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Drought resistance and recovery of photosystem II activity in a Mediterranean semi-deciduous shrub at the seedling stage

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Abstract

Phlomis fruticosa is one of the main species of post-fire Mediterranean communities, occupying areas with post-fire grazing or altered precipitation pattern and consequently water stress imposed on seedlings of co-occurring species. Seedlings of woody perennials often differ from their mature individuals regarding their resistance of photosynthetic performance to environmental stresses. Such differences have been reported for tree species but there is a lack of information regarding shrub species. In the present study, we tried to detect changes in (PSII) activity imposed by water stress in *P. fruticosa* seedlings as well as its capability for recovery after rehydration. Maximum PSII photochemical efficiency decreased only under severe water stress exactly as in mature plants in the field. However, leaf chlorophyll (Chl) content was almost stable regardless of leaf relative water content (RWC). We assume that the photoprotective chlorophyll loss process, reported for many mature Mediterranean species (including *P. fruticosa*), has not yet been developed at the seedling stage. On the other hand, photoprotection through an increase of the relative amount of non-Q_B-reducing centers was found during dehydration. Non-photochemical quenching (NPQ) contributed to protection from photodamage until moderate water stress but was significantly suppressed under severe water stress. Both processes were reversed after rehydration. The above characteristics enabled seedlings not only to survive during aggravating drought but also to maintain a considerable part of their effective quantum yield and perform significant electron transport even at extremely low relative water content (RWC). This was confirmed with measurements in a semi-natural environment (pots) and under real steady state conditions regarding adaptation of the photosynthetic machinery to prevailing light intensities.

Additional key words: non-Q_B-reducing centers; PSII quantum yield; recovery; seedlings; semi-deciduous shrub, steady state conditions; water stress.

Introduction

Photosynthesis is one of the most important physiological processes inhibited by water deficit (Kaiser 1987). Decrease of CO_2 assimilation rate has been attributed either to limited CO_2 diffusion to the intercellular spaces of the leaf because of stomatal closure or to impaired biochemical processes induced by loss of cellular water (Lawlor 1995). However, photosynthetic apparatus appears to be very resistant to drought (Giardi *et al.* 1996, Epron 1997, Bukhov and Carpentier 2004). Plant species exhibit different ranges of water contents within which photosynthetic apparatus can function effectively (Zunzunegui *et al.* 1999, Nogues and Baker 2000, Bukhov and Carpentier 2004, Yang *et al.* 2007). Poikilohydric plants such as green and red algae, lichens and mosses as well as a few higher plants, so-called “resurrection plants”, retain their photosynthetic activity under a wide range of internal water content. They often tolerate even complete desiccation (Scott 2000, Proctor and Tuba 2002). On the other hand, the majority of higher plants are drought sensitive plants which cannot survive under prolonged or/and severed water stress. These species close their stomata under moderate water stress (RWC $\geq$ 70 %) and thus suffer reduction of their normal photosynthetic rates. Under prolonged or severe water stress (RWC < 70 %), mesophyll metabolism is affected and decline in photosynthetic capacity has been found, while occasionally electron transfer seems to be affected as a response to coexisting heat or high light

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Abbreviations: ETR – apparent linear electron transport rate; F_0 and F_m – minimum and maximum fluorescence; F and F_m' – steady state and maximum fluorescence in light adapted state; F_i – fluorescence of the intermediate plateau in the fast induction phase; F_v/F_m – maximum PSII photochemical efficiency; $\Delta F/F_m'$ – PSII photochemical activity in the light adapted state; NPQ – non-photochemical quenching; PSII – photosystem II; PPFD – photosynthetically active photon flux density; Q_A and Q_B – quinones A and B; RWC – relative water content.

stress (Giardi *et al.* 1996). Recovery of photosynthetic activity when plants experience leaf water contents below 40 % is rare.

In the present work we investigated PSII resistance to water stress in seedlings of the malacophyllous, dimorphic, semi-deciduous Mediterranean shrub *Phlomis fruticosa* L. Overall photosynthesis (gas exchange measurements), as well as ecophysiology of mature plants of *P. fruticosa* have been thoroughly investigated in our laboratory and a big repertoire of adaptations (morphological, biochemical and physiological) against adverse Mediterranean summer has been found (Grammatikopoulos and Manetas 1994, Grammatikopoulos *et al.* 1995, Kyparissis *et al.* 1995, Kyparissis *et al.* 1997, Grammatikopoulos 1999, Karavatas and Manetas 1999, Kyparissis *et al.* 2000). However, seedlings and young plants are expected to be more sensitive to water stress than their mature individuals (Fenner 1987, Padilla and Pugnaire 2007, Fotelli *et al.* 2000). Besides, drought has been found to be responsible for 35 % of mortality in seedlings of at least 20 studied species (Moles and Westoby 2004). More specifically, photosynthetic resistance to water stress differs between seedlings and mature individuals in many woody perennials (Bond 2000).

