



Enhanced α -tocopherol quinone levels and xanthophyll cycle de-epoxidation in rosemary plants exposed to water deficit during a Mediterranean winter

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Summary

Photosynthesis operates in a constantly shifting balance between efficient capture of solar energy and its rapid dissipation when captured in excess. In an attempt to better understand the role of α -tocopherol in plant photoprotection, we examined the changes in α -tocopherol quinone (α -TQ), in parallel with those of other low-molecular-weight antioxidants, in rosemary plants exposed to water deficit during a Mediterranean winter. Relative leaf water content (RWC) decreased from about 85% to approximately 65% in drought, but plants did not show symptoms of oxidative damage, as indicated by constant F_v/F_m ratios and malondialdehyde (MDA) levels. α -TQ was present at concentrations of 20 mmol per 100 mol of chlorophyll, and represented less than 1% of total tocopherol content in non-stressed leaves. Although α -tocopherol levels were not significantly altered, α -TQ reached up to 36 mmol per 100 mol of chlorophyll under stress (under both high light and after exposure to increasing water deficit at lower light intensities). Furthermore, both α -TQ and xanthophyll cycle de-epoxidation were strongly negatively correlated with the relative efficiency of photosystem II photochemistry (ϕ_{PSII}) at midday. The biological significance of α -tocopherol and α -TQ in the network of photo- and antioxidative protection mechanisms evolved by plants to withstand stress is discussed.

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Abbreviations: α -TQ, α -tocopherol quinone; DPS, de-epoxidation state of the xanthophyll cycle; ϕ_{PSII} , relative efficiency of photosystem II photochemistry; F_v/F_m , maximum efficiency of photosystem II photochemistry; MDA, malondialdehyde; PPF, Photosynthetically-active photon flux density; ROS, reactive oxygen species; RWC, relative leaf water content

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Introduction

Water deficit, taken with high solar radiation and high temperatures during the summer has been considered the main limiting factor for plant growth in Mediterranean-type ecosystems (di Castri, 1981). However, climatological data presently available indicate that periods of water deficit are also common during Mediterranean winter, and models of climatic change predict an increase in the frequency and severity of droughts in the Mediterranean basin (Houghton et al., 2001). It is therefore of interest to study the response of Mediterranean vegetation to water deficit during the winter, since this may likely be a limiting factor for survival in the response of Mediterranean vegetation to climatic change.

Environmental stresses, such as water stress, may lead to an imbalance between antioxidant defenses and the amount of reactive oxygen species (ROS), resulting in oxidative stress (Smirnoff, 1993; Pastori and Foyer, 2002). Several ROS are thought to be involved at low concentrations in inter- and intracellular signaling in plant responses to stress (Foyer and Noctor, 1999; Op den Camp et al., 2003). They can also cause damage at various levels of organization, including damage to the chloroplasts, when present at high concentrations (Asada, 1999; Apel and Hirt, 2004). Apart from the xanthophyll cycle, photorespiration and other changes in metabolic activity which may protect the chloroplasts from oxidative damage (Demmig-Adams and Adams, 1996; Osmond et al., 1997), a number of enzymatic and non-enzymatic antioxidants that serve to control oxygen toxicity are present in chloroplasts. Of these, tocopherols (vitamin E), carotenoids, ascorbate and glutathione play an important role in maintaining the integrity of the photosynthetic membranes under oxidative stress (Havaux, 1998; Smirnoff and Wheeler, 2000; Munné-Bosch and Alegre, 2002; Munné-Bosch, 2005).

Although several studies have focused on the response of rosemary to summer drought stress (Munné-Bosch et al., 1999a,b; Munné-Bosch and Alegre, 2000; Werner et al., 2002; Ain-Lhout et al., 2004), no studies have evaluated the effects of drought on this species during winter. The aim of this study was to evaluate the effects of water deficit on rosemary plants during winter, with an emphasis on the role of carotenoids and tocopherols in the protection of the photosynthetic apparatus.

