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Photosynthetic response of poikilochlorophyllous desiccation-tolerant *Pleurostima purpurea* (Velloziaceae) to dehydration and rehydration

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Abstract

The poikilochorophyllous, desiccation-tolerant (PDT) angiosperm, *Pleurostima purpurea,* normally occurs in less exposed rock faces and slightly shady sites. Our aim was to evaluate the light susceptibility of the photosynthetic apparatus during dehydration-rehydration cycle in *P. purpurea.* In a controlled environment, the potted plants were subjected to water deficit under two different photosynthetic photon flux densities [PPFD, 100 and 400 μmol(photon) m^{-2} s^{-1}]. In the higher PPFD, net photosynthetic rate (P_N) become undetectable after stomata closure but photochemical efficiency of photosystem II, electron transport rate, and photochemical quenching coefficient were maintained relatively high, despite a partial decrease. The photochemical activity was inhibited only after the complete loss of chlorophylls, when leaf relative water content dropped below 72% and total carotenoids reached maximal accumulation. Nonphotochemical energy dissipation increased earlier in response to dehydration under higher PPFD. P_N and photochemical activity were fully recovered after rehydration under both light treatments. Our results suggested that the natural occurrence of *P. purpurea* should not be restricted by the light intensity during the complete desiccation-rehydration cycles.

Additional key words: chlorophyll fluorescence; gas exchange; photoprotective mechanisms; vegetative desiccation tolerance.

Introduction

Vascular, desiccation-tolerant (DT) plants, also named poikilohydrics or resurrection plants, endure their vegetative tissue dehydration with losses of more than 90% of the relative water content (RWC) without irreversible injuries. Dehydration is usually followed by leaf folding and contraction together with color changes and rigidness (Meirelles *et al.* 1997). The desiccated tissues return to their normal appearance and physiology within hours or few days after rehydration, depending on the species (Sherwin and Farrant 1996, Tuba *et al.* 1998).

During the dehydration-rehydration cycle, cellular mechanisms of protection and repair are induced, conferring the DT phenotype. Accumulation of solutes, synthesis of compounds that maintain membranes and

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Abbreviations: ATP – adenosine triphosphate; Car – carotenoids; Chl – chlorophyll; CO_2 – carbon dioxide; DD – days of deficit; DR – days of rehydration; DT – desiccation tolerant; *E* – transpiration; ETR – electron transport rate through the PSII; F_m – maximum fluorescence of the dark-adapted sample; F_m' – maximum fluorescence of the light-adapted sample; F_s – steady fluorescence; F_v/F_m – maximum photochemical efficiency of PSII; F_v'/F_m' – intrinsic photochemical efficiency of PSII; F_0 – minimal fluorescence of the dark-adapted sample; F_0' – minimal fluorescence of the light-adapted sample; g_s – stomatal conductance; HDT – homoiochorophyllous desiccation tolerant; HR – hours of rehydration; IR100 – PPFD of 100 μmol m^{-2} s^{-1}; IR400 – PPFD of 400 μmol m^{-2} s^{-1}; IRGA – infrared gas analyzer; LCF – leaf chamber fluorometer; NPQ – Stern-Volmer nonphotochemical quenching; $^1O_2$ – superoxide radical; P_N – net photosynthetic rate; PCO – photorespiratory carbon oxidation; PDT – poikilochorophyllous desiccation tolerant; PPFD – photosynthetic photon flux density; PS – photosystem; PVC – polyvinyl chloride; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; ROS – reactive oxygen species; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP – ribulose-1,5-bisphosphate; RWC – relative water content; SD – standard deviation; $x + c$ – xanthophylls and β-carotenes; Φ_{PSII} – actual photochemical efficiency of PSII.
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macromolecules integrity, and activation of antioxidant systems are among the most studied, protective mechanisms against osmotic, mechanical, and oxidative stress during dehydration (Rascio and La Rocca 2005).

Light can become excessive during the water deficit. Stomata closure limits CO_2 diffusion and restricts photosynthetic use of absorbed light energy and it leads to the formation of reactive oxygen species (ROS) (Dinakar *et al.* 2012). Generation of ROS can damage oxygen-evolving complex of photosystem (PS) II and the PSII reaction centers and inhibit photosynthesis (Smirnoff 1993). DT angiosperms avoid the toxic build-up of ROS by controlling the metabolism and they cease photosynthesis during desiccation (Sherwin and Farrant 1998). For this purpose, the DT plants perform two different strategies to deal with light in the desiccated state: (*1*) homoiochlorophyllous (HDT) plants maintain chlorophyll (Chl) content stable without major changes in the chloroplast membrane systems; and (*2*) poikilochlorophyllous (PDT) plants dismantle the chloroplast membrane systems and loose completely leaf Chl during dehydration and they restore all systems during the rehydration phase (Hambler 1961, Farrant *et al.* 2003). Nevertheless, both groups show increased activity of antioxidant enzymes and anthocyanin accumulation (Sherwin and Farrant 1998).

Although contrasting, the HDT and PDT plants constitute different strategies how to avoid damaging light–Chl interaction in the desiccated state and minimize free radical formation (Tuba *et al.* 1998, Farrant *et al.* 2003). The HDT plants are normally found at shady sites where leaf folding in their dried state complements the necessary shading to protect Chl from light. In this group, the highly preserved chloroplasts in the dried state allow the quick recovery of photosynthesis when moisture becomes available again (Péli *et al.* 2012) and only short wet periods to grow starts. On the other hand, the profound modifications of the PDT chloroplasts during dehydration require longer recovery time after rehydration. This strategy is obligatory for the plants that must endure several months in the dried state under high irradiance (Sherwin and Farrant 1996, Tuba *et al.* 1998, Tuba and Lichtenthaler 2011).