P. fruticosa is one of the main species of post-fire communities in Mediterranean ecosystems and regenerates both vegetatively and by seed germination (Arianoursou-Faraggitaki and Margaris 1981). In burned sites, grazed by herbivorous mammals, most of the seedlings of the other post-fire succession species are eliminated, forest re-establishment is interrupted and *P. fruticosa* dominates vast areas. However, even in protected from grazing regions, the normal post-fire succession of species is strongly dependent on water use efficiency of co-occurring seedlings (Archibold 1995). As an effect of climate change, the onset of summer drought occurs earlier and is prolonged until mid-autumn, leading to increased water stress in many of the different Mediterranean ecosystems. Consequently, differences of drought resistance range between species at the seedling stage could be critical for the community composition.

We address two main questions. First, if photosynthetic machinery of *P. fruticosa* seedlings is sensitive to water stress, what is the critical level for substantial reduction in photochemical yield and photosynthetic rates? And second, are reductions and/or impairments of photosynthetic apparatus reversible and to what extent? For this, we took advantage of our easily manipulated population of young plants growing in pots under field conditions. PSII function and characteristics were revealed from Chl fluorescence measurements derived from hundreds of leaves under really steady state light conditions.

Materials and methods

Experimental site and plants: Seeds of the Mediterranean semi-deciduous shrub *Phlomis fruticosa* L. were collected during summer from several plants growing in the field around the university campus. Seeds were stored for 3 months under dry conditions in the lab. At mid-October they were thoroughly washed and germinated in Petri-dishes in a growth chamber (12 h photoperiod, T = 15 °C, light intensity 25–30 μmol m^{-2} s^{-1}). 300 young seedlings (1–2 cm height) were transplanted in small pots and grew with almost full sunlight in a glasshouse for three months during winter. 150 seedlings similar in size, were finally transplanted in larger pots (25 cm depth, 20 cm in diameter) filled with soil collected from their natural habitat and placed under natural environmental conditions in the vicinity of the glasshouse and the laboratory. Plants were acclimated 3 months prior to treatments and measurements commencement. Uniform young plants were arranged in 3 groups according to height, leaf number and leaf area measurements (data not shown) in order to receive 3 regimes of irrigation (*ww*: well watered, *ms*: mild water stress, and *ss*: severe water stress). Adjustment of frequency and amount of irrigations among treatments was based on leaf RWC measurements monitored every week for each group of seedlings. Care was taken for control plants (*ww*) to ensure that they maintained relatively constant leaf RWC throughout the experiment. Measurements were made on days 7, 31 and 56 after the onset of different irrigation treatments. Recovery was measured 4 days (60th day of the experiment) after plants of drought treatments (*ms* and *ss*) were re-watered on a daily basis with the water amount used for control plants.

Relative water content (RWC) was calculated with the leaf disc floating method according to Turner (1981) using the formula RWC = (FM–DM) × 100/ (SM–DM), where FM is fresh mass, DM is dry mass and SM is saturated mass. 8–10 discs about 1 cm in diameter, were punched from 8–10 leaves (one leaf per plant, randomly sampled among 50 plants of each treatment) placed in a tiny sealed plastic bag and was immediately transferred to the lab (in about 1–2 min). FM was measured and then discs floated on distilled water in covered Petri dishes for 18 h to become fully turgid (Kyparissis and Manetas 1993). Saturated water mass was measured and discs were then oven dried at 80 °C for about 24 h. Finally their DM was measured.

Chl content: For Chl content estimation, a portable Chl meter (*SPAD 502, Minolta*, Osaka, Japan) was used (Manetas *et al.* 1998). Calibration curves of SPAD values versus actual Chl concentrations measured spectrophotometrically (*Shimadzu UV-160A* double beam spectrophotometer with 2 nm slit-width, *Shimadzu Scientific*

Instruments, Columbia, USA) were constructed. Concentration was estimated according to the equations of Lichthenthaler and Wellburn (1983).

Chl fluorescence was measured with a pulse-amplitude modulated fluorometer (*MINI-PAM, Walz*, Effeltrich, Germany) connected to a leaf clip (*Leaf-Clip Holder 2030-B*). The PSII photochemical efficiency in the light-adapted state [$\Delta F/F_m' = (F_m' - F)/F_m'$] was calculated according to Genty *et al.* (1989). The maximum (dark-adapted) PSII photochemical efficiency [$F_v/F_m = (F_m - F_0)/F_m$] was measured at predawn (about 8 h dark period) with the same instrument and with the same settings (gain, measuring light intensity). F_0 and F are fluorescence yields of dark and light-adapted samples before the application of a saturating light pulse. F_m and F_m' are maximum fluorescence yields of dark and light-adapted samples during the application of a saturating light pulse. The apparent linear electron transport rate through PSII (ETR) was calculated as ETR = $\Delta F/F_m' \times$ PPFD × A × 0.5, where PPFD is the photosynthetically active photon flux density falling on leaves, A is the absorptance (estimated as described below) of photosynthetic tissues and 0.5 holds for an assumed equal distribution of absorbed photons between the two photosystems. Calculation of non-photochemical quenching (NPQ) was based on the formula NPQ = $(F_m - F_m')/F_m'$ (Maxwell and Johnson 2000). During photosynthesis measurements, leaf temperatures were also monitored by thermocouple afforded by the leaf clip. Leaf temperatures changed significantly during a day as well as between sampling dates, but were not statistically different between treatments (data not shown).