Materials and methods

Plant material and growth conditions

Three-year-old rosemary (*Rosmarinus officinalis* L.) plants were grown at the Experimental Fields of the University of Barcelona under Mediterranean field conditions and receiving water exclusively from rainfall. Prior to the experiment, plants received the following monthly rainfall precipitation: September, 88.3 mm; October, 8.6 mm; and 1–10 November, 23.4 mm. From 10 November (day 0) to 16 December (day 36), plants were covered with a clear polyvinyl chloride (PVC) sheet when it rained, and were not watered at all so that the plant response to water deficit could be studied. Plants were not covered between 16 December (day 36) and 22 December (day 42), which allowed us to study the effects of water recovery on plants, as 12.4 mm of rain fell on 20 December (day 40). The environmental conditions were monitored throughout the study with a Weather Station (Delta-T Devices, Neumarket, UK) as described (Munné-Bosch et al., 1999b).

Plant water status, chlorophyll fluorescence, malondialdehyde (MDA) and chlorophyll contents, as well as levels of reduced and oxidized antioxidants, α -tocopherol, carotenoids and glutathione in fully-developed sun-exposed young leaves collected at midday (12 h solar time) were measured. For measurements of MDA, chlorophylls and antioxidants, samples were collected, frozen in liquid nitrogen and stored at -40°C until analysis.

Plant water status and chlorophyll fluorescence

Plant water status was estimated by measuring the relative leaf water content (RWC). Modulated chlorophyll fluorescence was measured by using a portable fluorimeter mini-PAM (Walz, Effeltrich, Germany) on attached leaves to estimate the maximum efficiency of PSII photochemistry (F_v/F_m) and the relative efficiency of PSII photochemistry (ϕ_{PSII}), which are indicative of chronic and dynamic photoinhibition of photosynthesis, respectively (Werner et al., 2002). F_v/F_m was estimated as $(F_m - F_o)/F_m$ after exposing the leaves to darkness for 1 h, while ϕ_{PSII} was estimated as $(F_{m'} - F_s)/F_{m'}$ in leaves exposed at incident photosynthetically-active photon flux (PPFD) in the field.

Estimation of lipid peroxidation

The extent of lipid peroxidation in leaves was estimated spectrophotometrically by measuring

the amount of MDA by the method described by Hodges et al. (1999). This method takes into account the possible influence of interfering compounds in the assay for thiobarbituric acid reactive substances.

Analyses of photosynthetic pigments and antioxidants

The extraction and HPLC analyses of chlorophylls, carotenoids, α -tocopherol and α -tocopherol quinone (α -TQ) were carried out as described by Munné-Bosch and Alegre (2003). The extraction and spectrophotometrical analyses of reduced and oxidized GSH were carried out as described by Noctor and Foyer (1998).

Statistical analyses

Analyses of variance (ANOVAs), Student's t-tests and regression analyses were conducted using SPSS (Chicago, IL, USA). Differences were considered significant at a probability level of $P < 0.05$.

Results

Rosemary plants withstand winter stress

Climatic conditions during the study period were characterised by a progressive reduction of the PPFD and air temperature. PPFD values decreased from $1100 \mu\text{mol m}^{-2} \text{s}^{-1}$, while maximum and minimum air temperatures decreased about 6°C during the experiment (Fig. 1). Plants were exposed to soil water deficit, which resulted in a significant reduction of RWC, which decreased from 85% to 65% after 36 days of stress. RWC values recovered after rainfall, attaining values similar to those observed in pre-drought conditions. F_v/F_m ratios and MDA levels, an indicator of lipid peroxidation, were kept unaltered throughout the experiment (Fig. 1). Glutathione levels were kept around $200 \text{ nmol (g DW)}^{-1}$ and no changes in its redox state were observed throughout the experiment (data not shown).