In the HDT species, the ability to halt and recover photosynthetic activity during the dehydration–rehydration cycle was proved to be dependent on the light levels (Degl'Innocenti *et al.* 2008, Georgieva *et al.* 2008). On the other hand, the PDT strategy has been less explored, namely the effects of light upon the physiological responses during the dehydration-rehydration cycle. The main studies concerning the PDT species and their response to light were done with south and southwestern Africa species. They were focused mainly on various mechanisms, such as leaf movement, changes in protective pigments, and the activity of some antioxidant enzymes, which would minimize damage caused by excessive light (Sherwin and Farrant 1998, Farrant *et al.* 2003).

Dicotyledons and ferns are always the HDT plants; monocotyledons include both types of the DT strategies (Tuba and Lichtenthaler 2011). Most of the PDT plants are comprised of Velloziaceae species, but the group involves also Cyperaceae, Anthericaceae, and Poaceae species (Tuba and Lichtenthaler 2011). In Brazil, the PDT plants are frequent at rock outcrops with occurrences registered in the southeastern region (Meirelles *et al.* 1999), the northeastern "Caatinga" (dry tropical forest) (Gaff 1987), and the Midwest "Cerrado" (Brazilian neotropical savanna) (Meirelles *et al.* 1997).

P. purpurea is the PDT, monocotyledon plant, endemic at rock outcrops of the Rio de Janeiro State, Brazil (Meirelles *et al.* 1997). The aim of this work was to evaluate the susceptibility of the photosynthetic apparatus of *P. purpurea* to different light intensities during dehydration-rehydration cycle. Contrary to other PDT species found in similar areas, *P. purpurea* normally occurs at less exposed rock faces and slightly shady sites. This raised the question if the interaction between light intensity and desiccation could be determinant to restrict its sites of occurrence. Our basic hypothesis was that photosynthetic ability during the recovery phase is not limited by the light regime during the dehydration phase. To test this, Chl fluorescence and gas-exchange analysis together with water status and photosynthetic pigment quantification in leaf tissues were determined during dehydration and rehydration in intact plants under two different light intensities.

Materials and methods

Plant material and culture condition: Eight-year-old (mature), potted individuals of *P. pupurea* (Hook.) Raf. (Velloziaceae) were grown according to Aidar *et al.* (2010). The plants were transferred to a growth chamber (*Fitotron PGR15, Conviron*, Canada) under a day/night cycle of 12/12 h with a PPFD of 400 μmol m^{-2} s^{-1} (IR400) at the leaf level (using both cool white fluorescent and incandescent lamps), 25°C temperature and 55–65% of relative humidity. Pots were watered daily up to field capacity using tap water applied directly in the soil, eventually wetting the leaves, during 60 d before the water-deficit treatment started.

Leaf curling was expected during water stress, because it reduces irradiation reaching the leaf surface. To prevent this interference, transparent plastic tabs were carefully positioned on the new, completely expanded leaves, aiming to maintain them fully opened and directly exposed to the light source (with the aid of rigid wires inserted at the edges of the pots). The tabs were constructed with transparent semirigid stripes of polyvinyl chloride (PVC) (0.3 mm thick), of 15 × 5 cm with holes of 6 mm in a diameter along the extension. At the leaf surface, the incident PPFD was reduced by covering the transparent tab with a screen of black nylon (75%). This resulted in values

of around 100 μmol m^{-2} s^{-1} of PPFD (IR100) at the upper leaf level, while the tabs without cover allowed IR400 at the leaf surface. All plastic tabs were installed 15 d before measurements started to allow leaf acclimation to the specific light intensity.

At the beginning, all the measurements were carried out on the leaves not detached of the intact plants under a fully hydrated condition (control) throughout dehydration and the rehydration phase. The water deficit was imposed by suspension of irrigation for 54 d (days of deficit, DD), because the PDT species are frequently subjected to drought periods of several months. This period was long enough to expose the plants substantially to the light regime in order to evaluate the effects during the desiccated state. This period was compatible with the drought periods experienced by *P. pupurea* in its natural habitats. Then, the rehydration phase started with daily watering up to field capacity by using tap water applied directly to the soil, eventually wetting the leaves. The rehydration treatment was carried out under continuous light during the first 24 h. Following this treatment, the standard, 12/12 h of light/dark photoperiod was maintained, the same cycle as at the beginning of the experiment, for 8 days.

Gas exchange and Chl fluorescence: Net photosynthetic rate (P_N), transpiration (E) and stomatal conductance (g_s) were measured with an infrared gas analyzer *IRGA* (*Portable Photosynthesis System LI-6400, LI-COR Inc.*, Lincoln, NE, USA) and calculated according to von Caemmerer and Farquhar (1981). Chl fluorescence induction kinetics was performed with a Leaf Chamber Fluorometer (*LI-6400-40, LI-COR Inc., Lincoln,* NE, USA) coupled to the IRGA. To simulate predawn conditions, minimal fluorescence of the dark-adapted sample (F_0) was always determined before the beginning of the light period. Then maximal fluorescence (F_m) was measured using saturation pulse (5,000 μmol m^{-2} s^{-1} PPFD, 0.8 s duration) and maximal quantum yield of PSII (F_v/F_m) was calculated as $(F_m - F_0)/F_m$ (Kitajima and Butler 1975).

