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Are symplast tolerance to intense drought conditions and xylem vulnerability to cavitation coordinated? An integrated analysis of photosynthetic, hydraulic and leaf level processes in two Mediterranean drought-resistant species

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ABSTRACT

In the last decades, plant resistance to drought conditions has been studied intensively both at whole-tree level and at tissue level. These studies have highlighted the role of xylem cavitation in the global resistance of plants to drought. In this paper, we investigate the coordination among several symplastic variables during intense drought conditions and its relationship to resistance to xylem cavitation. We selected 2-year-old seedlings of *Pistacia lentiscus* L. and *Quercus coccifera* L., two Mediterranean drought-resistant species with differences in both xylem vulnerability to cavitation and survival rates under field conditions. Drought provoked large decreases in photosynthetic rates and predawn F_v/F_m ratios, as well as less marked decreases in actual PSII efficiency (due to decreases in both intrinsic PSII efficiency and photochemical quenching). Photosynthetic pigment composition remained fairly unchanged down to water potentials of −8 MPa, despite inter-conversions within the xanthophyll cycle in both species. Cell membrane injury and proline accumulation followed similar patterns, and were much more intense in *P. lentiscus* than in *Q. coccifera*. Comparisons between variables revealed that both species: (i) followed a drought avoidance strategy, (ii) were very resistant to drought conditions at symplastic level, and (iii) showed an overall good relationship between apoplast (xylem cavitation) and symplast resistance (membrane stability, PSII functionality, proline accumulation and pigment composition). Differences between species in functional symplastic and apoplastic characteristics are discussed.

1. Introduction

Soil water availability is considered one of the most important factors affecting plant survival and species distribution. In dry and semiarid Mediterranean climates, plants are subjected to important intra- and inter-annual water oscillations. This scenario will worsen in the near future due to temperature increases and precipitation reductions, thus increasing the length, number and intensity of water deficit periods (Bates et al., 2008). An increment of recurrent droughts could affect both species survival and distribution in the coming decades (Tognetti et al., 1998; Ogaya and Peñuelas, 2007), but its effects would probably be most dramatic at the seedling stage (Vallejo et al., 2000).

It has been widely reported that drought affects many symplastic and apoplastic physiological processes with ultimate consequences for plant growth and survival (Levitt, 1980; Larcher, 1995). Under stressful conditions (high temperature, irradiance and water deficit), net photosynthesis, photosystem II (PSII) efficiency, photosynthetic pigment composition, cell membrane integrity and protein stability have been reported to play an important role in cell resistance to drought stress (Cornic and Massacci, 1996; Methy et al., 1996). Under these conditions, energy dissipation mechanisms could be promoted, mediated by changes in the de-epoxidation state of the xanthophyll cycle (Flexas and Medrano, 2002; Morales et al., 2006) and/or photoinhibition in the photosynthetic

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Abbreviations: *A*, net CO_2 uptake rate per unit leaf area; Chl, chlorophyll; Φ_{PSII} and Φ_{exc}, actual and intrinsic photosystem II efficiencies, respectively; F_o and F'_o, minimal Chl fluorescence yield in the dark or during light adaptation, respectively; F_m and F'_m, maximal Chl fluorescence yield in the dark or during light adaptation, respectively; FR, far-red; F_s, Chl fluorescence at steady-state photosynthesis; F_v and F'_v, $F_m - F_o$ and $F'_m - F'_o$, respectively; K_h, shoot hydraulic conductance; NPQ, non-photochemical quenching; PAR, photosynthetically active radiation; PPFD, photosynthetic photon flux density; PSI and PSII, photosystems I and II, respectively; qP, photochemical quenching; V + A + Z, violaxanthin + antheraxanthin + zeaxanthin; Ψ_{pd}, and Ψ_{xyl}, predawn and xylem water potential, respectively.

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apparatus of water-stressed leaves (Demmig-Adams and Adams, 1992, 2006; Morales et al., 2006). Moreover, under these conditions of intense water deficits and temperature stress, many phospholipids of biological membranes undergo phase transitions and membrane fusions that are disruptive to membrane structure and function. For these reasons, cell membranes are also considered one of the first targets of many stresses, and the maintenance of their integrity and stability under water stress conditions is a major component of drought tolerance in plants (Hsiao, 1973; Earnshaw, 1993; Lauriano et al., 2000).

Many works have regarded symplasmic tolerance/resistance to water deficit as a key step in plant productivity and survival. However, apoplasmic resistance (i.e., vulnerability to xylem cavitation) also plays a major role in drought resistance (Tyree and Sperry, 1989; Pockman and Sperry, 2000). Vulnerability to cavitation differs widely among species (Pockman and Sperry, 2000), and it has been reported that low vulnerability results in a higher drought tolerance in plants (Tyree and Ewers, 1991). In this sense, it has been suggested that vulnerability to cavitation determines the patterns of survival for the different species in Mediterranean and dryland ecosystems (Davis et al., 1998; Pockman and Sperry, 2000). Some species show an extraordinary resistance to lose hydraulic conductivity, corresponding to water potentials lower than −10 MPa (Davis et al., 1998; Vilagrosa et al., 2003). Previous works have shown relationships between the response of stomatal processes and the resistance to xylem cavitation (Salleo et al., 2000; Brodribb and Holbrook, 2003). In spite of the importance of vulnerability to cavitation for species survival, very few works have reported quantitative relationships between survival and resistance to xylem cavitation (Tyree et al., 2002; Brodribb and Cochard, 2009), and no information has been reported on symplastic processes suffered by plants at low water potentials, when loss of hydraulic conductivity is very high (i.e. >50%). After analyzing survival in two Mediterranean shrubs, Vilagrosa (2002) and Vilagrosa et al. (2003) found that higher resistance to xylem cavitation was not correlated with higher survival under field conditions. Therefore, the hypothesis of the present work was that processes other than xylem cavitation could determine both resistance to drought and survival under intense drought conditions. Moreover, previous observations of different patterns of leaf dieback between these two species could be related to differences in symplastic resistance at leaf level (Vilagrosa et al., 2003).

Thus, the main objectives of this study were: (i) to investigate drought symplastic resistance through changes in photosynthesis, PSII efficiency, photosynthetic pigment composition, cell membrane stability and proline accumulation, and (ii) to determine whether symplastic resistance to drought stress at leaf level was related to xylem vulnerability to cavitation. Finally, we tried to obtain a picture of the plant's overall resistance to drought stress, including both apoplastic and symplastic traits.

For this purpose, we analysed photosynthetic rates, PSII functionality and pigment composition, VAZ cycle**,** cell membrane stability and proline in two co-occurring Mediterranean shrub species, mastic tree (*Pistacia lentiscus* L.) and kermes oak (*Quercus coccifera* L.), during an intense drought period. Both species are typical of Mediterranean dry and semiarid climates, and are well adapted to intense drought conditions. In addition, we compared the results obtained here with those published in Vilagrosa et al. (2003) in relation to resistance to xylem cavitation.

2. Materials and methods

2.1. Plant material

Two hundred 2-year-old seedlings of each species, *P. lentiscus* L. and *Q. coccifera* L., were grown in 8.0 L containers filled with forest soil under full sunlight conditions. Seeds were obtained from the local Forest Service (Regional Government Seed Bank, Valencia, Spain), and the origin of the seeds was the same geographical area where the experiments were carried out (i.e., from a Mediterranean semiarid climate). Seedlings were watered and fertilized as needed during the nursery period.