Light curves under steady state conditions: For accurate recording of light-response curves, steady-state conditions at each step of increasing light intensity would be the ideal method (Rascher *et al.* 2000). Under field conditions, this is very difficult and usually, instant light curves are used where actinic light intensity increases gradually in rather short intervals. In any case, steady state conditions are preferable, so we measured effective quantum yields under ambient conditions on a diurnal basis (1–2 h intervals from sunrise to sunset) with a *MINI-PAM* fluorometer. Several hundred measurements of $\Delta F/F_m'$collected from plants of each treatment during a day were used in order to draw plots of $\Delta F/F_m'$ and ETR against light intensity as described below.

Curve fitting: The $\Delta F/F_m'$ versus PPFD plots were mathematically fitted using a double exponential decay function with zero-offset, as provided by $f(x) = m + ae^{-bx} + ce^{-dx}$. ETR versus PPFD plots were fitted using a single exponential function $f(x) = a(1-e^{-bx})$. In both equations a,b,c,d, and m are independent parameters (Rascher *et al.* 2000). High r^2 values support double exponential decay functions regarding curve fitting of $\Delta F/F_m'$ data points against PPFD (Fig. 3 inserts). From the results of the second equation the cardinal points of light-response curves for ETR can be determined with a = ETR_{max} and $PPFD_{sat}$ to be reached at 0.9 ETR_{max}.

Induction curves: Dark/light induction curves were measured on 8 plants per treatment, after dark adaptation overnight (about 8 h dark period), (actinic illumination of 500 μmol m^{-2} s^{-1}). Quantum yield, ETR and NPQ were calculated as previously described during both induction and recovery phase. The actual actinic light irradiance measured by the quantum sensor of the 2030-B leaf clip during the induction-recovery experiment never fell more than 4 % and can be considered stable.

Fast fluorescence induction kinetics measurement: Chl fluorescence of attached leaves was measured with a *Plant Efficiency Analyzer* (*PEA, Hansatech Instruments Ltd*., Norfolk, UK) after dark adaptation of 8 plants per treatment overnight (about 8 h dark period). Exciting red light of relatively low intensity (60 μmol m^{-2} s^{-1}) was provided by an array of six high intensity light emitting diodes (peak wavelength at 650 nm) with duration of 3 s. The relatively low intensity was used in order to record the F_i value of the intermediate plateau in the fast induction phase. It has been reported that the rise of fluorescence from F_0 to F_i is due to the reduction of a population of Q_A unable to transfer electrons to Q_B (Q_B-non-reducing PSII reaction centers) (Chylla and Whittmarsh 1989, Cao and Govindjee 1990). Subsequently, fluorescence rise from F_i to F_p is attributed to the reduction of plastoquinone pool in the so-called "Q_B-reducing" PSII centers (Hsu 1992). Then, the ratio $(F_i - F_0)/(F_p - F_0)$ denotes the relative amount of non-Q_B-reducing centers (Lu and Zhang 1998a,b, Kotakis *et al.* 2006). Increasing intensity of exciting light has been shown to affect the ratio, overestimating the relative amount of Q_B-non-reducing ("inactive") PSII centers and levels of ca 15–40 μmol m^{-2} s^{-1} are usually recommended (Tomek *et a*l. 2003). However, fluorescence yields with our instrument and plant material were unacceptably low at such levels and slightly higher exciting light intensity was used as mentioned above. Since our approach was comparative, we use the possibly overestimated values as an indication of actual differences between water stressed and control individuals.

Leaf absorptance (A) was calculated as A = 1 – R – T, where R and T are spectral reflectance and transmittance, respectively, integrated over the visible part of the spectrum. Both R and T were measured with an *Optronic OL 752* spectroradiometer (Orlando, FL, USA). A was estimated for each treatment and at each sampling date and was used as described above in order to calculate ETR values. However, values (data not shown) were almost stable throughout the experiment (A = 0,740 ± 0.030) except for severe stressed plants with RWC

(28 % ± 3) when A was 0.680 ± 0.020.

Statistics: Significance of differences in the measured parameters as well as in cardinal points of ETR versus PPFD between treatments was analyzed by a Student's *t*-test. An *F*-test assessing the null hypothesis for one curve fitting was used in order to compare the best fit lines of $\Delta F/F_m$' and ETR versus PPFD. (*SPSS 9.01, SPSS Inc.*, Chicago, USA). Only significant differences (with at least $p<0.05$) are discussed in the text.