The measurements of gas exchange and Chl *a* fluorescence in samples adapted to light were conducted always 2 h after the beginning of the light period on the same leaves sampled in the predawn measurements. Gas exchange was measured under steady-state conditions after positioning the leaf in the *IRGA* chamber adjusted previously with the same PPFD of the leaf light treatment (IR400 or IR100). The steady-state fluorescence (F_s) was measured under the continuous irradiation, followed by the application of a 2nd saturation pulse for 0.8 s to determine the maximum fluorescence (F_m') of the light-adapted sample. After the actinic light was turned off and the minimal fluorescence of the light-adapted sample (F_0') was determined, the leaf tissue was exposed to far red light of 740 nm for 3 s. The fluorescence parameters were used to calculate the intrinsic and effective quantum yield (F_v'/F_m' and Φ_{PSII}, respectively), electron transfer rate (ETR) (Genty *et al.* 1989), photochemical quenching (q_P) (Kooten and Snel 1990), Stern-Volmer-type nonphotochemical quenching (NPQ) (Bilger and Björkman 1990), and nonphotochemical quenching coefficient (q_N) (Kooten and Snel 1990).

Leaf and plant-soil relative water content: The leaf relative water content (RWC) was measured in detached leaves, other than those used for gas-exchange measurements. The leaves of similar rosette position and radiation exposure (IR400) were collected immediately after gas-exchange and fluorescence measurements. Sections of about 2 cm length were excised from the middle part of the leaf blade. Thereafter, the leaf sections were cut along the midrib resulting in two symmetric half pieces. One of them was used to estimate RWC; another was used for pigment extraction. Leaf RWC and plant-soil RWC were calculated according Turner (1981). These variables were estimated as the mass of the fully watered leaf piece or plant-soil unit (TM – saturated, "turgid" mass), in relation to the instantaneous mass attained for these samples throughout the experiment (FM – fresh mass) and to the lowest constant mass attained for these samples after water withdrawal (DM – dry mass). The RWC was calculated according Turner (1981) as $[(FM - DM)/(TM - DM)] \times 100$. Leaf instantaneous FM was taken before saturation. The saturation state was attained by floating leaf piece on distilled water. Leaf DM was measured after oven-drying overnight at 80°C. The plant-soil RWC was only the approximate value of the soil water status to have the reference of water lost during the dehydration process. The fully watered, plant-soil mass was taken after dipping the potted plant in water for 3 h and draining for 6 h. The instantaneous mass during the dehydration treatment was considered the FM of the potted plant. The minimum plant-soil DM was the lowest constant mass attained after water withdrawal.

Pigment extraction and quantification: Pigments were extracted from leaf sections in ethanol (96%). The leaf samples, collected immediately after gas-exchange and fluorescence measurements, were ground with pestle and mortar with some ethanol, then the remaining material and extracted pigments were suspended to 10 mL with the same ethanol used in the extraction. The suspension was centrifuged (3,000 rpm- for 300 s) and the pellet discarded. The absorbance of the supernatant was determined spectrophotometrically at 665, 649, 470 nm and used, respectively, to calculate chlorophyll (Chl) *a* and Chl *b*, and carotenoids (Car) concentration (including xanthophylls and β-carotenes, $x + c$), according to Lichtenthaler and Wellburn (1983). Total Chl ($a + b$) was obtained by the sum of Chl *a* and Chl *b*. The concentration was expressed in [μg g^{-1}(DM)]. All procedures for determination of photosynthetic pigments followed Aidar *et al.* (2010).

Experimental design and statistical analysis: The experiments were carried out in a completely randomized design. Four tabs, two transparent and two shaded, were allocated on four different leaves of each plant, in total of three plants (replications) per treatment. For the leaf gas exchanges and Chl *a* fluorescence, 6 replications were used under each irradiance. The other evaluations included only 3 replications (1 leaf per plant). Means and standard deviation (SD) were determined and compared by the analysis of variance (*ANOVA*) procedure using the *Tukey*'s test for multiple comparisons. A 5% of significance (p<0.05) level was used as statistically significant difference for result interpretation.

Results

The plant-soil RWC decreased promptly after ceasing watering. Leaf RWC values were higher than 70% until the plant-soil RWC reached 25% (10 DD; Fig. 1*A*). During 3 additional DD, the leaf RWC decreased to 16% (Fig. 1*A*). After 55 DD, leaf RWC reached 6% (Fig. 1*B*). After 12 h of rehydration (HR), the plant-soil RWC reached values near those of the control conditions (Fig. 2*B*).

During the progress of dehydration, Chl *a* and Chl *b* were maintained stable up to 10 DD (Fig. 2*A*). At this point, the leaves were partially rolled downwards along the

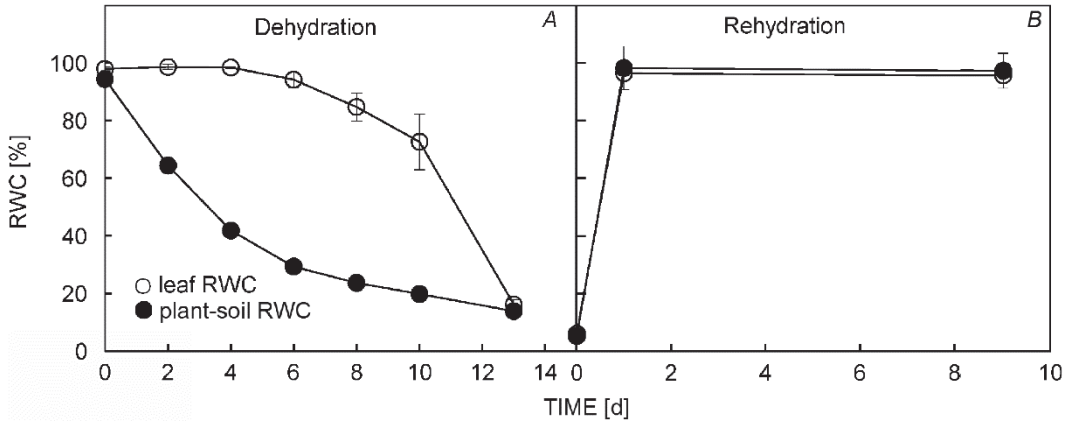

Fig. 1. Time progression of relative water content (RWC) in plant-soil (*solid circles*) and leaf pieces (*open circles*) of *P. purpurea* during dehydration (*A*) and rehydration (*B*) phases. Means ± SD, n = 3.

midrib. After this stage, the leaves turned yellow with the complete loss of Chl. It coincided with the period of the highest leaf dehydration and strong leaf rolling. The Car content increased about 7 times (Fig. 2*C*) during the entire dehydration period.