A drought period was imposed by withholding watering during the summer at full sunlight in the CITA (Centro de Investigación y Tecnología Agroalimentaria, Diputación General de Aragón, Spain) experimental fields. Seedlings were subjected to an intense drought period that covered from maximum turgor (predawn water potentials close to −0.1 MPa) to severe water deficit (predawn water potentials lower than −8 MPa). The plants reached the severe water deficit stage after about 30 days without watering.

2.2. Water potential and net photosynthesis

Predawn (Ψ_{pd}) water potentials were assessed in leafy shoots by means of a Scholander pressure chamber. To avoid tissue dehydration during measurements, the internal walls of the pressure chamber were covered with wet filter paper. In the same plants, net photosynthesis (A) was determined in three small twigs per seedling at mid-morning (07:00–08:00 solar time) with an infrared gas analyser (IRGA, ADC LCA 2, Hoddesdon, U.K.) at ambient CO_2 concentration (365 ppm), PPFD, relative humidity and temperature. Net photosynthesis was calculated according to the equations described by Von Caemmerer and Farquhar (1981).

2.3. Chlorophyll fluorescence and pigment composition

Chlorophyll (Chl) fluorescence was measured prior to sunrise and at 08:00 and 12:00 (solar time) with a PAM 2000 modulated portable fluorometer (Heinz Walz, Effeltrich, Germany). Measurements were taken on intact leaves. We used three leaves from each of the seedlings in which we had previously determined Ψ_{pd}. The experimental protocol for analysis of Chl fluorescence quenching was as in Morales et al. (2000) and references therein. Maximum potential PSII efficiency was estimated as $F_{\mathrm{v}}/F_{\mathrm{m}}$. Actual ($\Phi_{\mathrm{PSII}}$) and intrinsic ($\Phi_{\mathrm{exc.}}$) PSII efficiencies were estimated as $(F'_{\mathrm{m}} - F'_{\mathrm{s}})/F'_{\mathrm{m}}$ and $(F'_{\mathrm{v}} - F'_{\mathrm{m}})$, respectively. Photochemical quenching (qP) was estimated as $(F'_{\mathrm{m}} - F'_{\mathrm{s}})/F'_{\mathrm{v}}$. Non-photochemical quenching (NPQ) was estimated as $(F'_{\mathrm{m}} - F'_{\mathrm{m}}) - 1$.

For pigment analyses, samples were collected at predawn. We selected three leaves from a seedling in which we had previously measured the water potential. Three disks (0.2 cm^2) from each leaf were collected with a cork borer, wrapped in aluminum foil, frozen in liquid nitrogen and stored (still wrapped in foil) at −20 °C. Pigments were extracted in a mortar following the Abadía and Abadía (1993) procedure and analysed using an HPLC method described previously (Larbi et al., 2004).

2.4. Cell membrane injury

The electrolyte leakage technique has been extensively used to assess membrane integrity in relation to drought tolerance (Epron and Dreyer, 1992; Lauriano et al., 2000). The degree of water stress-induced cell membrane injury was estimated by measuring cell electrolyte leakage into an aqueous medium (Epron and Dreyer, 1992; Earnshaw, 1993). We used three leaves from a seedling in which we had previously measured the water potential. From each leaf, three disks (0.2 cm^2) were collected with a cork borer, and washed with distilled water (to remove electrolytes released at the disk edge). Leaf disks were incubated in 3 mL of distilled water in a polypropylene vial, and stored in the dark at 10 °C for 5 h (Solution 1, ECi). The disks were removed from the vial, plunged into liquid nitrogen and placed in another vial to be stored for 12 h at −25 °C.

We then added 3 mL of distilled water and again stored the disks in the dark at 10 °C for 5 h (Solution 2, ECf). It is assumed that this procedure removes all residual electrolytes from leaf tissues (Epron and Dreyer, 1992).

Initial (ECi) and final (ECf) electrical conductivities were measured at 25 °C with a conductivity meter (Crison CM 2202, Barcelona, Spain). Relative electrical conductivity (ECr, %) of each sample was calculated as: ECr = (ECi/ECi + ECf) × 100. Since the degree of solute leakage varies between species (Vasquez-Tello et al., 1990), values of ECr were expressed as relative values (100% would be the maximum value registered for each species).

2.5. Proline determination

Proline content was estimated using the acid-ninhydrin method (Bates et al., 1973). During the drought period, we selected three leaves from a seedling for which we had previously measured the water potential. From each leaf, we collected three disks (0.2 cm^2) which were immersed in liquid nitrogen and stored at −20 °C in a freezer until proline determination. Leaf tissue (0.5 g) was extracted with 3% sulfosalicylic acid (Panreac, Spain) using a homogenizer at maximum speed. After this, the samples were centrifuged at 4000 *g* and 2 mL supernatant was added to 4 mL of a mixture of glacial acetic acid and ninhydrin reagent in a 1:1 (v/v) ratio. The mixture was incubated in a water bath at 100 °C for 1 h. Cromophore was extracted with 4 mL of toluene. Absorbance was read in the organic phase at 520 nm. Proline concentration was calculated using a L-proline standard curve (Sigma–Aldrich, Spain).

2.6. Comparison of apoplastic and symplastic variables

In order to establish relationships among the physiological variables (Figs. 8 and 9; Tables 1 and 2), we selected several variables (A, F_v/F_m, A + Z/VAZ, ECr, proline concentration, K_h and loss of leaf area) which were quantitatively rated between 0 and 1 according to their minimum and maximum values during the drought period. By ranking the variables in this way, we were able to compare pairs of variables using the equation: $X - X_{\min}/X_{\max} - X_{\min}$, where X is the value of the target variable in a given water potential value, $X_{\min}$ corresponds to the minimum values registered in intense drought conditions and $X_{\max}$ corresponds to the maximum values registered in well-watered conditions (water potential values close to −0.1 MPa). Thus, the comparison between pairs of variables was carried out on a water potential basis, as derived from regression models between the values for each variable and the water potential.

Data on loss of hydraulic conductance (cavitation vulnerability curves) and loss of leaf area were extracted from Vilagrosa et al. (2003) in a parallel experiment with the same plant material (but another set of seedlings) and the same experimental settings. To determine the leaf area loss, the percentage of reduction in leaf area (i.e., leaf shedding) was computed as a function of water potential. Leaf area was determined by collecting the leaves and using scanner and specific software (e.g., Regent Inst., Canada) to determine the projected area of the leaves. Regression analyses (Tables 1 and 2) were used to establish relationships among the variables. Finally, all these data were plotted to illustrate the potential relationships and to clarify the existing trends between them. As a result of the rating index introduced, the data ranged from 0 (minimum relative value registered) to 1 (maximum relative value registered). For A (net photosynthesis), F_v/F_m (maximum potential PSII efficiency) and K_h (shoot hydraulic conductance), values close to 1 correspond to the maximum values registered (optimal) and values close to zero correspond to the minimum values (deficient) when plants were near death. For ECr (cell membrane damage), Pro (proline) and A + Z/VAZ (de-epoxidation state of the VAZ cycle), values close to zero correspond to the minimum values registered (optimal) whereas values close to 1 correspond to values far from optimal (deficient).

