reduction of PSI end acceptors; PI_{TOT}, Performance Index Total (potential) for energy conservation from photons absorbed by PSII to the reduction of PSI end acceptors; R123, ozone-resistant snap bean genotype; RC/CS_0, number of active RCs to one inactive RC for a PSII cross-section; RE/RC, electron flux reducing end electron acceptors at the PSI acceptor side, per RC; S156, ozone-sensitive snap bean genotype; TR/RC_0, maximal trapping rate of PSII; V_0^*, relative variable fluorescence of the second PF transient (PF*); V_I, relative variable fluorescence at 30 ms, $V_I = (F_I - F_0)/(F_M - F_0)$; V_J, relative variable fluorescence at 3 ms, $V_J = (F_J - F_0)/(F_M - F_0)$; v_{ox}, rate of P700 and PC oxidation, calculated as the maximum slope decrease of MR_t/MR_0; v_{red}, rate of P700 and PC re-reduction, calculated as the maximum slope increase of MR_t/MR_0; φ_{Eo}, maximum quantum yield of electron transport; φ_{Po}, maximum quantum yield of primary photochemistry; Ψ_{Eo}, probability that a photon trapped by the PSII reaction centre enters the electron transport chain; φ_{Ro}, quantum yield of an electron reaching the end acceptor of PS I; $\Delta I/I_0$, amplitude of the MR signal measured during the FR pulse; ΔV_{I-P}, amplitude of the I–P phase of the OJIP fluorescence transient; δ_{Ro}, probability that an electron is transported from reduced PQ to the electron acceptor side of PS I.

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1. Introduction

Tropospheric ozone (O_3) is a widespread phytotoxic air pollutant, which is known to affect plant photosynthetic function in different ways. The first interaction between O_3 and plants occur at the level of the guard cells of stomata, in which O_3 triggers the production of Reactive Oxygen Species (ROS), that activate a signalling cascade inducing an overall efflux of anions and K^+ (Vahisalu et al., 2010; Vainonen and Kangasjärvi, 2015). The consequent loss of guard cells turgor, leading to stomatal closure, reduces the gas uptake through stomata; this mechanism restricts the O_3 flux into the leaves ("avoidance mechanism", Castagna and Ranieri, 2009) but, at the same time, also limits the photosynthetic CO_2 assimilation (Astorino et al., 1995). The O_3 molecules that enter the sub-stomatal chamber can directly peroxidate the membrane lipids of the mesophyll cells (Vitale et al., 2008) or, in the apoplastic space, can generate toxic ROS (like OH^-, O_2^-, H_2O_2), which can then move inside the cells (Vainonen and Kangasjärvi, 2015). Furthermore, O_3 triggers the ROS production from different endogenous, enzymatic sources, a process known as "oxidative burst" (Jaspers and Kangasjärvi, 2010). Despite acting as signals molecules, that leads to the activation of several plant defence responses (Vainonen and Kangasjärvi, 2015), ROS are also responsible of direct oxidative damages to different molecules involved in the photosynthetic process, such as chlorophylls a and b, and Rubisco, whose activity and content has been reported to decline under O_3 stress (Goumenaki et al., 2010). Moreover, detrimental effects of O_3 on the photosynthetic electron transport, and in particular on Photosystem II (PSII) function, have been demonstrated (Guidi et al., 2002; Pellegrini, 2014). A reversible, photoprotective down regulation of PSII photochemistry is, however, the most commonly observed PSII response to O_3, being a consequence of the reduced demand of NADPH and ATP from the Calvin cycle; the latter can be caused by both stomatal closure and biochemical limitations (Bussotti et al., 2011; Mereu et al., 2011; Salvatori et al., 2013). Also, the role of Photosystem I (PSI) in plant photosynthetic response to O_3, has received increasing attention in the last years. In fact, the measurement of prompt chlorophyll "a" fluorescence (PF) and the JIP test analysis (Strasser et al., 2004, 2010) have shown that the I–P part of the PF transient, which correlates to PSI content and activity (Ceppi et al., 2012; Schansker et al., 2005), is particularly sensitive to O_3 and other oxidative stress factors (Oukarroum et al., 2009; Bussotti et al., 2011; Pollastrini et al., 2014; Bernardini et al., 2015). In particular, the amplitude of the I–P phase of the PF (ΔV_{I-P}) was reduced by O_3 stress in many studies, indicating a negative effect of this pollutant on the efficiency of electron transport through PSI, to reduce the end acceptors beyond PSI (Bussotti et al., 2011; Mereu et al., 2011; Pollastrini et al., 2014). More recently, a novel approach has been introduced, consisting in the simultaneous measurement of PF and Modulated Reflection (MR) signal at 820 nm, which specifically detects the changes in the redox state of P700 and plastocyanin (PC) (Strasser et al., 2010; Gao et al., 2014). This new technique has proven to be a powerful tool to investigate the combined stress response of the two Photosystems, and the few studies performed so far have pointed out that PSI is involved in all the investigated stress responses (Strasser et al., 2010; Oukkarroum et al., 2013; Salvatori et al., 2014; Fusaro et al., in press). Thus, there is the need to raise further insights about the role of PSI in the oxidative stress response in *vivo*, in particular for what concerns O_3 stress.