Light-induced dynamic photoinhibition in plants

Despite the fact that F_v/F_m ratios were kept above 0.75 throughout the experiment, the ϕ_{PSII} was significantly smaller at the beginning of the experiment than during recovery. Major climatic differences between start and the end of the

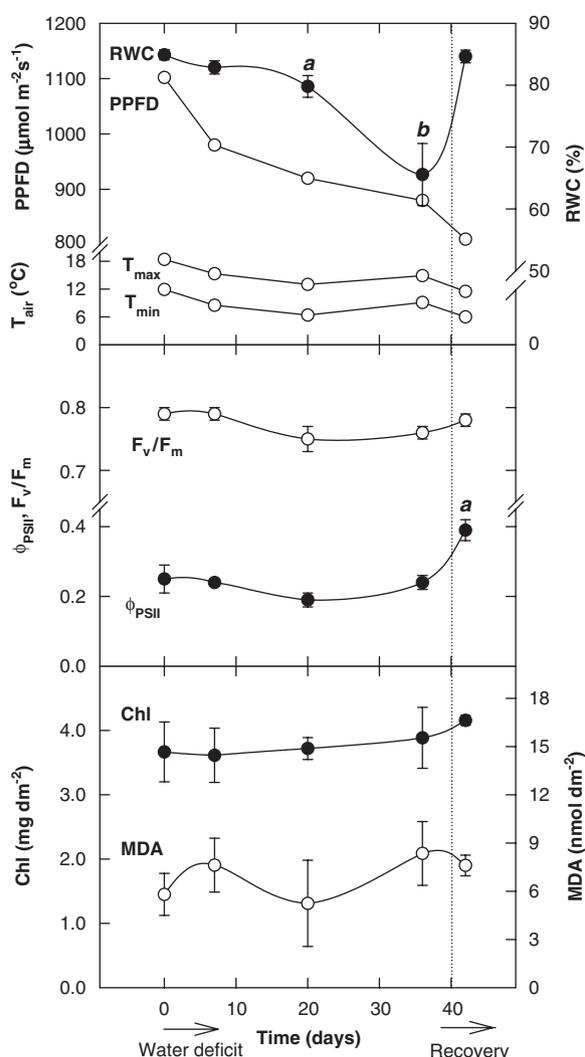


Figure 1. Changes in meteorological conditions (maximum and minimum air temperature and PPFDs), relative leaf water content (RWC), the maximum and relative efficiencies of PSII photochemistry (F_v/F_m and ϕ_{PSII} , respectively), and levels of chlorophyll a+b (Chl) and malondialdehyde (MDA) in leaves of rosemary plants exposed to water deficit during a Mediterranean winter. Error bars indicate SEM. $n = 8$ plants for RWC, F_v/F_m and ϕ_{PSII} , and $n = 3-4$ plants for Chl and MDA. Letters indicate statistical significant differences at $P \leq 0.05$.

experiment were PPFD and air temperature values, while RWC was kept at approximately 85%. The highest PPFD values observed at the beginning of the experiment were associated with smaller ϕ_{PSII} values (Fig. 1). In turn, this was associated with higher α -TQ levels and de-epoxidation state of the xanthophyll cycle (DPS) values, while no significant differences were observed for chlorophyll and β -carotene levels throughout the experiment (Figs. 2 and 3). α -TQ was present at concentrations of 36 mmol per 100 mol of chlorophyll, which