2.7. Hydraulic measurements and leaf shedding

Data on loss of conductance (K_h) due to xylem cavitation and leaf shedding were computed from the findings of a previous study (Vilagrosa et al., 2003). Thus, in the present experiment we used both the same seedlings and the same imposed drought conditions as in the previously cited experiment to determine vulnerability to cavitation. This use of the same plant material in both experiments has allowed us to compare the results of the two studies.

The methodology employed to determine vulnerability to cavitation was based in the hydraulic method (Tyree and Sperry, 1989). Vulnerability to embolism was measured in current-year twigs by constructing curves through the dehydration method. Initial hydraulic conductivity was calculated as the mass flow rate of the solution through the twig segment divided by the pressure gradient along the segment ($kg\,m^{-1}\,s^{-1}\,MPa^{-1}$). After the initial hydraulic conductivity was measured, the twig segments were flushed with a pressurized solution (100 kPa for 10 min) to remove any air emboli, and the maximum hydraulic conductivity was measured. The percent loss of conductivity (K_h) was computed as: $(1 - K_{h\ \mathrm{initial}}) \times 100/K_{h\ \max}$. A more detailed explanation can be found in Vilagrosa et al. (2003).

Table 1
Best regression models for the variables plotted in Fig. 8 for *Q. coccifera*. The models are: linear ($y = b_o + b_1x$), exponential ($y = b_o \exp b_1 x$) and inverse ($y = b_o + b_1/x$).

Independent	Dependent	Best model	b_o	Slope b_1	*F*	*df*	R^2	*P*
K_h	*A*	Exp.	−9.01	8.26	56.5	31	0.65	<0.001
	F_v/F_m	Lin.	−0.303	1.37	7035.6	31	0.99	<0.001
	A + Z/VAZ	Lin.	1.46	−1.57	1606.6	31	0.98	<0.001
	ECr	Lin.	0.600	−0.552	1864.6	31	0.98	<0.001
	Pro	Lin.	0.043	−0.044	4667.7	31	0.99	<0.001
A	F_v/F_m	Inv.	0.994	−0.005	102.2	31	0.77	<0.001
	A + Z/VAZ	Inv.	−0.016	0.006	71.2	31	0.70	<0.001
	ECr	Inv.	0.071	0.002	169.2	31	0.84	<0.001
	Pro	Inv.	0.001	0.0002	134.1	31	0.81	<0.001
F_v/F_m	A + Z/VAZ	Lin.	1.116	−1.15	3103.1	31	0.99	<0.001
	ECr	Lin.	0.094	−0.401	1537.7	31	0.98	<0.001
	Pro	Lin.	0.033	−0.032	5174.5	31	0.99	<0.001
A + Z/VAZ	ECr	Lin.	0.088	0.341	554.7	31	0.95	<0.001
	Pro	Lin.	0.002	0.027	1042.4	31	0.97	<0.001
ECr	Pro	Lin.	−0.006	0.089	6933.0	31	0.99	<0.001

Table 2
Best regression models for the variables plotted in Fig. 9 for *P. lentiscus*. The models are: linear ($y = b_o + b_1x$), exponential ($y = b_o \exp b_1x$) and inverse ($y = b_o + b_1/x$).

Independent	Dependent	Best model	b_o	Slope b_1	*F*	*df*	R^2	*P*
K_h	*A*	Exp.	−8.79	8.48	360.0	27	0.93	<0.001
	F_v/F_m	Lin.	0.371	0.655	993.0	27	0.97	<0.001
	A + Z/VAZ	Exp.	0.111	−7.51	169.7	27	0.78	<0.001
	ECr	Lin.	0.574	−0.542	1268.0	27	0.98	<0.001
	Pro	Exp.	−0.056	−6.48	154.7	27	0.85	<0.001
A	F_v/F_m	Inv.	0.927	−0.0005	385.3	27	0.93	<0.001
	A + Z/VAZ	Inv.	−0.021	0.0004	84.4	27	0.74	<0.001
	ECr	Inv.	0.117	0.0004	388.4	27	0.93	<0.001
	Pro	Inv.	−0.017	0.0004	97.5	27	0.76	<0.001
F_v/F_m	A + Z/VAZ	Lin.	0.724	−0.813	205.8	27	0.87	<0.001
	ECr	Lin.	0.866	−0.808	45084.0	27	0.99	<0.001
	Pro	Lin.	0.748	−0.833	250.8	27	0.89	<0.001
A + Z/VAZ	ECr	Lin.	0.165	0.860	178.8	27	0.86	<0.001
	Pro	Lin.	0.0074	1.012	28076.8	27	0.99	<0.001
Ecr	Pro	Lin.	−0.142	1.02	215.4	27	0.88	<0.001

2.8. Statistical analyses

Regression analyses were used to fit net photosynthesis, maximum potential PSII efficiency, pigment composition and electrolyte leakage, with water potential. Differences between species were tested with analysis of covariance (ANCOVA), since this technique combines regression analysis with analysis of variance (Underwood, 1997). Water potential was the concomitant variable and species comparisons were established for the independent term and the equality of slopes through the interaction of the concomitant variable with each factor. When necessary, variables were logarithmically transformed to reach the assumptions of the ANCOVA.

Linear and non-linear regression analyses were used for establishing relationships among the main symplastic variables (*A*, F_v/F_m, A + Z/VAZ, ECr, Pro) and also when decreases in shoot hydraulic conductivity (loss of K_h) due to xylem cavitation were observed.

All statistical analyses were performed with SPSS version 12.0 statistical package (SPSS Inc., Chicago, Illinois, USA).

3. Results

3.1. Changes in photosynthetic rates and maximum potential PSII efficiency during water stress

Both species showed similar decreases in photosynthetic rates (*A*) as a function of Ψ_{pd} ($F_{1,20} = 3.7$, $P = 0.07$; Fig. 1). Maximum *A* values were between 5 and 7 μmol CO$_2$ m^{-2} s^{-1} with high water availability. These rates decreased as drought developed and both species reached *A* values very close to zero with Ψ_{pd} values of ca. −4 MPa.

The dark-adapted maximum potential PSII efficiency (estimated from the modulated Chl fluorescence parameter F_v/F_m) curves plotted as a function of Ψ_{pd} were similar for both species ($F_{1,20} = 0.22$, $P = 0.64$; Fig. 2C and D). Both species maintained relatively high predawn F_v/F_m values, ranging between 0.70 and 0.75, down to −4 MPa. Under conditions of intense drought (Ψ_{pd} lower than −4 MPa), predawn F_v/F_m values decreased steeply. Both species showed predawn F_v/F_m values very close to 0–0.2 at Ψ_{pd} of ca. −8 MPa. In both species, the decreasing values of F_v/F_m can be attributed to important decreases in F_m values when drought developed, whereas F_o maintained relatively constant values throughout the drought period (Fig. 2A and B). Only *P. lentiscus* showed slight increases in F_o values at predawn and at very low water potential values (Fig. 2B).

