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Diurnal changes in photosystem II photochemistry, photoprotective compounds and stress-related phytohormones in the CAM plant, *Aptenia cordifolia*

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Acclimation of photosynthetic light reactions to daily changes in solar radiation requires adjustments in photosystem II photochemistry and may be affected by environmental stresses, such as drought. In this study, we examined the effects of a short-term, severe water deficit on diurnal variations in photosystem II photochemistry, photoprotective compounds (tocopherols and carotenoids, including the xanthophyll cycle) and stress-related phytohormones (abscisic acid and salicylic acid) in the CAM plant, *Aptenia cordifolia* L. f. Schwantes. Violaxanthin was rapidly converted to zeaxanthin under high light, the de-epoxidation state of the xanthophyll cycle reaching maximum levels of 0.95 at midday in irrigated plants. Under a higher photoprotective demand caused by water deficit, plants showed significant increases in abscisic acid and γ-tocopherol levels, which were followed by decreases in β-carotene and the F_v/F_m ratio at later stages of stress. Decreases in this ratio below 0.70 correlated with sustained increases in the de-epoxidation state of the xanthophyll cycle, which kept above 0.90 at night after 15 days of water deficit. In contrast to abscisic acid, salicylic acid levels kept constant under water deficit and showed a sharp decrease during the day both under irrigated and water stress conditions. We conclude that the CAM plant, *A. cordifolia* showed several strategies of acclimation to short-term water deficit, including abscisic acid and γ-tocopherol accumulation, as well as sustained increases in the de-epoxidation state of the xanthophyll cycle, which was tightly coupled to daily variations in photosystem II photochemistry. The differential accumulation of tocopherol homologues under water deficit and the diurnal fluctuations of salicylic acid levels in this CAM plant will also be discussed.

1. Introduction

Crassulacean acid metabolism (CAM) is considered to be an adaptive mechanism allowing many terrestrial and epiphytic succulents to survive in periodically droughted habitats. In CAM plants, carbon dioxide uptake occurs during the night, when stomata open; carbon dioxide is combined with phosphoenolpyruvate to yield oxaloacetate, which is reduced to malate. Malate is transported into the vacuole and malic acid accumulates during the night. Nocturnal acid accumulation and nocturnal stomatal aperture are the main diagnostic features of CAM. During the day malate is decarboxylated in the cytoplasm, providing Rubisco with increased partial pressures of carbon dioxide during C_3 photosynthesis. The net outcome of the functioning of CAM is that carbon dioxide is fixed with significant water saving relative to C_3 photosynthesis, since the process occurs at times of lower evaporative demand and a larger air-leaf carbon dioxide concentration gradient [1–3].

In C_3 plants, water deficit may lead to an imbalance between the production and consumption of reducing equivalents and ATP in photosynthesis by inducing stomatal closure and consequently decreasing carbon dioxide assimilation during the day. This may enhance excess excitation energy in thylakoids of stressed plants, which must activate adequate photoprotection mechanisms, as those described for high light-acclimated plants, to dissipate excess energy and afford protection to photosystem II (PSII) [4]. Osmond suggested that high daytime partial pressures of carbon dioxide inside the leaves and re-fixation of respiratory CO_2 might contribute to the protection of the photosynthetic machinery against high irradiance with closed stomata in CAM plants [5]. Since then, some evidence has been gathered, most of it in facultative CAM plants, in favor of this hypothesis (reviewed by [3]). However, it has been shown that photoprotection through the xanthophyll cycle may not necessarily be more effective in CAM than in C_3 species, as shown in a comparison of *Clusia multiflora* (obligate C_3) and *Clusia minor* (facultative CAM). Zeaxanthin

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Abbreviations: ABA, abscisic acid; CAM, crassulacean acid metabolism; Chl, chlorophyll; DPS, de-epoxidation state of the xanthophyll cycle; DW, dry weight; ϕ_{PSII}, relative efficiency of PSII photochemistry; F_v/F_m, maximum efficiency of PSII photochemistry; IR, irrigated; PPFD, photosynthetically active photon flux density; PSII, photosystem II; SA, salicylic acid; WS, water-stressed.

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content and non-photochemical quenching of chlorophyll fluorescence were equally large in both species subjected to high irradiance, precluding a specific role of CAM in energy dissipation. Nevertheless, leaves of *C. multiflora* became necrotic and died when plants were maintained for a long period under high irradiance, whereas leaves of *C. minor*, by switching to CAM, did not [6].

Baby sun rose (*Aptenia cordifolia* L. f. Schwantes, family Aizoaceae) is a perennial herb, spreading over ground and neighboring vegetation, with small, heart-shaped, dark green succulent leaves interspersed with small, axillary, many-petaled, bright pink to purple flowers. Baby sun rose, which is native to the coastal deserts of southern Africa, was first identified as a C_3 plant that induces CAM under salt stress [7], but later it was found to be an obligate CAM plant, both under water deficit and well-watered conditions [1,8]. In perennial obligate CAM plants, where up to 99% of CO_2 assimilation occurs during the night [9], our knowledge on the response of photoprotective mechanisms under water deficit is still very limited, although several studies have shown the importance of carotenoids, and particularly of xanthophyll cycle-dependent energy dissipation, in acclimation to high light stress [reviewed by 10].