After 24 HR of rehydration (DR), leaves recovered *ca*. 64% of the control Chl content (Fig. 2*B*). Complete recovery of Chl (*a*+*b*) content was reached after 9 days (Table 1). During the same period, Car decreased progressively, but maintained the concentration about 3 times larger compared with the control conditions (Fig. 2*D*, Table 1).

In the control condition, the P_N average was 8.7 μmol m^{-2} s^{-1} under the IR400, a value 6 times larger than that observed under IR100 (Fig. 3*A*). After water withdrawal, P_N, g_s, and E showed a partial decrease even when the leaf RWC was still 98%, while plant-soil RWC was 42% (Fig. 1*A*). When leaf and plant-soil RWC reached 94% and 29%, respectively, P_N became slightly negative. Then, P_N was maintained around zero (Fig. 3*A*) up to the maximum dehydration of leaf tissues (6% of RWC) (Fig. 1*A*). After 12 HR under continuous light (1st day), P_N became initially negative (Fig. 3*B*), corresponding to a respiration phase, even with values of g_s and E around zero (Fig. 3*D*). Within 24 HR, P_N became positive (Fig. 3*B*) with a low increase in g_s (Fig. 3*D*) and E (Fig. 3*F*). The maximum recovery of P_N (Fig. 3*B*), g_s (Fig. 3*D*), and E (Fig. 3*F*) was observed on 9 DR.

The maximum (F_v/F_m, Fig. 4*A*), intrinsic (F_v'/F_m', Fig. 4*C*), and actual (Φ_{PSII}, Fig. 4*E*) photochemical efficiencies of PSII, and the electron transport rate (ETR) (Fig. 4*G*) showed a dual phase pattern of decreasing before the establishment of apparently anabiotic state.

After suspension of irrigation, these parameters were maintained high as long as any P_N occurred. With P_N cessation on 6 DD, F_v'/F_m', Φ_{PSII} and ETR decreased only partially and established a new phase of stable values that were maintained until 10 DD. This decrease was more pronounced under IR400.

F_v/F_m decrease started first under IR400. All the fluorescence parameters decreased to zero after 10 DD. It was accompanied by the complete loss of Chl.

Table 1. Comparisons of physiological parameters of *P. purpurea* before suspension of water supply (control) and after recovery from desiccation (9 d of rehydration) under photosynthetic photon flux densities (PPFD) of 400 μmol m^{-2} s^{-1} and 100 μmol m^{-2} s^{-1}. Means ± SD followed by *different superscript letter* in the same row are significantly different (p<0.05). RWC – relative water content; Chl – chlorophyll; Car – carotenoids; P_N – net photosynthetic rate; g_s – stomatal conductance; E – transpiration; F_v/F_m – maximum photochemical efficiency of PSII; F_v'/F_m' – intrinsic photochemical efficiency of PSII; Φ_{PSII} – actual photochemical efficiency of PSII; q_P – photochemical quenching coefficient; q_N – nonphotochemical quenching coefficient; NPQ – Stern-Volmer nonphotochemical quenching; ETR – electron transport rate through the PSII.

PPFD		Control	After recovery	% of recovery
400 [μmol m^{-2} s^{-1}]	Leaf RWC [%]	97.96 ± 0.7[a]	95.45 ± 1.03[b]	97.48 ± 0.9
	Chl (*a* + *b*) [μg g^{-1}]	1537.23 ± 111.25[a]	1506.42 ± 266.7[a]	98 ± 19.6
	Car [μg g^{-1}]	31.07 ± 2.42[a]	81.85 ± 27.45[b]	263.42 ± 110.8
	P_N [μmol(CO_2) m^{-2} s^{-1}]	8.57 ± 2.596[a]	8.2 ± 1.296[a]	95.68 ± 9.1
	g_s [mol(H_2O) m^{-2} s^{-1}]	0.147 ± 0.083[a]	0.169 ± 0.073[a]	114.56 ± 118
	E [mmol(H_2O) m^{-2} s^{-1}]	2.08 ± 1.05[a]	2.48 ± 0.98[a]	119.34 ± 98.5
	F_v/F_m	0.81 ± 0.012[a]	0.83 ± 0.011[b]	102.09 ± 2.9
	F_v'/F_m'	0.57 ± 0.08[a]	0.65 ± 0.067[a]	113.39 ± 21.7
	Φ_{PSII}	0.27 ± 0.042[a]	0.33 ± 0.058[a]	122.89 ± 39.8
	q_P	0.47 ± 0.032[a]	0.52 ± 0.106[a]	109.13 ± 29.8
	q_N	0.62 ± 0.244[a]	0.42 ± 0.197[a]	68.54 ± 40.8
	NPQ	1.4 ± 1.05[a]	0.59 ± 0.47[a]	42.07 ± 62.9
	ETR [μmol m^{-2} s^{-1}]	46.15 ± 7.23[a]	56.74 ± 9.79[a]	122.93 ± 39.8
100 [μmol m^{-2} s^{-1}]	P_N [μmol(CO_2) m^{-2} s^{-1}]	0.93 ± 0.541[a]	2.16 ± 0.648[b]	231.42 ± 286.9
	g_s [mol(H_2O) m^{-2} s^{-1}]	0.009 ± 0.003[a]	0.047 ± 0.025[b]	549.71 ± 262
	E [mmol(H_2O) m^{-2} s^{-1}]	0.14 ± 0.05[a]	0.75 ± 0.39[b]	513.43 ± 235.4
	F_v/F_m	0.84 ± 0.012[a]	0.83 ± 0.005[a]	99.23 ± 1.6
	F_v'/F_m'	0.62 ± 0.032[a]	0.68 ± 0.028[b]	109.68 ± 6.6
	Φ_{PSII}	0.54 ± 0.034[a]	0.55 ± 0.041[a]	101.9 ± 8.9
	q_P	0.86 ± 0.036[a]	0.8 ± 0.044[b]	92.89 ± 6.9
	q_N	0.67 ± 0.072[a]	0.46 ± 0.114[b]	69.65 ± 16.9
	NPQ	1.31 ± 0.38[a]	0.63 ± 0.261[b]	48.52 ± 22.9
	ETR [μmol m^{-2} s^{-1}]	22.95 ± 1.47[a]	23.3 ± 1.8[a]	101.54 ± 9.1