3.2. Changes in modulated chlorophyll fluorescence parameters

Fig. 3 shows the main variables related to modulated Chl fluorescence under direct sunlight conditions. In general and in both species, Φ_{PSII}, $\Phi_{exc.}$ and qP values decreased with increased drought conditions. In *Q. coccifera*, the NPQ values increased from 1.5 to 2.5 up to values of ca. 3 down to −4 MPa. From this point, lower values of water potential (between −6 and −8 MPa) produced a decrease in the NPQ values. In *P. lentiscus*, the NPQ values remained fairly constant.

3.3. Changes in photosynthetic pigment composition

Although predawn F_v/F_m values decreased to nearly zero (see Fig. 2), the decrease was not due to alterations in the Chl content of the leaves. Both species maintained a similar pigment composition during the whole drought period, until Ψ_{pd} reached values lower than −8 MPa (Fig. 4). Chlorophyll *a* and *b*, β-carotene and neoxanthin concentrations were fairly constant during the whole drought period (Fig. 4A–D). *P. lentiscus* showed more marked increases in lutein concentrations, whereas *Q. coccifera* maintained similar values during the whole drought period (Fig. 4C and D). The Chl *a*/Chl *b* ratios did not change during the development of

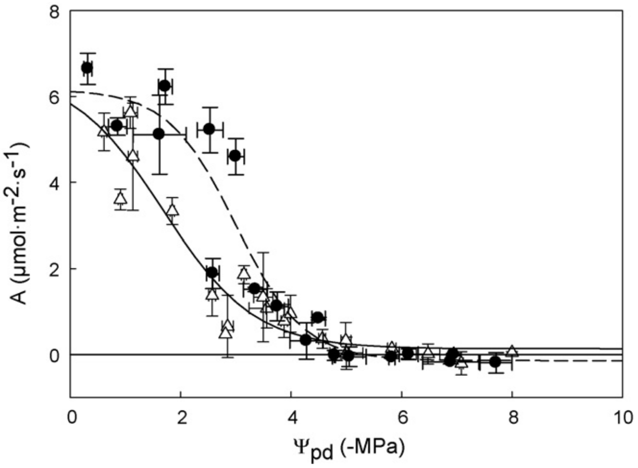

Fig. 1. Relationship between net photosynthetic rates (*A*) and predawn water potential (Ψ_{pd}) for *Q. coccifera* (circles) and *P. lentiscus* (triangles) in the early morning during the intense drought period. Pairs of points were adjusted to sigmoid regression where: $A = 0.07 + 6.3/(1 + \exp.(+(\Psi_{pd} - 2.43)/0.44))$ ($R^2 = 0.95$, $P < 0.01$) for *Q. coccifera* and, $A = 0.22 + 4.8/(1 + \exp(+(\Psi_{pd} - 1.98)/0.5))$ ($R^2 = 0.91$, $P < 0.01$) for *P. lentiscus*. Data are mean ± SE.

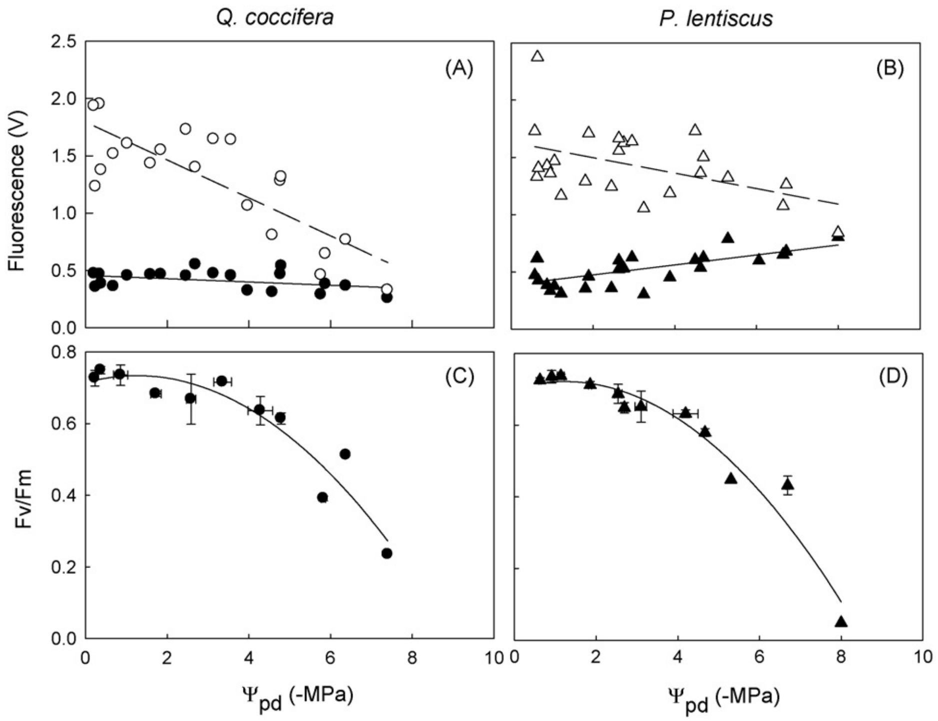

Fig. 2. Relationship between minimal Chl fluorescence (F_o, closed symbols) and maximal Chl fluorescence (F_m, open symbols) and predawn water potential (Ψ_{pd}) (A and B), and relationship between the maximal potential PSII efficiency (F_v/F_m) and predawn water potential (Ψ_{pd}) (C and D). Data are mean ± SE (C and D). Regression lines for F_v/F_m were adjusted to the sigmoid function: $y = a + b/(1 + \exp(-x - c)/d)$ where $a = -23.6$, $b = 24.3$, $c = 16.7$, $d = -2.5$, $R^2 = 0.96$, $F_{3,8} = 72.5$, $P < 0.01$ for *Q. coccifera* and $a = -1.9$, $b = 2.70$, $c = 10.3$, $d = -1.97$, $R^2 = 0.91$, $F_{3,7} = 24.1$, $P < 0.01$ for *P. lentiscus*.

water stress in either of the species investigated (Fig. 4E and F). *P. lentiscus* showed lower values of Chl *a* and *b* concentrations than *Q. coccifera*, but this difference was a consequence of lower specific leaf mass, and no differences were found when Chl concentrations were expressed on a leaf dry weight basis (data not shown).

However, being a mechanism to avoid photodamage, the VAZ pool increased slightly with drought in both species (Fig. 4G and H). The de-epoxidation state at predawn maintained low values until Ψ_{pd} reached values lower than −6 MPa (Fig. 5). When Ψ_{pd} was close to −8 MPa, A + Z were maintained during the night, with A + Z/VAZ values of approximately 0.9. No differences between species were found ($F_{1,20} = 0.48$, $P = 0.50$).

3.4. Changes in electrolyte leakage and proline accumulation

Cell membrane injury, evaluated as electrolyte leakage (ECr), was sensitive to drought stress, and differences between species were observed ($F_{1,28} = 7.25$, $P = 0.01$; Fig. 6). In both species, ECr maintained low values until the water potential reached −4 MPa. Based on this water potential, ECr reflected better membrane stability at lower water potentials in *Q. coccifera* than in *P. lentiscus*. At −8 MPa, values of ECr in *P. lentiscus* reflected a high degree of membrane destabilization (ca. 90%), whereas at the same water potential in *Q. coccifera,* ECr reached only 50% of its maximum value.

