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Quenching partitioning through light-modulated chlorophyll fluorescence: A quantitative analysis to assess the fate of the absorbed light in the field

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ABSTRACT

Plants use a small part of the total absorbed light energy for net carboxylation, while the remaining amount is dissipated via alternative pathways involving thermal processes, fluorescence and non-carboxylation photochemistry in order to limit the formation of reactive oxygen species (ROS) and other photooxidative risks. The commonly used analysis of the Photosystem II (PSII) fluorescence signals gives qualitative information about absorbed light energy management by plants, but it is difficult to appreciate the relative contribution of each pathway in energy partitioning.

This study reports the application of quenching partitioning through a chlorophyll fluorescence approach performed on peach leaves subjected to three different light intensities for four durations of exposure in absence of recovery from photo-damage. This methodology was compared with the P_{700} redox kinetic method for determining the functional PSII fraction in leaves. In the absence of recovery processes the active PSII concentration decayed with an increase in photon exposure (the product of irradiance and the time of exposure), following an exponential pattern according to the reciprocity law. The photoprotective thermal dissipation (Φ_{NPQ}) was proportional to irradiance up to 30 min of photoinhibitory treatment. Afterwards Φ_{NPQ} was limited by the increasing competition for the absorbed energy re-emitted by the inactive PSII (Φ_{NF}). Φ_{NF} increased with the photon exposure dissipating up to 70% of the total incoming energy. The energy funnelled to photochemistry (Φ_{PSII}) decreased with increasing exposure time or intensity, becoming zero after 120 min of photoinhibitory treatment at the maximum irradiance (2100 μmol photon m^{-2} s^{-1}). The relation between the fraction of energy dissipated by the inactive PSII (derived from the quenching partitioning) and the inactive PSII fraction (measured with the P_{700} redox kinetic method) was linear.

The quenching partitioning through light-modulated chlorophyll fluorescence is a useful tool to analyse plant energy management and gives also a reasonable estimation of the active PSII fraction. This methodology can easily be used in the field as measurements are rapid, non-destructive and detection devices are portable.

1. Introduction

Plants can be considered as low-efficiency organisms, as in full light they convert only 5–10% of the total absorbed energy in accumulated dry matter (Long et al., 1994), while the remaining amount is dissipated via alternative mechanisms and/or can drive photoinactivation processes. Small changes in carboxylation efficiency, light harvesting and photosynthate allocation could, therefore, modify primary net carbon gain and commercial plant productivity (Flore and Lakso, 1988). The energy absorbed by the leaf is utilized or dissipated through several mechanisms involving thermal dissipative processes, fluorescence emission and photochemistry, all of which are competitive mechanisms. Energy partitioning analysis offers insights into how plants manage these competitive processes to maximize photosynthetic carboxylation, limit the formation of reactive oxygen species (ROS) and other photooxidative risks (Niyogi, 1999).

Light-dependent thermal dissipation can be divided into photoprotective quenching (commonly called non-photochemical quenching, *NPQ*) and quenching associated with PSII photoinactivation (Björkman and Demmig-Adams, 1994). The former is accomplished by a trans-thylakoid pH gradient that, by determining the PsbS protein conformational changes and the xanthophyll cycle activation (Ort, 2001), can dissipate up to 75% of the total

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absorbed energy (Demmig-Adams et al., 1996); this percentage can rise up to about 90% when other abiotic stresses, such as drought stress, occur (Hendrickson et al., 2004a; Flexas and Medrano, 2002). The key enzyme of this mechanism, violaxanthin de-epoxidase (VDE), is restricted to the thylakoid lumen and its activity is strictly dependent on the trans-thylakoid ΔpH (Ort, 2001). The maximum VDE activity is reached when the lumen pH is lower than 6.5 (Bratt et al., 1995). PSII photoinactivation quenching is an uncontrolled process, caused by thermal re-emission of photoinactivated PSII complexes (Walters and Horton, 1993; Müller et al., 2001; Lee et al., 2001; Matsubara and Chow, 2004). Recent research suggests that this dissipation pathway could be considered as a slowly reversible, protective mechanism, since inactive PSII complexes could act as protective shields from photon pressure for the surviving PSII complexes. On the other hand, the remaining functional PSII centers, by splitting water, create the optimal pH level for activation of enzymes promoting the new protein synthesis in chloroplasts, used for repair (Sun et al., 2006). Thermal dissipation via Photoinactivated PSII, however, contributes to only a small extent if the complexes are quickly repaired (Aro et al., 1994; Mattoo and Edelman, 1987; Mattoo et al., 1984).

Light-independent thermal dissipation also occurs amounting to approximately 20% of the absorbed light (Cailly et al., 1996; Hendrickson et al., 2005; Kornyeyev and Hendrickson, 2007). This contribution to the loss of photochemical efficiency of PSII has been argued as an inevitable consequence of the equilibrium between the radical pair and excitons in the light-harvesting antenna (Hendrickson et al., 2005).