Results

Plant water status: Water status of the 3 groups varied significantly during the experimental period due to different irrigation regimes (Fig. 1*A*). From diurnal measurements, it was evident that RWC was stable throughout the day, with a small decline during the first 1–2 h of the day (Fig. 1*B*,*C*). This is the reason why we used the midday values of RWC in order to refer to water status of the plant during the experimental period.

The first measurement represents water status at time zero of the experiment, when plants were divided to three regimes of irrigation. The almost identical water status confirms a proper arrangement of individuals into the three groups. Once water was withheld in the two (*ms* and *ss*) groups, RWC started to decline. Decline in RWC for leaves of moderately stressed plants was more gradual compared with that of severely stressed ones. Finally, RWC of severely stressed plants dropped off to extremely low values (~28 %), while RWC of mildly stressed plants maintained at relatively low values (60–70 %) during the experiment. Plants of both treatments, when re-irrigated, regained water content approaching the levels of control individuals (~70 %). Leaves of plants that experienced very low water content did not show any signs of necrotic areas (Grammatikopoulos 1999).

PSII under water stress and recovery: Maximum PSII photochemical efficiency (F_v/F_m) remained unaffected (0.835–0.790), even after 2 months of sustaining moderate drought (60–70 %) (Table 1). A relatively significant reduction (0.680–0.730) was found when leaf RWC dropped below 30 %. Four days after re-watering a rapid, though not complete recovery of maximum PSII photochemical efficiency was detected in severely stressed plants (0.740–0.785). The relative amount of non-Q_B-reducing $(F_i-F_0)/(F_p-F_0)$ centers seemed to be more sensitive to leaf water content reduction, as it increased by 50 % even with moderate water stress (from control ratio ~0.220 at RWC 80–90 % to 0.334 at RWC 60–70 %). With further reduction of leaf RWC (25–35 %) the ratio was doubled (0.469). Four days after re-watering, the ratio from both water stress treatments (*ms* and *ss*) recovered at control levels (0.230–0.270) following the corresponding recovery of RWC (Table 1). Total amount of Chl expressed on a leaf area basis showed a trend for reduction with progressive water deficit throughout the experiment but differences were not statistically significant ($p>0.05$) among irrigation treatments as well as inside the treatment with time variation (Table 1).

Fig. 2 shows recordings of dark-light induction curves (light intensity: 500 µmol m^{-2} s^{-1}) followed by light–dark relaxation kinetics in dark adapted plants (overnight adaptation). The effective quantum yield ($\Delta F/F_m'$) was

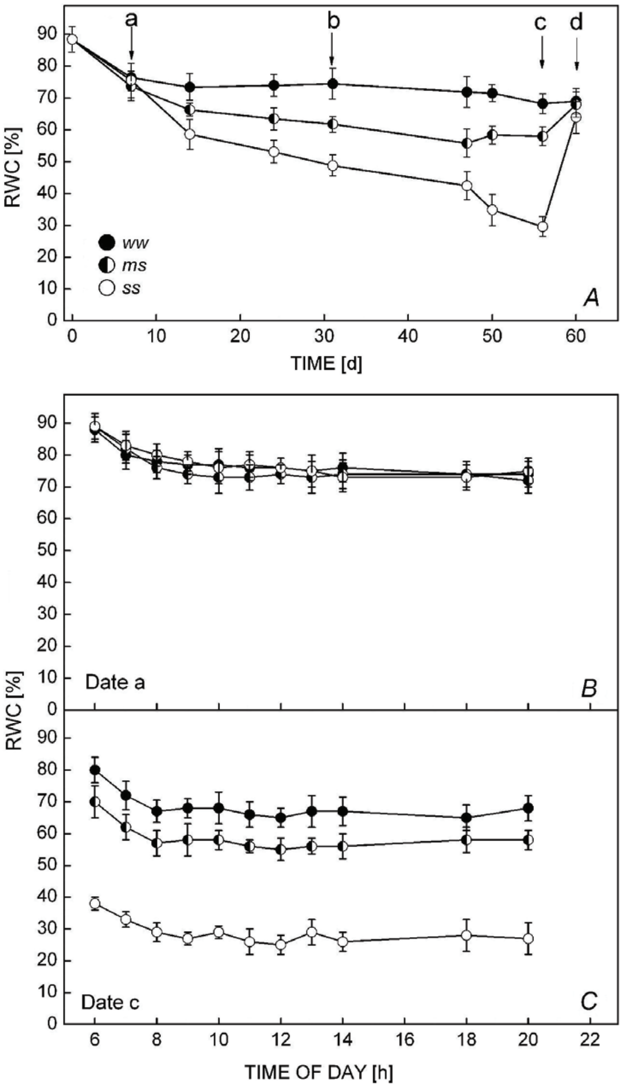

Fig. 1. Time course of the changes in relative water content (RWC) in *P. fruticosa* seedlings under different irrigation treatments (● *ww*: well watered plants, ◐ *ms*: mild stress, ○ *ss*: severe stress). *A*: Changes during dehydration and rehydration experiment. Letters (*a*,*b*,*c*,*d*) indicate the sampling dates for chlorophyll fluorescence measurements. *B*,*C*: Diurnal changes of RWC at the onset and at the end of the dehydration experiment. Data are means ± SD from 8–10 independent measurements. Rehydration started on 56th day.