Proline accumulation followed a similar trend to that of electrolyte leakage, especially in *P. lentiscus*. *P. lentiscus* showed higher proline content at lower water potentials than *Q. coccifera* ($F_{1,15} = 7.55$, $P = 0.01$; Fig. 7). The latter species showed low values of proline content during the whole drought period, with a slight tendency to increase when the water potential reached −8 MPa ($R^2 = 0.37$, $F_{1,8} = 4.7$, $P = 0.06$).

3.5. Relationships among symplastic variables and with xylem cavitation

Relationships between symplastic and apoplastic (xylem cavitation) variables were different depending on the species and the processes involved (Figs. 8 and 9). Three types of relationships between symplastic and apoplastic variables were determined in the regression analysis: inverse, exponential and linear (Tables 1 and 2). The inverse and exponential types respond to asymptotic relationships where one variable decreases suddenly without significant changes in the other variable (i.e., relationships between *A* and the other variables). In all cases, the coefficients of determination (R^2) were higher than 0.65. This result points to a good synchronization between the different processes at cell level in *P. lentiscus* (Fig. 9). In *Q. coccifera*, the picture was somewhat different as long as a higher tolerance to drought conditions did not produce the same response among the symplastic variables (Fig. 8). In *Q. coccifera*, ECr and Pro were more resistant to drought conditions than the same variables in *P. lentiscus*. On the contrary, F_v/F_m and A + Z/VAZ showed the same response patterns in both species regardless of their tolerance to drought (Figs. 8 and 9). Regressions predominantly showed an exponential or linear relationship among symplastic variables and vulnerability to cavitation depending on the species involved (Tables 1 and 2). In fact, there was a good fit between the overall resistance of the xylem to drought-induced cavitation and other functional processes like loss of leaf area, mortality limits for each species, cell membrane injury and F_v/F_m.

To further explore the relationships between symplastic and apoplastic damages, we compared the F_v/F_m in several species with their PLC_{50} (50% loss of hydraulic conductivity) by using experimental data from the literature as well as unpublished data (Fig. 10). The results showed that the F_v/F_m are close to optimum values when the PLC_{50} are high (higher than −4 MPa), and they

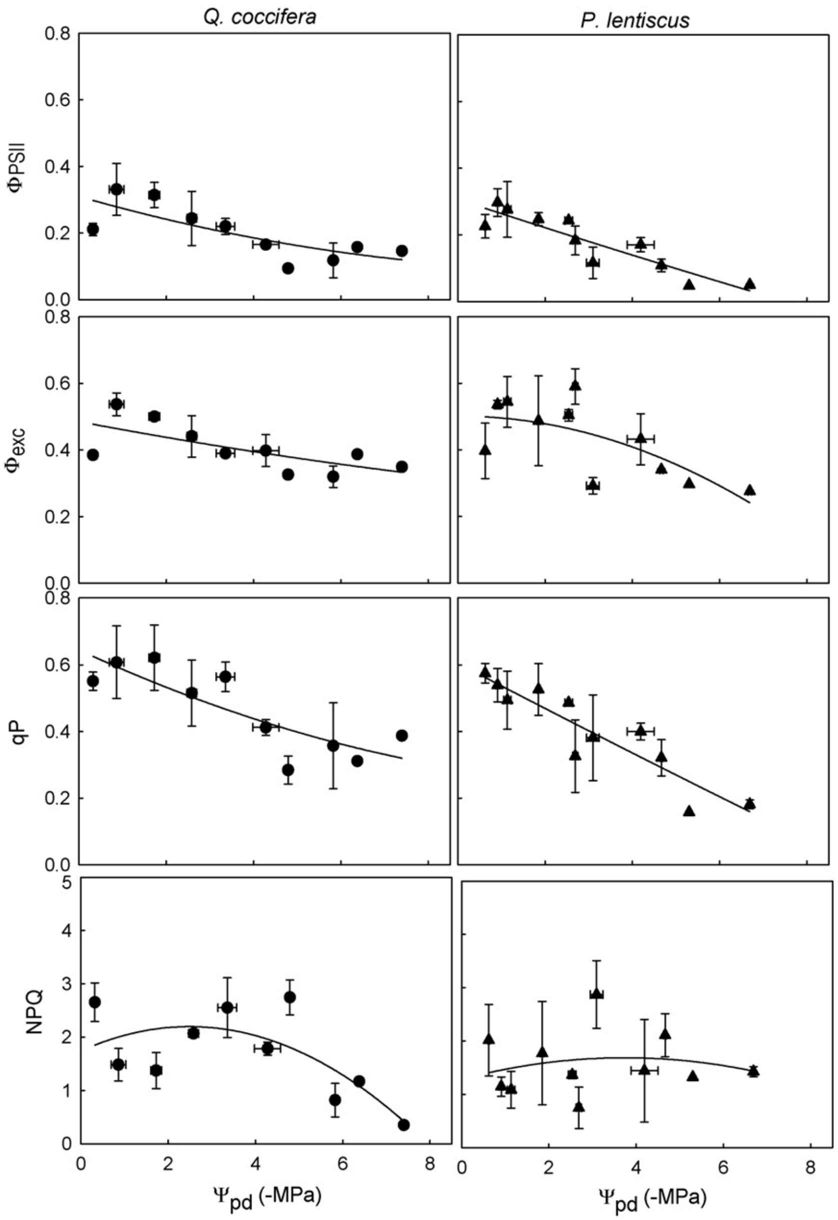

Fig. 3. Actual (Φ_{PSII}) and intrinsic ($\Phi_{exc.}$) PSII efficiency, photochemical (qP) and non-photochemical (NPQ) quenching as a function of predawn water potential (Ψ_{pd}) for *Q. coccifera* (circles) and *P. lentiscus* (triangles) in the early morning during an intense drought period. Regression lines were plotted to illustrate tendencies. Data are mean ± SE.

decrease at lower PLC_{50} values, indicating that this symplastic variable responds to intense drought conditions when xylem resistance to cavitation has reached a certain limit (i.e., below −4 MPa, the water potential at which other species have died with optimal F_v/F_m ratios).

4. Discussion

The results of this work show that both *Q. coccifera* and *P. lentiscus* had a very high resistance to drought conditions at symplastic level. This high resistance is clearly seen in their photosynthetic pigment composition, which remained fairly unchanged down to water potentials close to −8 MPa, despite slight increases in the VAZ pool (and inter-conversions within the cycle) in both species, as well as increases in lutein in *P. lentiscus* as drought stress progressed. Moreover, variables related to PSII efficiency (i.e., F_v/F_m, Φ_{PSII} and $\Phi_{exc.}$) showed similar resistance in both species, with slight decreases when photosynthetic rates approached zero. It should be noted that the NPQ of water-stressed leaves was higher than the values reported. From Fig. 2 it can be seen that F_m values of water-stressed leaves were quenched when compared to control leaves. This would result in an underestimation of NPQ, which should be referred to as “apparent NPQ” (Corcuera et al., 2005). This phenomenon has previously been described for winter leaves (Corcuera et al., 2005, and references therein).