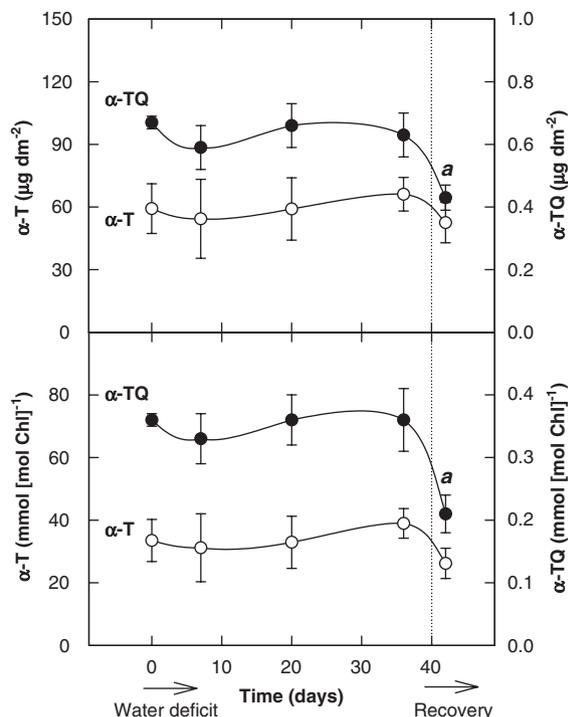


Figure 2. Changes in α -tocopherol (α -T) and α -tocopherol quinone (α -TQ), given per leaf area and per chlorophyll unit, in leaves of rosemary plants exposed to water deficit during a Mediterranean winter. Error bars indicate SEM $n = 3$ –4 plants. Letters indicate statistical significant differences at $P \leq 0.05$.

corresponds to $0.36 \text{ mmol mol Chl}^{-1}$, at the beginning of the experiment (at the highest PPFDs), while it was found at $20 \text{ mmol per } 100 \text{ mol}$ of chlorophyll at the end of the experiment (at lowest light intensities).

Influence of drought on mechanisms of photo- and antioxidant protection

A significant reduction of RWC was observed after 20 days of soil water deficit, reaching minimum values after 36 days of stress (Fig. 1). To evaluate the effects of drought we may compare the effect of this RWC reduction (day 36) with recovery (day 42). During this period (16–22 December), RWC increase significantly (from 65% to 85%) as a result of rainfall, while PPFD and air temperature values changed only slightly ($70 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 3°C , respectively). ϕ_{PSII} values were smaller, while α -TQ levels were higher, during water stress than after recovery followed by the period of rainfall (Figs. 1 and 2). Further, the highest DPS values were observed after 36 days of water deficit (Fig. 2).