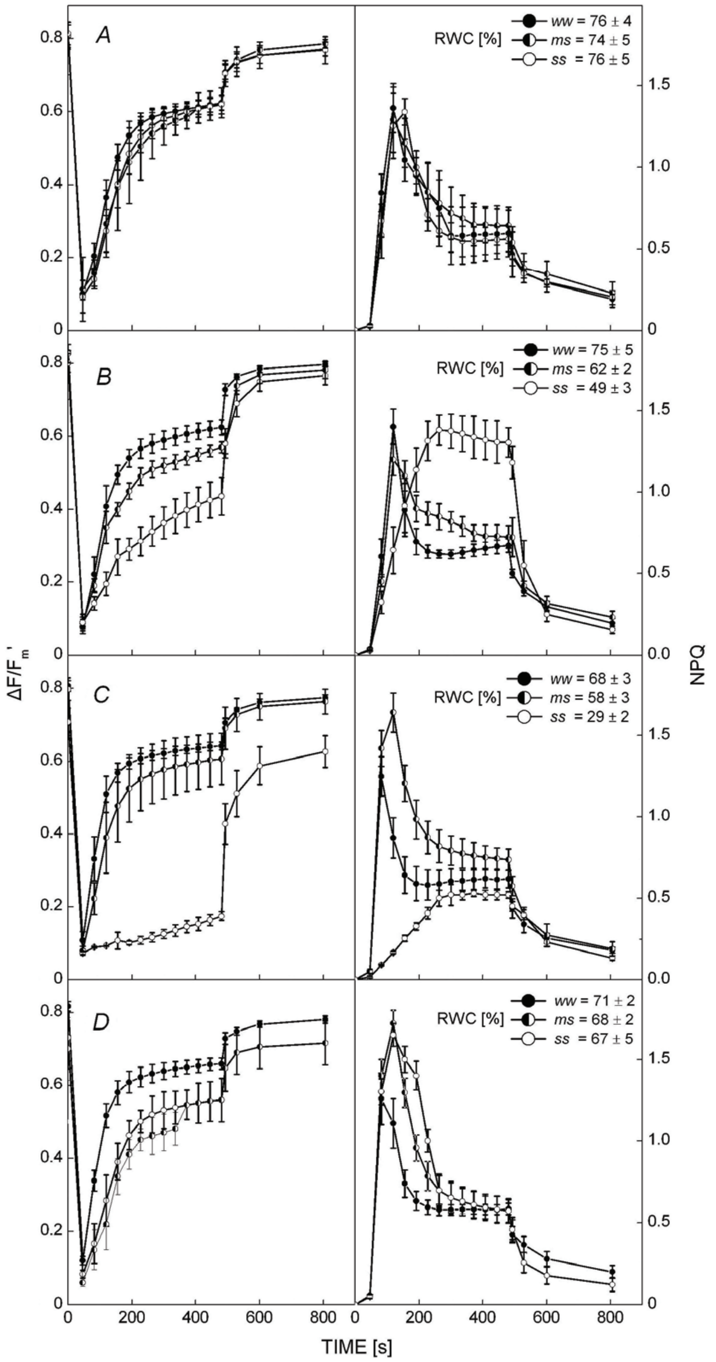

Fig 2. Dark-light induction and recovery (after 8 min) curves by the saturation pulse method for the three different irrigation treatments (● *ww*: well watered plants,◐ *ms*: mild stress, ○ *ss*: severe stress). Actinic light intensity was 500 µmol m^{-2} s^{-1}. *Left panels*: quantum yields $\Delta F/F_m'$. *Right panels*: non-photochemical quenching (NPQ). Data are means ± SD (*n* = 8). Letters (*A*,*B*,*C*,*D*) indicate the sampling dates as illustrated in Fig. 1*A*.

relatively high even with mild water stress (60–70 % RWC) falling significantly at 50 % RWC and reaching very low values ($\Delta F/F_m'$ ~0.150) when RWC dropped down below 30 %. ETR induction kinetics, as expected, are characterized by close to parallel increase with that of $\Delta F/F_m'$ reaching steady state value within the 8 min of induction curve programme (data not shown). At the beginning of the experiment (Fig. 2*A*, *right*), NPQ showed acute development (2–3 min) after the onset of illumination (~1.3±0.2) as well as slower relaxation (6–8 min) at steady state values (~0.6±0.1) simultaneous with the achievement of maximum effective quantum yield ($\Delta F/F_m'$) for our experimental actinic light (500 µmol m^{-2} s^{-1}). This time pattern of NPQ induction was similar (no statistical differences) among the three treatments. However, at 50 % RWC, NPQ development was slightly lower but remarkably stabilised at high values until termination of the illumination period (Fig. 2*B*, *right*). When RWC dropped down to 30 % (Fig. 2*C*, *right*) NPQ maximum values were not higher than 0.8±0.1 and achieved only after 6 min from the onset of illumination. The non-photochemical quenching development was almost the same among treatments when plants re-watered.