Previous works pointed out that chloroplast is well-protected under stress conditions maintaining high PSII stability (Epron and Dreyer, 1992; Cornic and Massacci, 1996; Gallé et al., 2007). Results reported in this study agree with these previous observations and seem to indicate that drought resistance at chloroplast level would be independent of the resistance of other symplastic and apoplastic compartments. In this sense, chloroplasts in Mediterranean species under drought and high light conditions have been shown to have several strategies to avoid photoinhibitory processes, e.g., mechanisms to prevent or dissipate excessive light absorption, or mechanisms to consume the reducing power generated by PSII

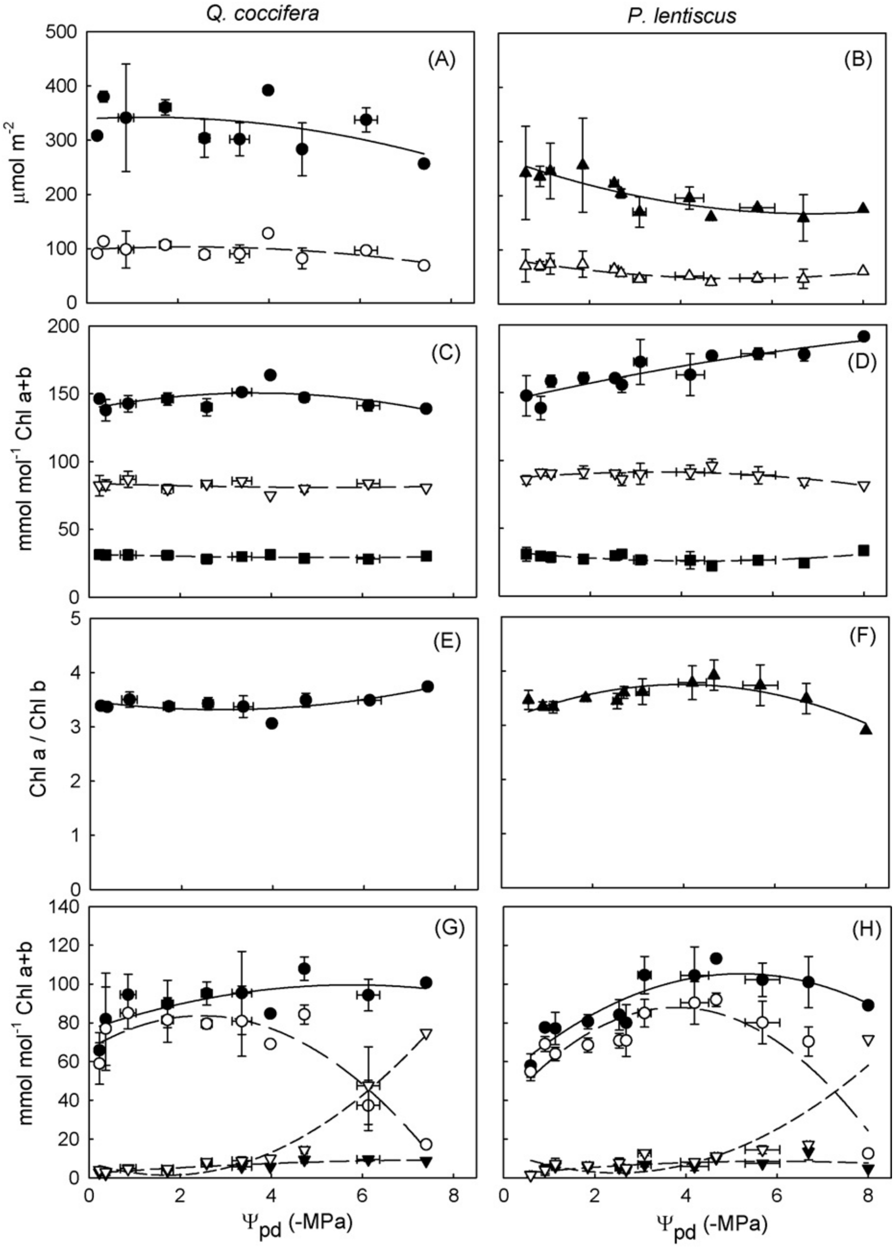

Fig. 4. Changes in pigment composition at predawn as a function of predawn water potential (Ψ_{pd}) for *Q. coccifera* (left) and *P. lentiscus* (right). (A and B) Chl *a* (closed symbols) and Chl *b* (open symbols). (C and D) Lutein (closed circles), β-carotene (open triangles) and neoxanthin (closed squares). (E and F) Chl *a*/*b* ratio. (G and H) VAZ cycle (closed circles), violaxanthin (open circles), anteraxanthin (closed triangles) and zeaxanthin (open triangles). Regression lines were plotted to illustrate tendencies. Data are mean ± SE.

(Flexas and Medrano, 2002; Demmig-Adams and Adams, 2006). From these results, we postulate that symplastic stress (cell injury) could be compartmentalized in plant cells (see below).

In spite of the high resistance at chloroplast level in both species, some differences in the other symplastic variables were found. Cell membrane injury (estimated through electrolyte leakage, ECr) and proline accumulation reflected a higher level of stress in *P. lentiscus* than in *Q. coccifera*. *P. lentiscus* showed significant increases in ECr values between −6 and −8 MPa (values were very close to 100% at −8 MPa) and in proline accumulation, just before plants died (Vilagrosa et al., 2003). For the same range of water potentials, *Q. coccifera* ECr values were only around 50% with very low proline accumulation. Several works have previously reported high values of electrolyte leakage – reflecting membrane destabilization – just before plants died, before photochemical damage appeared or in plant species with lower tolerance to drought (Epron and Dreyer, 1992; Gibon et al., 2000). In the same way, our results with *P. lentiscus* agree with other studies reporting that species increase proline content under intense stress conditions (Lansac et al., 1994; Sofo et al., 2004). *Q. coccifera,* in contrast, maintained low proline accumulation values during the whole range of water potentials, up to −8 MPa, in parallel with lower electrolyte leakage damage.

4.1. Symplast and apoplast relationships

Vulnerability to cavitation is considered one of the most important variables determining drought tolerance in plants (Tyree and Ewers, 1991), and it has been pointed out that plants with higher resistance to cavitation show greater tolerance to water scarcity and higher survival under drought conditions (Tyree et al., 2003; McDowell et al., 2008; Brodribb and Cochard, 2009). In fact, Maherali et al. (2004) observed a relationship between PLC_{50}

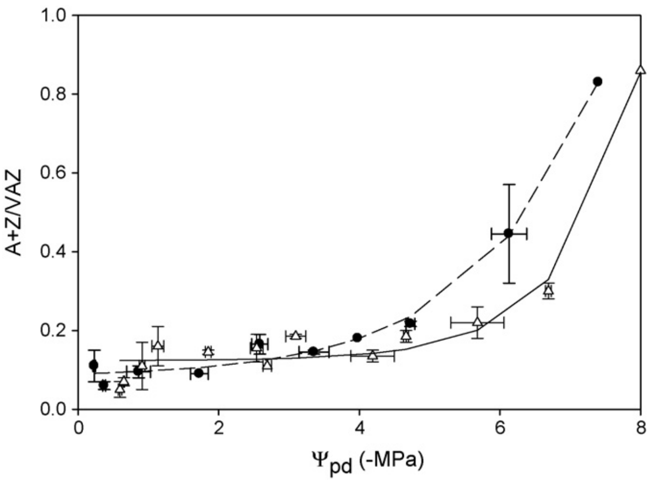

Fig. 5. Relationship between the de-epoxidation state of the VAZ cycle (A + Z/VAZ) at predawn as a function of predawn water potential (Ψ_{pd}) for *Q. coccifera* (circles) and *P. lentiscus* (triangles). Data registered were adjusted to the sigmoid function: $y = a + b/(1 + \exp(-(x - c)/d))$, ($R^2 = 0.99$, $P < 0.01$) and ($R^2 = 0.97$, $P < 0.01$) for *Q. coccifera* and *P. lentiscus*, respectively. Data are mean ± SE.