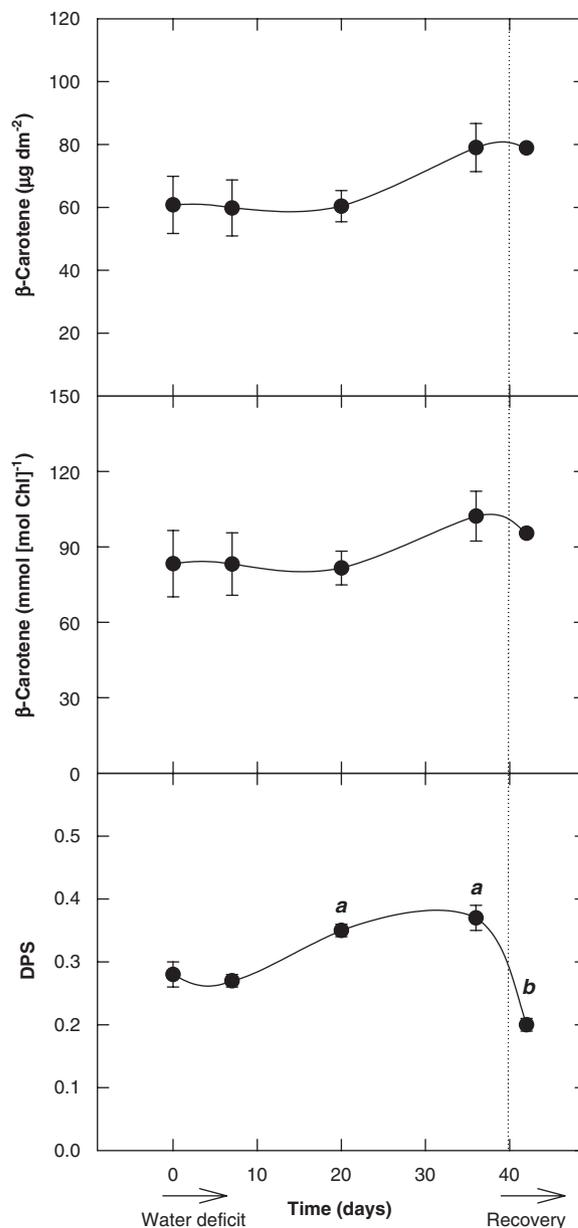


Figure 3. Changes in β -carotene (β -Car) levels, given per leaf area and per chlorophyll unit, and in the de-epoxidation state of the xanthophyll cycle (DPS), in leaves of rosemary plants exposed to water deficit during a Mediterranean winter. Error bars indicate SEM $n = 3$ –4 plants. Letters indicate statistical significant differences at $P \leq 0.05$. DPS was calculated as $(Z + 0.5A)/(V + Z + A)$, where Z is zeaxanthin, A is antheraxanthin and V is violaxanthin.

Discussion

Rosemary plants did not suffer from oxidative damage, as indicated by constant F_v/F_m ratios and MDA levels throughout the study. Plants showed enhanced xanthophyll cycle de-epoxidation and

higher α -TQ levels under water stress. The extent of xanthophyll cycle de-epoxidation is related to the capacity of plants to dissipate excess excitation energy as heat in the thylakoid membranes, conferring protection to the photosynthetic apparatus (Demmig-Adams and Adams, 1996). α -Tocopherol, in cooperation with other antioxidants, contributes to the preservation of an adequate redox state in chloroplasts and to maintaining thylakoid membrane structure and function during plant responses to stress (Munné-Bosch, 2005).

α -Tocopherol can physically quench, and therefore deactivate $^1\text{O}_2$ in photosynthetic membranes (Trebst et al., 2002). It has been estimated that before being degraded, one molecule of α -tocopherol can deactivate up to 120 $^1\text{O}_2$ molecules by resonance energy transfer (Fahrenholtz et al., 1974). In addition, α -tocopherol can chemically scavenge $^1\text{O}_2$ and lipid peroxy radicals. The chemical scavenging of $^1\text{O}_2$ by α -tocopherol irreversibly leads to their quinones and epoxides, while the scavenging of lipid peroxy radicals results in the formation of tocopheroxyl radicals, which can be recycled back to α -tocopherol by the ascorbate–glutathione cycle (Munné-Bosch and Alegre, 2002; Munné-Bosch, 2005). In the present study, enhanced α -TQ levels were observed during water stress in rosemary, and this occurred in conjunction with reductions in ϕ_{PSII} . Our results are in agreement with previous studies, which report on enhanced levels of α -TQ in plants exposed to conditions favouring $^1\text{O}_2$ formation in chloroplasts (Yamauchi and Matsushita, 1979; Munné-Bosch and Alegre, 2003). These studies indicate that α -tocopherol is irreversibly degraded, in part, to α -TQ under stress, and this may serve a photo- and antioxidant protective function.

α -TQ was present at concentrations of 20 mmol per 100 mol of chlorophyll, and represented less than 1% of total tocopherol content in non-stressed leaves (at the end of the experiment). Although α -tocopherol levels were not significantly altered, α -TQ reached up to 36 mmol per 100 mol of chlorophyll under stress (both under high light at the beginning of the experiment or after exposure to increasing water deficit at lower light intensities). The intracellular localisation of α -TQ is limited to chloroplasts, either in the envelope, where it is synthesised, in plastoglobuli of the stroma, where it is stored, and in thylakoid membranes (Kruk and Strzalka, 1995). In the present study, α -TQ levels correlated with ϕ_{PSII} , suggesting a putative role for α -TQ in dynamic photoinhibition of photosynthesis. ϕ_{PSII} was negatively correlated with both DPS and α -TQ values, and regression coefficients of 0.691 and 0.896,

respectively, were obtained. α -TQ has been related to photosynthetic electron transport in thylakoids, and it has been shown that cyclic electron transport around PSII is stimulated by α -TQ (Kruk et al., 2000; Kruk and Strzalka, 2001). It is therefore suggested that enhanced α -TQ levels during periods of low precipitation in winter may contribute to the dissipation of excess energy in thylakoids, thereby conferring photoprotection onto the photosynthetic apparatus.

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