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The mechanism(s) involved in the photoprotection of PSII at elevated CO_2 in nodulated alfalfa plants

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Abstract

In a previous study, we found that enhanced CO_2 subjected to nodulated alfalfa plants grown at different temperatures (ambient and ambient + 4 °C) and water availability regimes could protect PSII from photodamage. The main objective of this study was to determine the mechanism(s) involved in the photoprotection of PSII at elevated CO_2 levels in this plant. Elevated CO_2 reduced carboxylation capacity-induced photosynthetic acclimation and reduced enzymatic and/or nonenzymatic antioxidant activities, suggesting that changes in electron flow did not cause any photooxidative damage (which was also confirmed by H_2O_2 and lipid peroxidation analyses). Enhanced nonphotochemical quenching and xanthophyll cycle pigments revealed that plants grown at 700 μmol mol^{-1} CO_2 compensated for the reduction in energy sink with a larger capacity for nonphotochemical dissipation of excitation energy as heat, i.e., modulating the status of the VAZ components. Elevated CO_2 induced the de-epoxidation of violaxanthin to zeaxanthin, facilitating thermal dissipation and protecting the photosynthetic apparatus against the deleterious effect of excess excitation energy.

Keywords: Alfalfa; Antioxidants; Elevated CO_2; *Medicago sativa*; Photosystem II; Photoprotection; Nonphotochemical quenching; Xanthophyll cycle

1. Introduction

The global atmospheric CO_2 concentration is increasing and, according to the Intergovernmental Panel on Climate Change, it is expected to reach 700 μmol mol^{-1} by the end of this century (IPCC, 2001). Most experiments analysing the effect of CO_2 increase on vegetative processes in the context of climate change have studied the effect of CO_2 or CO_2–temperature interaction under optimal conditions of water supply. However, when studying the effect of climate change on Mediterranean environments, it should be kept in mind that circulation models also predict drier conditions for the Mediterranean basin due to an increase in temperature and water deficit (IPCC, 2001; Alley et al., 2007). Furthermore, it is well known that the effect of combined stresses on plant growth causes alterations that cannot be predicted from the effects of the stresses alone because of synergism and antagonism phenomena (Valladares and Pearcey, 1997). Chaves and Pereira (2004) observed that, although photochemical processes are resistant to low water availability conditions, they induce downregulation of the photosynthetic apparatus when they interact with elevated temperature and irradiation levels.

Abbreviations: A, anteraxanthin; A_{sat}, light-saturated rate of CO_2 assimilation; APX, ascorbate peroxidase; ASA, ascorbate; CAT, catalase; DHA, dehydroascorbate; F_v/F_m, maximal photochemical efficiency; g, leaf conductance; GR, glutathione reductase; GSSG, oxidized glutathione; GSH, reduced glutathione; H_2O_2, hydrogen peroxide; IPCC, Intergovernmental Panel on Climate Change; J_{max}, electron transport rate contributing to RuBP regeneration; Je(PSII), electron transport through Photosystem II; Je(PCR), electron transport through photosynthetic carbon reduction; Je(PCO), electron transport through photorespiratory carbon oxidation; Ja, electron transport through the alternative pathway; l, stomatal limitation; PPFD, photosynthetic photon flux; PSII, Photosystem II; NPQ, nonphotochemical quenching; O_2^-, superoxide anion; (OH), oxygen hydroxyl radical; OEC, oxygen evolving complex; R_d, rate of day respiration; pO_2, ambient partial pressure of O_2; ROS, reactive species of oxygen; SOD, superoxide dismutase; Sr, relative specificity of Rubisco; TGT, temperature gradient tunnel; V, violaxanthin; V_C, rate of carboxylation by Rubisco; V_{cmax}, maximum photosynthetic rate; V_o, rate of oxygenation by Rubisco; Z, zeaxanthin; ϕ_{PSII}, quantm yield of Photosystem II electron transport; θ_v, soil volumetric water content.

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Although the influences of increases in CO_2 levels on plant growth and physiology have been studied by a number of workers (Wolfe et al., 1998; Urban, 2003; Long et al., 2004; Aranjuelo et al., 2005a, 2006; Erice et al., 2006a), the effect of elevated CO_2 on plant photochemistry and its influence on PSII have received little attention. Furthermore, the results of previous studies reveal significant discrepancies and variability. For example, Hymus et al. (2001) observed that plants exposed to elevated CO_2 concentrations exhibited increased (photochemical) requirements for light-saturated electron flow through Photosystem II, whereas Scarascia-Mugnozza et al. (1996) reported that CO_2 increase had a depressive effect on plant photochemistry. According to previous studies, elevated CO_2 levels will increase the photosynthetic carbon reduction cycle (the major sink for the reducing equivalents generated by the primary chemical reactions) and consequently the electron flow that drives it (Hymus et al., 2001). In addition, elevated CO_2 will competitively suppress the photorespiratory carbon oxidation cycle and the resulting electron flow. However, several studies on C_3 plants grown under elevated CO_2 concentration have observed that, after an initial stimulation of photosynthetic rates, the carboxylation capacity of plants decreases after long-term exposure (Ainsworth et al., 2004; Long et al., 2004; Aranjuelo et al., 2005b). As observed by Hymus et al. (2001), changes in C assimilation at elevated CO_2 require modifications in the partitioning of the absorbed energy between heat dissipation and photochemistry. When photosynthesis decreases and light excitation energy is in excess, overexcitation of the photosynthetic pigments in the antenna can occur. Impairment of photosynthetic function will lead to excessive excitation energy in Photosystem II (PSII), leading to an accumulation of reactive oxygen species (ROS) and thereby resulting in oxidative stress.