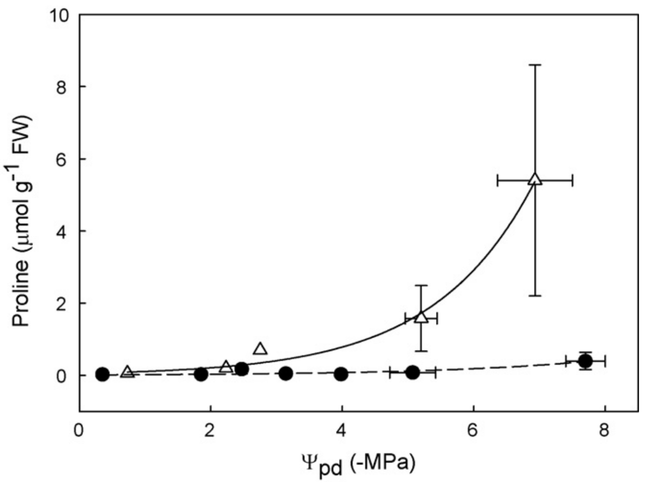

Fig. 7. Proline content on a fresh weight basis as a function of leaf water potential for *P. lentiscus* (triangles) and *Q. coccifera* (circles). Data were adjusted to the exponential function: $y = a\exp(bx)$, where ($R^2 = 0.99$, $P < 0.01$) and ($R^2 = 0.80$, $P < 0.01$) for *P. lentiscus* (solid line) and *Q. coccifera* (dashed line), respectively. Data are mean ± SE.

and vegetation types, with the lowest values for Mediterranean species. However, these authors also called attention to the lack of studies relating tolerance to desiccation, plant and soil water content, xylem cavitation and wilting symptoms (see Tyree et al., 2002; Brodribb and Cochard, 2009 for example). In a previous study, Vilagrosa et al. (2003) reported vulnerability curves to xylem cavitation, patterns of leaf dieback and survival capacity as a function of xylem water potential for two co-ocurrring Mediterranean species (*P. lentiscus* and *Q. coccifera*). The same authors observed that the resistance to xylem cavitation did not correspond to the patterns of survival in the field because *P. lentiscus* showed higher survival than *Q. coccifera* (Vilagrosa, 2002; Vilagrosa et al., 2003). These results disagree with results from previous works (McDowell et al., 2008; Brodribb and Cochard, 2009). Thus, one possibility to explore was the extent of symplastic resistance to drought in *Q. coccifera*. The results obtained in the present work do not support the hypothesis of a drought-mediated decrease in survival in *Q. coccifera* due to a higher symplastic vulnerability. On the contrary, *Q. coccifera* showed a generally good relationship between apoplast (xylem cavitation) and symplast resistance (membrane stability, PSII functionality, proline accumulation and pigment composition). Alternative explanations for the lower survival in *Q. coccifera* might be found in some other functional aspects related to root vulnerability to cavitation and rooting patterns, as shown in different studies (Vilagrosa, 2002; Martínez-Vilalta and Piñol, 2002; Costa-e-Silva et al., 2004; Pereira et al., 2006), but these possibilities deserve further investigation.

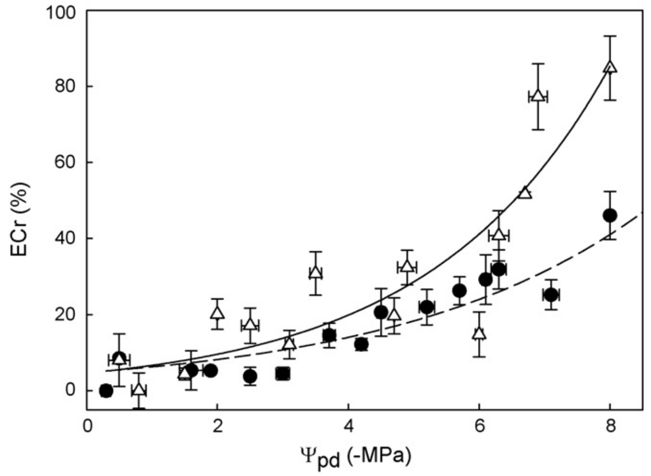

Fig. 6. Cell membrane injury (% of relative conductivity, ECr) measured by means of the electrolyte leakage technique as a function of predawn water potential (Ψ_{pd}) for *Q. coccifera* (circles) and *P. lentiscus* (triangles). Data were adjusted to the exponential function: $y = a\exp(bx)$, where ($R^2 = 0.92$, $P < 0.01$) and ($R^2 = 0.82$, $P < 0.01$) for *P. lentiscus* (solid line) and *Q. coccifera* (dashed line), respectively. Data are mean ± SE.

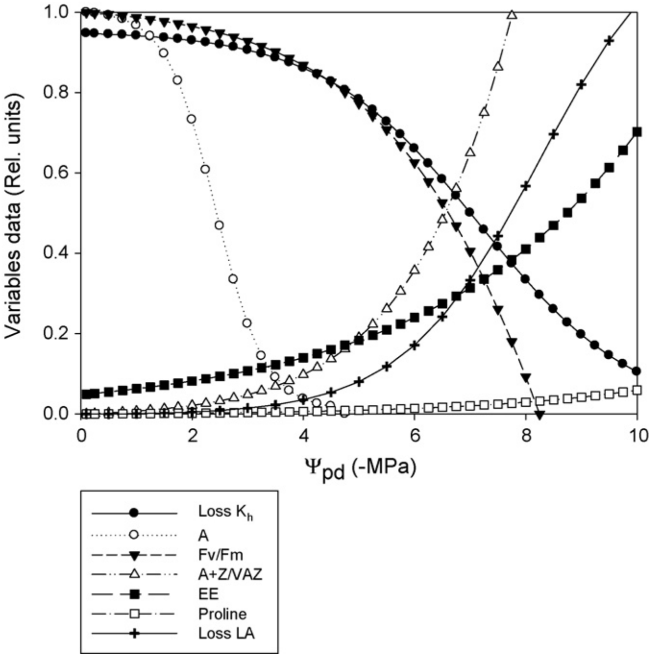

Fig. 8. Comparison between apoplastic (Loss of hydraulic conductivity, K_h), main symplastic (A, F_v/F_m, A + Z/VAZ, ECr, Pro) and loss of leaf area (LA) variables for *Q. coccifera*. For comparison purposes, the data range was from 0 (minimum relative value registered) to 1 (maximum relative value registered). For A, F_v/F_m, loss of LA and loss of K_h values close to 1 correspond to maximum values registered (optimal) and values close to zero correspond to minimum values (deficient). For ECr, Pro and A + Z/VAZ representing cell membrane damage, proline content and de-epoxidation state of the VAZ cycle, respectively, values close to zero correspond to maximum values (optimal) whereas values close to 1 correspond to values far from optimal. K_h values were obtained from Vilagrosa et al. (2003).