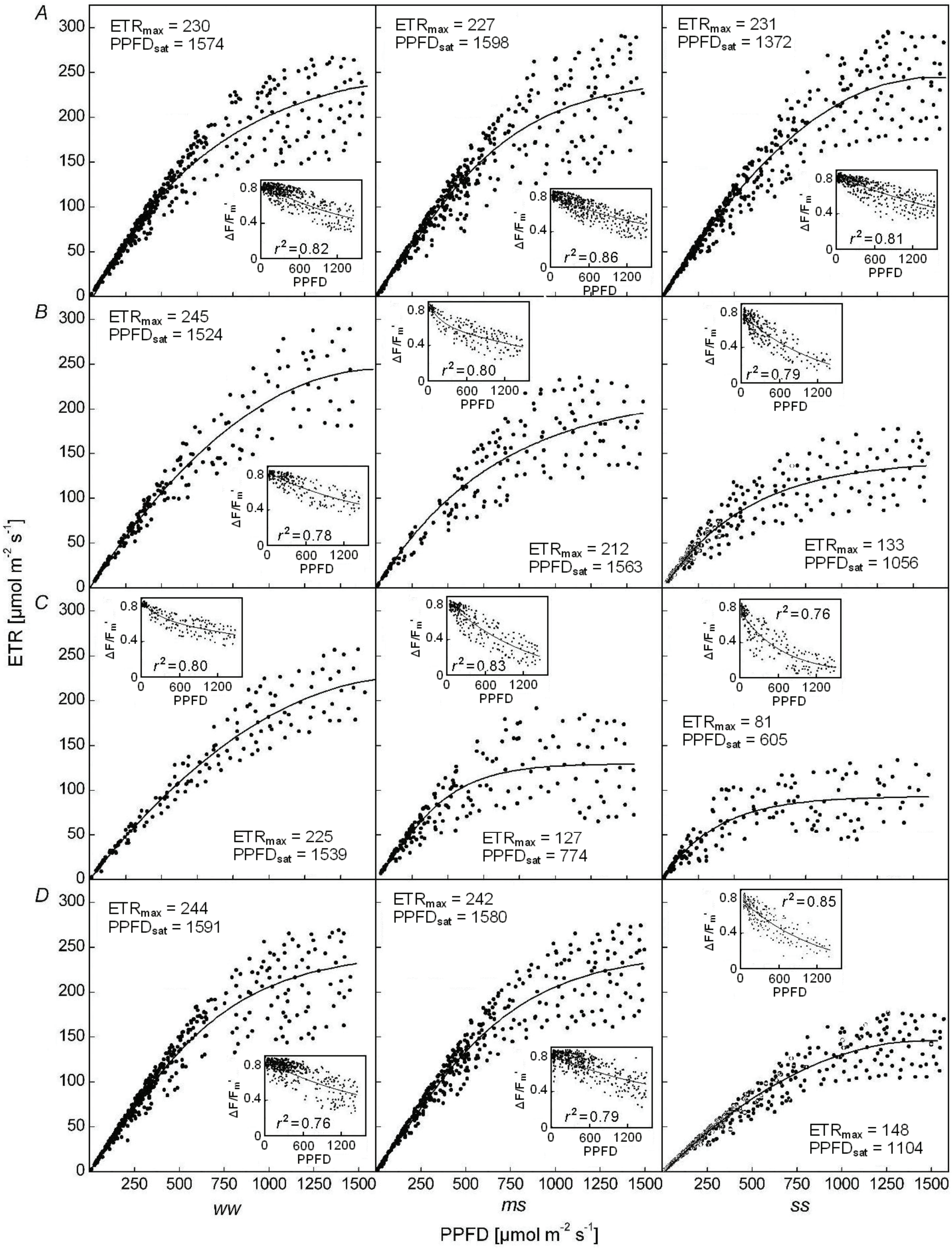

Fig. 3. Light response curves of electron transport rate (ETR) under steady state ambient conditions. Data collected from several measurements during the experimental dates and fitted against a single exponential function (lines). *Left panels*: well watered plants (*ww*), central panels: mild stress (*ms*), *right panels*: severe stress (*ss*). For each light curve, the cardinal points ETR $_{max}$ and PPFD$_{sat}$ are given numerically. *Inserts*: corresponding effective quantum yield ($\Delta F/F_m'$) values fitted against a double exponential decay function with zero-offset (lines; r^2 – regression coefficient). Letters (*A*,*B*,*C*,*D*) indicate the sampling dates as illustrated in Fig. 1*A*.