In this work, we have tested the effects of acute O_3 stress on PSII and PS I photochemistry of a "model plant system", i.e. the O_3 sensitive (S156) and O_3 resistant (R123) genotype pairs of *Phaseolus vulgaris* L. (snap bean), that is currently used for O_3 biomonitoring under the UNECE-ICP Vegetation Programme (Burkey et al., 2005). Our previous experiments have suggested that PSI plays a determinant role in the response of both genotypes to chronic, as well as to acute, O_3 stress (Salvatori et al., 2013, 2014). Here, we have further investigated how PSI activity is modulated under O_3 stress, testing the hypothesis that this modulation differs between the two genotypes, coherently with their known degree of O_3-sensitivity.

2. Methods

2.1. Plant material and O_3 fumigations

The experiment was conducted in a "walk-in" chambers facility, consisting of two closed chambers (2.5 m × 3.9 m × 3.0 m h), one used as control and one for O_3 fumigation (Salvatori et al., 2013). Air temperature was maintained at 25.7 ± 0.24 °C and 22.9 ± 0.3 °C during day and night, respectively, relative humidity was 68.8 ± 2.5%, and VPD was 1.1 ± 0.2 kPa. A PAR of approximately 700 μE m^{-2} s^{-1} (350 μE $m^{-2}s^{-1}$ at sample leaf height) was provided for 11 h per day by 6 metal halide lamps (1000 W, Philips HPI-T). Microclimatic conditions were monitored at 6-min interval, and did not differ significantly between the chambers.

Ten seeds of the ozone-sensitive (S156) and ozone-resistant (R123) genotypes of *P. vulgaris* L. (Burkey et al., 2005), provided by the ICP Vegetation Coordination Centre (2012 seed batch), were sown duiring Julian Day (JD) 44 in 7 l pots filled with garden soil and sand (1:0.5), added with a slow releasing fertilizer (Nitrophoska Blue, 12-12-17 and microelements, 3 g per pot). At the pod filling stage (33 days after sowing, JD 77), plants were randomly divided in two experimental sets: 5 ozone-sensitive and 5 ozone-resistant plants were exposed for 3 consecutive days (JD 78, 79, 80) to a mean hourly O_3 concentration of 136.3 ± 6.5 ppb, 5 h per day (total: 2044.2 ppb), simulating a photochemical pollution episode in a Mediterranean rural area (Paoletti, 2009; Fares et al., 2010). The other 10 plants were instead kept in the control chamber under filtered air (O_3 = 0 to maximal 4.2 ppb). Cumulative exposure was expressed as AOT40, i.e. the sum of the differences between the hourly mean ozone concentration in ppb and 40 ppb for each hour when the concentration exceeds 40 ppb, accumulated during daylight hours (Mills et al., 2011). O_3 in the fumigation chamber was generated by flowing pure oxygen on a UV light source (Helios Italquartz, Milan, Italy), and then added to the chamber air inlet by a Teflon tube. The O_3 concentration at plant height was continuously monitored with a photometric O_3 detector (Model 205, 2B Technologies, Boulder, CO, USA). In each chamber, plants were relocated randomly every day to reduce possible position effects. All plants were watered daily at field capacity with a sub-irrigation system, in which water was delivered from below the pots (Salvatori et al., 2013).

2.2. Assessment of visible ozone symptoms

Plants were inspected daily in both chambers, assessing the extent of ozone-like foliar symptoms. Following the ICP Vegetation experimental Protocol 2011 (ICP Vegetation, 2011), the total number of trifoliate leaves was counted for each plant, and those with 1–5%, 5–25%, >25% of injured area were recorded. The number of dead or senescent leaves remaining on the plant was also recorded. These data were then combined to calculate a Plant Injury Index (PII), derived as PII = (LA*AA)/100, where LA is the percentage of affected leaves per plant, and AA is the mean percentage of area affected for the symptomatic leaves (Calatayud et al., 2007).

2.3. Gas exchanges measurements

Net CO_2 assimilation (A, μmolCO_2 m^{-2} s^{-1}), leaf transpiration (E,