Abscisic acid (ABA) is known to modulate key aspects of plant responses to water deficit such as transpiration efficiency, hydraulic conductivity and leaf expansion [11]. Furthermore, it has been shown that exogenous ABA can induce CAM metabolism in a number of species (reviewed by [12]) and it has been recently suggested that ABA might have a role in photoprotection in CAM plants, since exogenous ABA can induce chloroplast clumping in the leaf cells of two species of *Kalanchoë* under light [13]. Although it is well known that ABA levels increase in plant responses to drought stress, including some CAM plants (reviewed by [3]), to our knowledge no studies have evaluated diurnal variations in endogenous ABA contents in CAM plants exposed to water deficit. Although salicylic acid (SA) has also been shown to participate in plant responses to abiotic stress, including drought [14,15], no studies have been performed thus far to evaluate the role of this compound in CAM plants.

In a recent study, the influence of a short-term drought on diurnal gas exchange, malic and citric acid contents, water relations and chlorophyll fluorescence were evaluated in *A. cordifolia* [8]. In the present study, we aimed at better understanding how CAM plants respond to water deficit, and more particularly to what extent daily variations in PSII photochemistry, photoprotective compounds (tocopherols and carotenoids, including the xanthophyll cycle) and stress-related phytohormones (ABA and SA) may be influenced by short-term drought in the CAM plant, *A. cordifolia*.

2. Materials and methods

2.1. Plant material and growth conditions

Thirty plants of baby sun rose (*A. cordifolia*) were purchased in Verdflor, S.L. (Barcelona, Spain) on 28 June 2007. Plants, which were ∼20 cm tall and were grown in pots of 100 mL capacity containing a mixture of soil:peat:perlite (1:1:1, by vol.), were immediately transferred outdoors at the experimental fields of the Faculty of Biology of the University of Barcelona, where they acclimated to full sunlight for 11 days until the experiment started, on 9 July 2007. Then, two water regimes were imposed on plants: irrigated (IR) plants were kept watered twice a week with Hoagland's solution, while water-stressed (WS) plants were not watered at all for up to 15 days. During the experiment (9–24 July 2007), plants were exposed to the following climatic conditions (maximum daily photosynthetically active photon flux density [PPFD] of ∼2000 μmol m^{-2} s^{-1}, photoperiod of ∼15/9 h [day/night], and daily air temperature and relative humidity ranging 19–29 °C and 36–87%, respectively, Table 1). Environmental conditions were monitored by means of a weather station situated 100 m from plants and consisted of a PPFD pyranometer sensor CM11 (KIPP and ZONEN, Delft, The Netherlands) and a HMP35AC thermohygrometer (Vaisala, Helsinki, Finland).

Leaf hydration, chlorophyll fluorescence, photosynthetic pigments (chlorophylls and carotenoids, including the xanthophyll cycle), tocopherols, ABA and SA were measured in fully expanded young leaves on clear sunny days at pre-dawn (1 h before sunrise) at 0, 4, 10 and 15 days of experiment, and every 2–3 h at day 15 in both IR and WS plants. For measurement of photosynthetic pigments, tocopherols and phytohormones, leaves were collected, frozen in liquid nitrogen and stored at −80 °C until analysis.

2.2. Leaf hydration and chlorophyll fluorescence

Leaf hydration was determined as (FW − DW)/DW, where FW is the fresh weight and DW is the dry weight after oven-drying the leaves at 80 °C to constant weight. Chlorophyll fluorescence measurements were performed by using a mini-PAM (Walz, Effeltrich, Germany) as previously described [16]. The relative and maximum efficiencies of PSII photochemistry (ϕ_{PSII} and F_v/F_m, respectively) were calculated from chlorophyll fluorescence data obtained from leaves exposed to light during the day or

Table 1
Climatological conditions (maximum daily photosynthetically active photon flux density [PPFD], maximum and minimum daily air temperature, and maximum and minimum daily relative humidity), during the measurement days of the experiment; and PPFD, air temperature and relative humidity on sampling times (solar time) of 24 July.

Day of experiment	Days of water deficit	PPFD$_{max}$ (μmol m^{-2} s^{-1})	T_{max} (°C)	T_{min} (°C)	RH$_{max}$ (%)	RH$_{min}$ (%)
9 July	0	2086	28.6	21.1	87	36
13 July	4	1950	27.0	19.0	83	54
19 July	10	1872	28.3	21.9	84	52
24 July	15	1988	26.9	21.0	85	59

24 July	Time of day	PPFD (μmol m^{-2} s^{-1})	T (°C)	RH (%)
	2:00	0	22.3	83
	4:00	0	22.2	82
	6:00	268	23.8	75
	9:00	1540	24.8	63
	12:00	1988	26.0	61
	15:00	1316	25.4	67
	18:00	124	23.4	74
	20:00	0	22.5	79
	23:00	0	22.9	73