Production of ROS, including superoxide (O_2^{-}), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\bullet}OH$), is an inevitable consequence of life in an oxygen-rich environment (Polle et al., 1990). ROS are formed within the plant as a consequence of (a) photochemical production of H_2O_2 in the atmosphere from air pollution; (b) donation of electrons directly to oxygen during photosynthesis, especially with high light; and (c) in response to environmental stresses such as drought/heat, etc. ROS damage plant cells by oxidizing membrane lipids, including the photosynthetic apparatus (Foyer and Harbinson, 1994); inhibit protoplast regeneration (Marco and Roubelakis-Angelakis, 1996); and damage proteins, chlorophyll, and nucleic acids (Foyer and Harbinson, 1994).

Plants have developed three main mechanisms to diminish photooxidation: (a) to prevent the production of ROS by diminishing the electron transport chain; (b) to scavenge ROS formed by an integrated system of enzymatic and nonenzymatic antioxidants (Asada, 1999). The ascorbate-glutathione cycle is the most important antioxidant cycle in plants (Alscher et al., 1997). The first ROS produced in the plant cells is the superoxide radical anion (O_2^{-}), which is dismutated to H_2O_2 by superoxide dismutase (SOD). The H_2O_2 is then reduced to H_2O by ascorbate peroxidase (APX). The ascorbate oxidized by APX is reduced by the reduced form of glutathione, and the glutathione is yet again reduced by glutathione reductase (GR) (Alscher et al., 1997). Secondly, the chloroplastic water–water cycle, which is related to the electron flow from the water in Photosystem II to O_2 reduction in PSI without any release of superoxide and hydrogen peroxide, has been proposed as an effective mechanism to dissipate excess excitation under environmental stress (Asada, 1999; Ort and Baker, 2002; Zhou et al., 2006). (c) To diminish photooxidation through xanthopyll cycle-dependent thermal dissipation, an important photoprotective process in the light-harvesting antenna of Photosystem II (Gilmore, 1997; Verhoeven et al., 1999). In this process, the formation of a pH gradient across the thylakoid membrane activates the de-epoxidation of violoxanthin (V) to zeaxanthin (Z) and anteraxanthin (A), facilitating the thermal dissipation of excess excitation energy (Demming et al., 1987). Xanthophyll cycle-dependent energy dissipation downregulates the photochemical efficiency of PSII, thereby protecting the reaction centres from photooxidation.

Several responses of photoinhibition to elevated CO_2 concentration have been reported. In wheat plants grown under elevated CO_2 conditions, a greater proportion of the absorbed light is used in photochemistry at high light (Habash et al., 1995). Such an increase was reflected in larger photochemical energy dissipation with the consequent reduction in photoinhibition. Other authors showed decreased photochemistry and increased photoinhibition in plants exposed to drought (Scarascia-Mugnozza et al., 1996) and heat stress conditions (Roden and Ball, 1996). An increase or decrease in photochemistry and photoinhibition depends on whether photosynthetic downregulation occurs (Hymus et al., 2001). In a previous study carried out by our group with alfalfa plants grown under elevated CO_2 conditions, it was observed that, although plants grown at 700 μmol mol^{-1} had photosynthetic acclimation, CO_2 increase protected PSII from higher excess in excitation energy as indicated by the higher F_v/F_m (Aranjuelo et al., 2005a). Hymus et al. (2001) also observed that elevated CO_2 enhanced nonphotochemical quenching to compensate for the reduction in energy sink, thus contributing to the alleviation of excessive excitation energy in the PSII. The influence of elevated CO_2 on PSII photoprotection has also been highlighted by other authors (Carvalho and Amâncio, 2002; Kurasova et al., 2003; Kitao et al., 2005).

In a previous study, we observed that, although elevated CO_2 resulted in significantly lower values of PSII efficiency in the light (ϕ_{PSII}), higher values of F_v/F_m were measured in dark-adapted leaves (Aranjuelo et al., 2005b). As a follow up, in the present work, we examine the photoprotective effect of elevated CO_2 (at different temperatures and water availability regimes) on PSII in nodulated alfalfa plants. This experiment was performed in temperature gradient tunnels (TGTs) placed over plants under standard field conditions. The objective was to analyse the mechanisms involved in CO_2-induced photoprotection of the photochemistry of PSII (Aranjuelo et al., 2005a) and to determine which processes could be linked to the protection of reaction centres. To do so, the following processes associated with photodamage were studied: (a) the electron transport chain; (b) scavenging of ROS by antioxidant molecules, enzy-