PSII function under steady state conditions: Several measurements of effective quantum yield ($\Delta F/F_m'$) at different light intensities (steady state ambient conditions) during the experimental dates are plotted in Fig. 3 (inserts). $\Delta F/F_m'$ was always lower in water stressed plants compared to control ones, especially at higher irradiances (400–1600 µmol m^{-2} s^{-1}). Correspondingly, ETR is plotted versus PPFD and maximum electron transport rate (ETR$_{max}$) as well as saturating photosynthetically active radiation (PPFD$_{sat}$) values are given numerically in the graphs (Fig. 3). ETR, ETR$_{max}$ and PPFD$_{sat}$ were calculated as described in Materials

Table 1. Effects of withholding water supply and re-watering on maximum PSII photochemical efficiency (F_v/F_m), relative amount of non-Q_B-reducing centers ($(F_i–F_0)/(F_p–F_0)$) and chlorophyll (Chl) amount per leaf area, measured on leaves of *P. fruticosa* seedlings among three irrigation regimes (*ww* – well watered plants, *ms* – mild stress, *ss* – severe stress). Data are mean ± SD from 30–40 independent measurements for F_v/F_m and Chl amount, and from 10–12 independent measurements for $(F_i–F_0)/(F_p–F_0)$. Different letters (a,b,c) within each date indicate statistically significant differences among the three irrigation treatments. Different numbers (1,2,3) within each treatment indicate statistically significant differences among different dates.

	Treatment	F_v/F_m	$(F_i–F_0)/(F_p–F_0)$	Chl [μg cm^{-2}]
Time from the onset of treatments [d]				
7	*ww*	$0.806 \pm 0.030^{a\,1}$	$0.219 \pm 0.031^{a\,1}$	$34.4 \pm 4.7^{a\,1}$
	ms	$0.808 \pm 0.037^{a\,1}$	$0.225 \pm 0.043^{a\,1}$	$32.8 \pm 2.7^{a\,1}$
	ss	$0.813 \pm 0.031^{a\,1}$	$0.226 \pm 0.021^{a\,1}$	$34.1 \pm 3.2^{a\,1}$
31	*ww*	$0.830 \pm 0.005^{a\,1}$	$0.220 \pm 0.047^{a\,1}$	$32.2 \pm 1.8^{a\,1}$
	ms	$0.827 \pm 0.025^{a\,1}$	$0.334 \pm 0.037^{b\,2}$	$32.5 \pm 2.1^{a\,1}$
	ss	$0.801 \pm 0.026^{a\,1}$	$0.391 \pm 0.043^{b\,2}$	$33.1 \pm 3.8^{a\,1}$
56	*ww*	$0.806 \pm 0.015^{a\,1}$	$0.252 \pm 0.034^{a\,1}$	$30.1 \pm 2.9^{a\,1}$
	ms	$0.797 \pm 0.008^{a\,1}$	$0.312 \pm 0.039^{b\,2}$	$30.0 \pm 3.1^{a\,1}$
	ss	$0.708 \pm 0.037^{b\,2}$	$0.469 \pm 0.040^{c\,3}$	$29.5 \pm 3.6^{a\,1}$
Time after re-watering [d]				
4	*ww*	$0.816 \pm 0.013^{a\,1}$	$0.237 \pm 0.023^{a\,1}$	$32.4 \pm 1.9^{a\,1}$
	ms	$0.811 \pm 0.014^{a\,1}$	$0.255 \pm 0.027^{a\,1}$	$33.7 \pm 2.9^{a\,1}$
	ss	$0.765 \pm 0.032^{b\,3}$	$0.260 \pm 0.024^{a\,1}$	$30.9 \pm 3.4^{a\,1}$

and methods. ETR_{max} and $PPFD_{sat}$ values of all the three groups of plants were identical (230 and 1550 correspondingly) after 7 days from the onset of experiment, when RWC was still the same among treatments (Fig. 3*A*). They were significantly lowered at 50 % RWC (ETR_{max} = 133, $PPFD_{sat}$ = 1056) and further reduced (ETR_{max} = 81, $PPFD_{sat}$ = 605) at 30 % RWC in severely stressed plants (Fig. 3*B*,*C*). After re-watering ((Fig. 3*D*) values increased to intermediate values (148 and 1104 correspondingly). In mildly stressed plants ETR_{max} dropped down to 127 and $PPFD_{sat}$ to 774 at 58 % RWC (Fig. 3*C*) but after 56 days of water withholding. Values in this group fully recovered when plants re-watered (Fig. 3*D*).