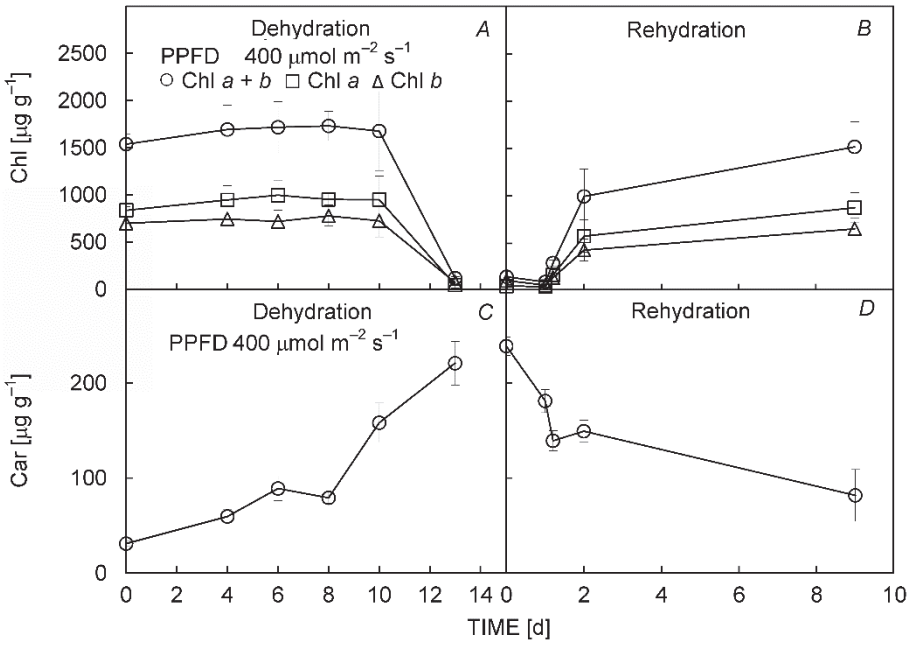

Fig. 2. Chlorophyll (Chl) *a*, Chl *b*, and Chl (*a*+*b*) (*A,B*) and carotenoids (*C,D*) concentration in leaf pieces of *P. purpurea* during dehydration (*A,C*) and rehydration (*B,D*) phases under PPFD of 400 μmol m^{-2} s^{-1}. Means ± SD, n = 3.

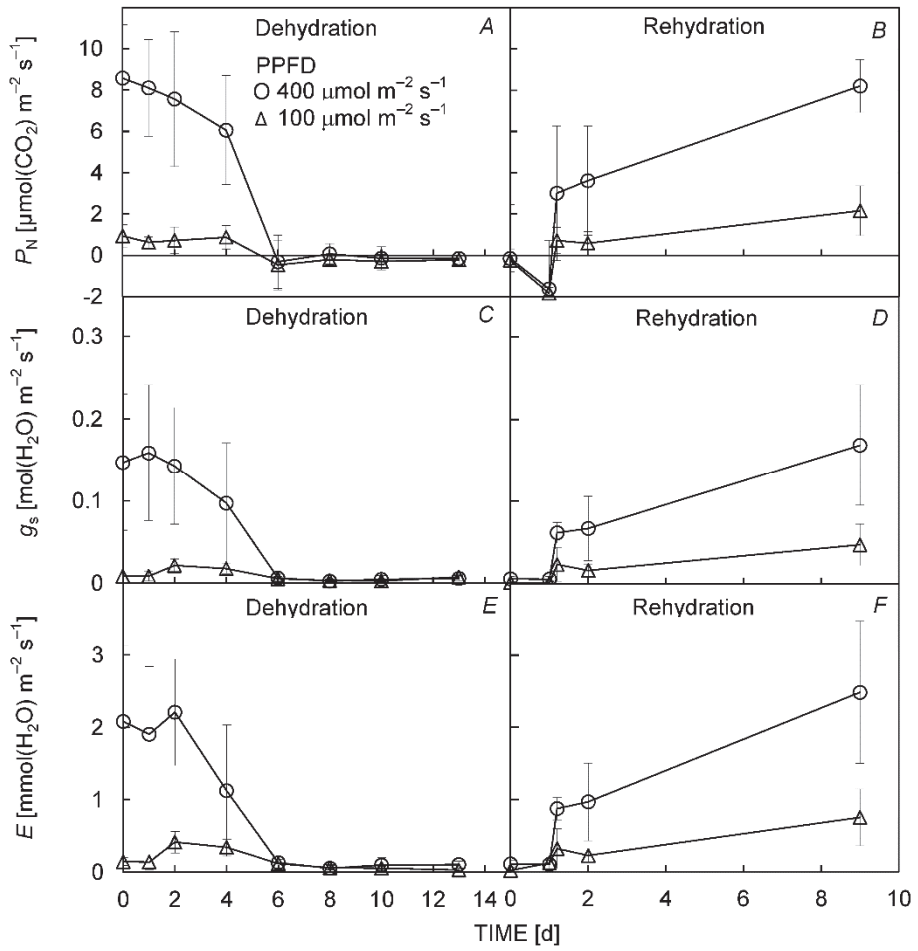

Fig. 3. Net photosynthetic rate (P_N) (*A,B*), stomatal conductance (g_s) (*C,D*), and transpiration rate (*E*) (*E,F*) during the progress of dehydration (*A,C,E*) and rehydration (*B,D,F*) in *P. purpurea* under PPFD of 400 μmol m^{-2} s^{-1} (○) and 100 μmol m^{-2} s^{-1} (Δ). Means ± SD, n = 6.