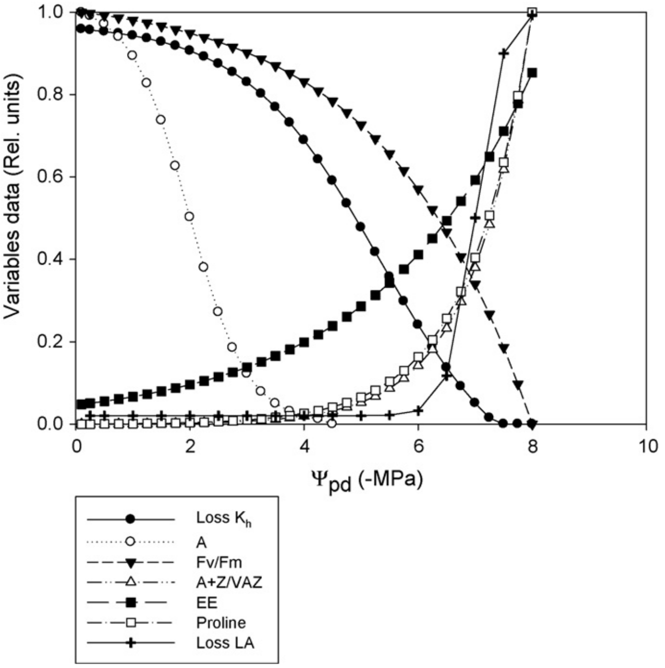

Fig. 9. Comparison between apoplastic (Loss of hydraulic conductivity, K_h) and main symplastic (*A*, F_v/F_m, A + Z/VAZ, ECr, Pro) and loss of leaf area (LA) variables for *P. lentiscus*. Legend as in Fig. 8.

Although resistance to xylem cavitation and symplastic resistance were related, both compartments followed different patterns of response (Figs. 8 and 9), as was expected. In fact, xylem cavitation (loss of K_h) showed an exponential relationship with symplastic variables (Pro, Ecr, F_v/F_m, A + Z/VAZ), but it followed a linear trend for other physiological variables (Tables 1 and 2). From this pattern of responses it can be deduced that the various symplastic compartments respond differently according to their capacity to tolerate the water deficit. In fact, when the PLC_{50} was compared with the counter-value of F_v/F_m in several species (Fig. 10), it was observed that the F_v/F_m values remained high regardless of the PLC_{50} until severe stress (lower than −4 MPa) was reached and the $F_v\underline{F}_m$ began to decrease suddenly. This behavior was found only in species very resistant to intense drought conditions (Peguero-Pina et al., 2008, 2009), whereas most species were reported to die under intense drought with unaltered F_v/F_m ratios (Morales et al., 2006; Peguero-Pina et al., 2009). From a functional and evolutionary point of view, it would not make sense to have a xylem that has very high resistance to cavitation but very low tolerance at leaf level to drought conditions (Hsiao, 1973; Pereira et al., 2006). In fact, high resistance to cavitation has been related to several traits associated with high investments in construction costs in the xylem network (Pratt et al., 2007). Therefore, plants would be expected to be able to maintain at least the same resistance at leaf level than at xylem level by means of some adaptive processes, multi-gene expression or regulatory networks (Shinozaki et al., 2003; Valliyodan and Nguyen, 2006).

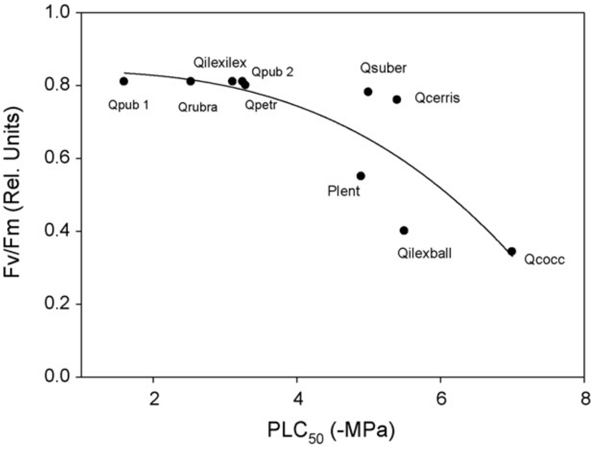

Fig. 10. Relationships between water potential at 50% loss of hydraulic conductivity (PLC_{50}) and the maximal potential PSII efficiency (F_v/F_m) at the water potential for several Mediterranean species (*Quercus coccifera*, *Quercus suber*, *Quercus ilex ilex*, *Quercus ilex ballota*, *Quercus pubescens*, *Quercus cerris* and *Pistacia lentiscus*) from data from the present study, experimental data published previously (Cochard et al., 1992; Methy et al., 1996; Tognetti et al., 1998; Vilagrosa et al., 2003; Peguero-Pina et al., 2009) and unpublished data from E. Gil-Pelegrin (see text for details).

Recently, several works (Brodribb and Holbrook, 2003; Brodribb and Cochard, 2009) have analysed the concept of security margins between stomatal closure and xylem cavitation. Wide security margins are common in plants living in environments with intense water limitations (Hacke et al., 2000). In fact, the species treated in the present work maintained wide security margins (between 4 and 6 MPa) between the point of stomatal closure and the water potentials at which plants died (Vilagrosa et al., 2003 and the present work). The results obtained in the present work would show another security margin between symplast resistance at leaf level and xylem cavitation. The delay between PLC_{50} and some of the symplastic variables (for example, until 2 MPa for ECr and Proline in *Q. coccifera*, or PLC_{50} and F_v/F_m in *P. lentiscus*) would reflect a mechanism to maintain high structural and functional stability at leaf level (Figs. 8 and 9). The functional significance would lie in the fact that in the Mediterranean climate these species experience important water oscillations during the year with periods of very intense drought stress. During these periods, high temperatures and high radiation loads can, jointly with xylem vessel cavitation and declines in leaf water supply, generate high levels of stress in plants. It is also plausible that the delay between PLC_{50} and symplastic variables could also be related to the possibility of xylem refilling during the autumn rains. Therefore, the capacity to maintain costly structures (cell membranes, metabolic and photosynthetic machinery, etc.) would be an important factor for the survival and competitiveness of this species in water-limited environments.

In summary, this paper provides a coordinated analysis of plant responses to drought at the whole-plant level (i.e., photosynthetic and hydraulic processes). The symplastic and the apoplastic compartments showed good convergences during an intense drought period. However, it seems that the different symplastic compartments may respond differently according to their capacity to tolerate the water deficit or regulatory networks, especially at very low water potentials. Within the symplast, the photosynthetic machinery appears to behave independently due to differences with the other compartments and the similar pattern of response between both species. These processes cannot explain the differences found in field condition survival under drought, but they can provide worthwhile data for coordinating processes at plant and leaf level in response to stress conditions.

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