Discussion

Our main question had to do with the degree of drought resistance of *P. fruticosa* seedlings. The need to understand physiological changes better over the life cycle of woody plants has been increasing (Bond 2000), while the limiting effect of summer drought on seedling establishment has long been acknowledged in Mediterranean environments (Herrera 1992). We intended to examine the possible implications of photosynthetic machinery in drought resistance mechanisms, independent of root diversion, as not only soil moisture but also the role of rooting depth affects plant establishment (Padilla and Pugnaire 2007). Therefore, we grew our seedlings in pots to avoid any significant variation of rooting depth between individuals. We also used soil from their natural habitat to mimic field conditions.

The results presented here indicate that PSII function is progressively suppressed under prolonged drought and the first symptoms on its functionality have been detected at moderate water stress. However, more serious dysfunctions were found at very low water contents. Maximum PSII photochemical efficiency (F_v/F_m) was reduced only under severe water stress (RWC < 30 %). The reduction was not sustained after re-watering and control levels were measured after four days. An analogous steep transient drop during summer and recovery after the first autumn rain was detected in mature plants under field conditions (Karavatas and Manetas 1999). We can assume that concerning maximum PSII photochemical efficiency there is no difference between seedlings and mature plants.

One of the major photoprotective responses of plants to avoid photoinhibition under water stress is harmless thermal dissipation of excess excitation energy through the xanthophyll cycle function usually estimated by NPQ measurement (Demmig-Adams *et al.* 1996). Changes of NPQ process development in our case indicated that decrease of F_v/F_m was not due to photodamage and that heat dissipation process though still persistent under severe water stress is not fully functional. Under similar RWC, Kyparissis *et al.* (2000) recorded the highest photoprotective potential (xanthophyll pool size) of mature *P. fruticosa* plants in the field. Therefore, seedlings appeared to be less efficient than the mature plants against the increased demand for thermal energy dissipation during severe water stress, but similar regarding recovery after re-watering (Kyparissis *et al.* 2000, Montanaro *et al.* 2007). However, even moderate water stress induced a significant increase in the proportion of Q_B-non-reducing PSII reaction centers. The main feature of a Q_B-non-reducing PSII reaction center is its inability to transfer electrons from Q_A to Q_B (Chylla and Whittmarsh 1989, Cao and Govindjee 1990, Melis 1985, Tomek *et al.* 2003). The same pattern was found either in senescent leaves of maize plants (Lu and Zhang 1998b) or in twigs chlorenchyma of *Eleagnus angustifolia* under a microenvironment of extremely deficient linear electron transport activity (Kotakis *et al.* 2006). In our study, the relative increase of Q_B-non-reducing PSII reaction centers is possibly a mechanism for down-regulation of photosynthetic electron transport in order to equilibrate ATP and NADPH production with their decreased demand in the Calvin cycle of water stressed leaves.

Contrary to previous studies that have related decrease in leaf Chl with reduced risk of over-excitation and photodamage in functionally different Mediterranean species such as perennial grasses (Balaguer *et al.* 2002), evergreen sclerophylls (Kyparissis *et al.* 1995), pines (Elvira *et al.* 1998) as well as in mature plants of *P. fruticosa* (Kyparissis *et al.* 1995, 2000), we did not find any reduction of leaf chlorophyll content even under severe water stress. We assume that the process of Chl loss against photodamage, possibly, has not already been developed at the seedling stage of our plants. This is also supported by the fact that seedlings of *P. fruticosa* do not exhibit the same leaf demography, characteristics and flower period of mature plants (Grammatikopoulos and Manetas 1994, Grammatikopoulos *et al.* 1995, Grammatikopoulos 1999, Kyparissis *et al.* 1995, 1997). Sporadic measurements in some of these characteristics during the experiment did not reveal such differences in leaves of our plants (data not shown). Presumably, we can claim that PSII resistance mechanisms to water stress differ between seedling stage and maturity in *P. fruticosa*. Similar observations have been reported for woody plants (Bond 2000).

Our results suggest that the effects of drought on PSII function in seedlings of *P. fruticosa* are almost completely reversible even after severe water stress. Suppression of electron transport rates and effective quantum yields under steady state field conditions appears only as a transient interruption of PSII normal function. Down-regulation mechanisms, mainly through the redistribution of ‘active’ and ‘inactive’ PSII reaction centers and partly through harmless thermal dissipation of excess excitation energy contributed to the rapid re-establishment of PSII function after rehydration. Remarkably, the photoprotective mechanism of Chl loss, met not only in many Mediterranean species but also in mature plants of *P. fruticosa*, was absent at the seedling stage of this species. Previous observations indicate possible antagonistic interactions between environmental stresses, with one constraint enhancing the tolerance of photosynthesis toward another, superimposed constraint (Havaux 1992, Epron 1997, Nogues and Baker 2000). Individuals of all the three irrigation treatments experienced exactly the same daily courses of irradiation, while, although temperatures varied strongly during a day as well as between sampling dates, they were never statistically different between treatments. Consequently, detected differences in PSII function among treatments should be attributed to water stress. It is possible however, that co-existing water stress, heat and high irradiation contribute to maintenance of PSII activity of *P. fruticosa* seedlings under field conditions.

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