With rehydration, F_v/F_m (Fig. 4*B*), Φ_{PSII} (Fig. 4*D*), F_v'/F_m' (Fig. 4*F*), and ETR (Fig. 4*H*) increased significantly within the first 24 h and, after nine days, all parameters achieved complete recovery compared with the control conditions (Table 1).

The q_P (Fig. 5*A*) decreased, while the q_N (Fig. 5*C*) and NPQ (Fig. 5*E*) increased during dehydration, all of them in the dual transition phase as observed for the quantum efficiency parameters (Fig. 4). On 13 DD, some q_P and q_N replication values changed abruptly, clearly due to computation of higher values of basal fluorescence compared to maximum fluorescence under very low Chl content, which has no physiological significance. This effect did not occur with NPQ, which approximated zero after complete dehydration.

After 9 DR, q_P recovered completely (Fig. 5*B*), but q_N (Fig. 5*D*) and NPQ (Fig. 5*F*) became lower than those observed in the control under fully hydrated condition under both light treatments.

Discussion

The general pattern of leaf gas exchange during desiccation-rehydration cycle was generally in agreement with earlier reports on the same and others PDT species (Meguro *et al.* 1977, Tuba *et al.* 1994, Aidar *et al.* 2010). Restrictions of g_s seemed to be the principal cause for the initial decrease in P_N during progressive dehydration of *P. purpurea*. This was caused probably due to limited gas diffusion to the intercellular spaces as some authors also suggest for HDT species (Schwab *et al.* 1989, Peeva and Cornic 2009). Concomitantly, early decrease of F_v'/F_m' under the higher PPFD and under partial stomata closure was associated with the increase of nonphotochemical quenching, indicating the decrease in the sink capacity for electrons. Under conditions of low CO_2 assimilation, light becomes excessive causing an excess of protons in the lumen and activating nonphotochemical quenching as heat dissipation by xantophyll cycle (Saccardy *et al.* 1998). Despite the enhanced nonphotochemical quenching in the