The remaining absorbed energy is engaged to mobilize the electrons through several photochemical processes, including photosynthesis. Electrons exiting from the PSII core complex can be used to create reducing power for photosynthesis, photorespiration nitrogen and sulfur metabolism; otherwise, they can be dissipated via alternative transports such as the water–water cycle, cyclic electron transport around PSI and the glutathione–ascorbate cycle (Niyogi, 1999). Moreover, these alternative energy users pump supplementary H^+ into the lumen, thus contributing to the proton gradient across the thylakoid membrane that supports VDE activity (Heber and Walker, 1992; Asada, 1999; Noctor and Foyer, 1998; Cruz et al., 2005).

Despite this broad array of photoprotective mechanisms, plants are not able to avoid photoinactivation. In fact, in many species during the course of a sunny day, the PSII complex pool can be almost entirely destroyed and quickly repaired (Chow and Aro, 2005). In nature the recovery rate should be similar to the photoinactivation rate since it is often difficult to detect a *net* loss of PSII activity (Chow et al., 2005). PSII photoinactivation *in vivo* is not restricted to high light but also occurs at low irradiance (Anderson and Aro, 1994; Keren et al., 1995; Park et al., 1995; Tyystjärvi and Aro, 1996). By inhibition of D1 protein synthesis, it is possible to appreciate the real PSII inactivation (Aro et al., 1994). In the absence of repair the decrease of PSII activity is a function of photon exposure (the product of irradiance and time of exposure), following the reciprocity law (Jones and Kok, 1966; Park et al., 1995; Lee et al., 1999).

The management of the energy entering the leaf arouses great interest among scientists. The classical analysis of fluorescence parameters gives qualitative information about plant energy management, although it is difficult to appreciate the relative importance of each pathway. For example, while Φ_{PSII} (quantum yield of photochemical conversion in PSII) or F_v/F_m (maximum quantum yield of PSII) are efficiency parameters indicating the energy fraction used by those processes, NPQ (light-dependent non-photochemical quenching) is not an efficiency indicator, as attested by the fact that it can attain values much higher than 1. Several approaches have been attempted to evaluate the relative importance of all these mechanisms (Cailly et al., 1996; Demmig-Adams et al., 1996; Kramer et al., 2004; Kornyeyev and Hendrickson, 2007). Kornyeyev and Hendrickson (2007) modified an approach to partitioning suggested by Cailly et al. (1996). This analysis allows us to quantify the fraction of absorbed energy that is (1) utilized for photochemistry (net photosynthesis and photochemical processes), (2) quenched by light-dependent or light-independent thermal dissipation or (3) dissipated by inactive PSII. Moreover, with concurrent fluorescence and gas exchange measurements a further partitioning of photochemistry pathways has been proposed (Cheng et al., 2001; von Caemmerer, 2000; Galmés et al., 2007).

The aims of this study were to validate the quenching analysis method in peach tree leaves and to characterize the light management and the photoinactivation process as a function of photon exposure in the absence of repair in this fruit tree species.

2. Materials and methods

2.1. Plant material

The trial was carried out in 2007 at the Research School of Biological Sciences of the Australian National University, Canberra (Australia), on one year old nectarine (*Prunus persica* L. Batsch var. laevis) trees of the cultivar Red Gold, grafted on seedling rootstocks, grown in pots and receiving abundant water and fertilizer. During summer (January 2007) the day before the measurements, after the sunset (~19.30 h), well expanded attached leaves were labelled and covered with aluminium foil to avoid any possible photoinactivation and leaf temperature increase.

2.2. Photoinhibitory treatment

Early in the morning (~7.30 h) leaf discs were floated with their abaxial side up, in order to facilitate gas exchange (Sun et al., 2006), on a 1 mM lincomycin solution (an inhibitor of the D1 protein repair; Aro et al., 1994) for 2 h in darkness. Uptake of lincomycin to inhibit PSII recovery in leaf discs allowed us to observe the photoinactivation of PSII without the complication of concurrent repair. Leaf discs continued to float with the abaxial side up on the lincomycin solution (23 °C) during illumination on the adaxial side. Photoinhibiting light was directed vertically upwards, through a transparent water bath, to a clear Petri dish in which the leaf discs were floated. Leaf discs were exposed to different photon exposure levels obtained by the combination of 3 irradiances (500, 1000 and 2100 μmol photon m^{-2} s^{-1}) and 4 exposure times (30, 60, 90 and 120 min) to create various stages of photoinactivation of PSII.

2.3. Experiment 1: Functional PSII determination

Before the photoinhibitory treatment, 9 leaf discs (3 for each irradiance) were used as non-photoinhibited controls. On these discs, the functional PSII content (μmol m^{-2}) was quantified by flash-induced oxygen evolution, assuming that each functional PSII evolves one O_2 molecule after four saturating, single-turnover flashes (Chow et al., 1989, 1991). For each irradiance/exposure time combination the relative functional PSII (normalized to the value of the non-photoinhibited control) was quantified on 3 leaf segments by the measurement of the redox kinetic of P_{700} according to Losciale et al. (2008), with a dual wavelength (820/870 nm) unit (ED-P700DW) attached to a phase amplitude modulation fluorometer (Walz, Effeltrich, Germany). Assuming that in the non-photoinhibited state the relative functional PSII amount is 1, multiplying the functional fraction of PSII by the absolute functional PSII content of a non-photoinhibited sample (obtained by flash-induced oxygen evolution measurements) the functional