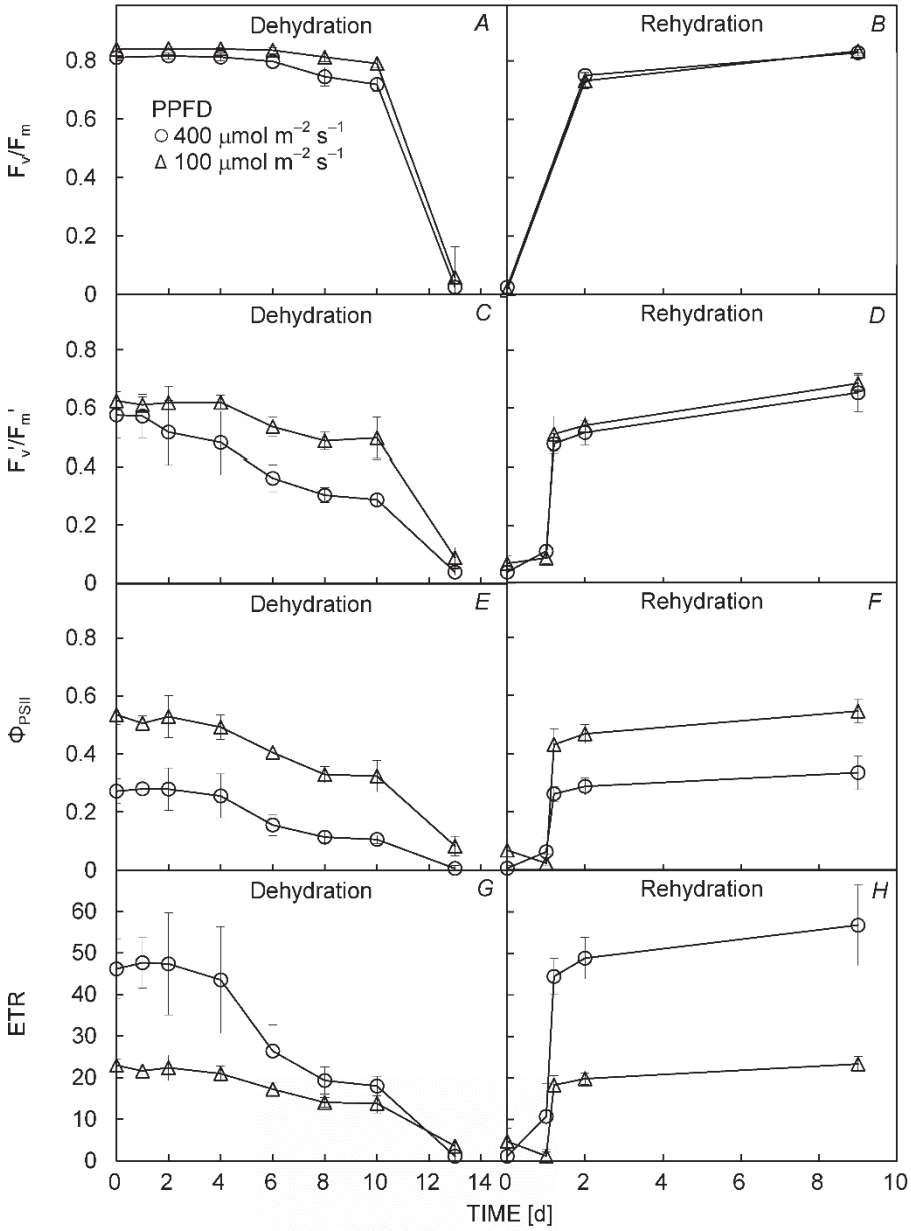

Fig. 4. Maximum quantum yield of the PSII photochemistry (F_v/F_m) (*A,B*), intrinsic quantum yield of the light-adapted state (F_v'/F_m') (*C,D*), actual quantum yield of PSII photochemistry (Φ_{PSII}) (*E,F*), and electron transport rate (ETR) (*G,H*) during the progress of dehydration (*A,C,E,G*) and rehydration (*B,D,F,H*) in *P. purpurea* under PPFD of 400 μmol m^{-2} s^{-1} (○) and 100 μmol m^{-2} s^{-1} (Δ). Means ± SD, $n = 6$.

light, high F_v/F_m measured in predawn conditions indicated the reversibility of the heat dissipation processes and the effective protection against photoinhibition during the first days of irrigation ceasing.

After complete stomata closure, low intercellular concentration of CO_2 limited carboxylation by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and, consequently, the regeneration of ribulose-1,5-bisphosphate (RuBP). Since the decrease in the RuBP regeneration can limit the operation of Calvin cycle (Medrano *et al.* 2002), it is expected a decrease in the proportion of opened PSII reaction centers as a consequence of an impediment to the flow of electrons from PSII to PSI. This was confirmed by partial decrease of q_P and ETR, respectively. However, q_P sustained relatively high after stomata closure. Probably, this could be explained by reassimilation of the CO_2 lost by respiration, at least until the 10 DD. This could be supported by the maintenance of Φ_{PSII} values that indicate the proportion of absorbed energy used in photochemistry and correlate with the quantum yield of CO_2 (Fryer *et al.* 1998). However, under CO_2 limitation, the photorespiratory carbon oxidation (PCO) cycle must be also involved in the maintenance of electron flow (Lawlor and Cornic 2002). Besides this, alternative electron flow, such as cyclic electron flow around PSI and water-water reactions, can also occur as additional ways to prevent oxidative stress during dehydration caused by excessive light (Endo and Asada 2002). The high resistance of electron transport chain in thylakoids was consistent with papers reporting the electron transport insensitive to a 50% decrease in the

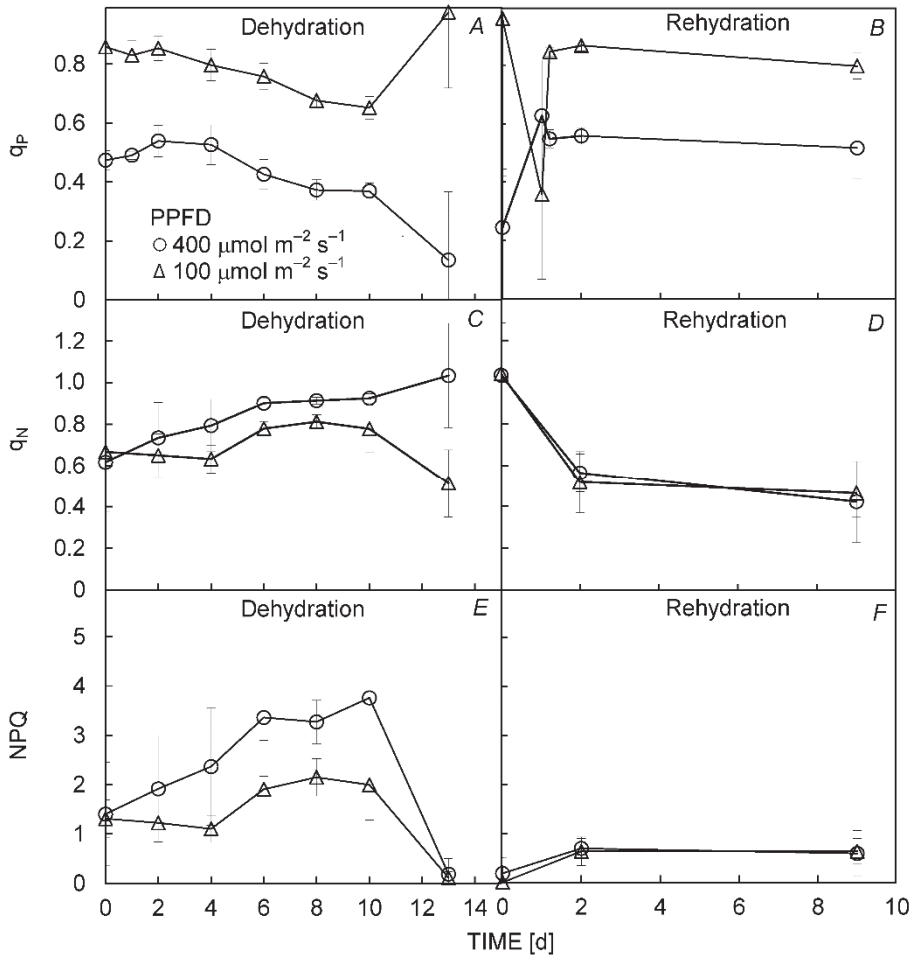

Fig. 5. Photochemical quenching coefficient (q_P) (*A,B*), nonphotochemical quenching coefficient (q_N) (*C,D),* and Stern-Volmer nonphotochemical quenching (*E,F*) during the progress of dehydration (*A,C,E*) and rehydration (*B,D,F*) in *P. purpurea* under PPFD of 400 μmol m^{-2} s^{-1} (○) and 100 μmol m^{-2} s^{-1} (Δ). Means ± SD, n = 6.

leaf RWC (Cornic *et al.* 1992, Lawlor and Cornic 2002). Studies on resurrection plants also suggested that Calvin cycle enzymes are more affected by dehydration than membrane-bound, electron transport reactions (Schwab *et al.* 1989, Georgieva *et al.* 2007).

Photochemistry maintained for an extended time after stomata closure enables rapid P_N recovery if water becomes available and stomata reopen. Obviously, if water deficit persists, components of the photosynthetic apparatus can be damaged. It was verified by the decrease of predawn F_v/F_m early under the high PPFD, indicating the occurrence of chronic photoinhibition (Osmond 1994). When potted, *P. purpurea* usually showed a lower rate of dehydration than others sympatric PDT species, such as *Vellozia candida* (Velloziaceae) (under similar contidions of body size, soil volume, and climatic condition). This could be possible probably due to a thicker wax layer on *P. purpurea* leaves, or lower g_s, or both, compared with *V. candida*. Consequently, *P. purpurea* has higher probability to benefit from next rainfall and recover from the incomplete desiccation faster than *V. candida*. This implies that *P. purpurea* kept the metabolism active longer after stomata closure during dehydration, although it predisposes the leaves to be damaged by light if the Chl content is maintained. Thus, the higher irradiance the plants are exposed to, the higher probability of photooxidative damage under conditions of incomplete desiccation. As photooxidation of photosynthetic apparatus can decrease the efficiency of carbon gain, its repetitive occurrence during incomplete dehydration-rehydration cycles could be one of the reasons why *P. purpurea* is excluded from more light-exposed sites.

The plant can avoid dehydration with low g_s only if different mechanisms can be induced for the desiccation tolerance. Thus, the transiently negative P_N after stomata closure is consistent during dehydration with the process called desiccation respiration and it can be explained by an effective rise in respiration rate according to Tuba *et al.* (1997). These authors suggest that this phenomenon is related to energy supply to control disassembly of the internal membrane structures in PDT species. However, as detected immediately after stomata closure, desiccation respiration also starts providing energy to induce desiccation tolerance mechanisms.

Chl loss and dismantling photosynthetic apparatus seems to be the last process to prepare tolerant leaf tissues for desiccation. *P. purpurea* occurs naturally at the sites subjected to periods of water deficit for several months. Thus, the complete loss of Chl during dehydration is important to prevent its interaction with light and

photooxidative damage in desiccated leaf tissues. In general, PDT plants are found in intermittently arid habitats under high irradiances (Tuba and Lichtenthaler 2011). Thus, the advantage of Chl loss to prevent pigment-light interaction in the desiccated state is consistent with the needs of DT plants to colonize more exposed sites in rock outcrops (Sherwin and Farrant 1996). On the other hand, HDT plants retain most of their photosynthetic apparatus and they usually survive relatively shorter drought periods in the desiccated state (Tuba and Lichtenthaler 2011). In this case, light may cause a cumulative damage in the dried green leaves decreasing gradually the ability to recover after long drought periods. Therefore, HDT plants are found in more shady habitats than PDT species. Concluding, Chl loss in PDT plants is important for the maintenance of leaf tissues viability in the desiccated state in long-term, preventing accumulative photooxidation.

During rehydration, all components of photosynthetic apparatus together with Chl must be renewed for the reconstitution of functional PSII antennae and reaction centers in PDT species (Pérez *et al.* 2011). Any damage caused by light during dehydration phase must be repaired to recover fully the photosynthetic apparatus during rehydration. Our experiments proved that higher irradiance, and therefore more intense photoinhibition, during dehydration phase did not affect the ability to recover P_N during rehydration. On the other hand, q_P and ETR recovered more rapidly under higher PPFD during 24 h of rehydration, probably due to a higher rate of Chl resynthesis under such conditions. These results indicate that PDT mechanism is effective in light protection and recovery of *P. purpurea* from desiccation.

Progressive accumulation of Car during dehydration indicated their protective role against light damage in leaf tissues of *P. purpurea*. These pigments can absorb excessive light instead of Chl and act as antioxidants preventing the formation of superoxide radical (Smirnoff 1993). Contrary to our present results, Car content remained constant despite dehydration-rehydration under direct sunlight (PPFD of 1,600 μmol m^{-2} s^{-1}) in our previous work (Aidar *et al.* 2010). It seems that Car content in *P. purpurea* depends on the PPFD under which the plants grow. However, when they grow under PPFD below a certain limit, the Car content can be regulated by dehydration. In others PDT species studied, Car also did not increase during dehydration (Sherwin and Farrant 1998, Farrant *et al.* 2003). This might be associated with growth conditions or with the presence of other protective pigments.

Interestingly, while Car content did not decrease to the control values after several days of rehydration, q_N and NPQ became comparatively lower suggesting acclimation. As Car are quenching triplet-state Chl, maintaining their high content could be important even after rehydration phase due to safe recovery of Chl content until the steady state is established.

Despite the susceptibility of photosynthesis to photoinhibition during dehydration, two different light intensities did not affect the ability of *P. purpurea* to recover normal photosynthetic activity after rehydration. PDT poikilochlorophylly strategy is important both as the way to prevent light-Chl interaction in the desiccated state for a long period, as well as to renew the photosynthetic apparatus during rehydration regardless of photoinhibition suffered during dehydration. Further research is needed for a detailed characterization of the exact mechanisms involved in light protection of *P. purpurea*.

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