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Drought responses in Aleppo pine seedlings from two wild provenances with different climatic features

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Abstract

Global warming will likely exacerbate the negative effects of limited water availability in the Mediterranean area. The Italian Aleppo pine (*Pinus halepensis* Mill.) provenances are distributed along the coasts except Otricoli provenance growing in an unusual location between 300 and 1,000 m a. s. l., in Umbria (central Italy). The aim of the present study was to investigate the photosynthetic response to a 28-day-long drought and to a subsequent reestablishment of water availability in Otricoli and North Euboea (Greece) provenances, representing different locations along a rainfall gradient in the natural range of this species. Six-month-old seedlings were used in this experiment since at this age Aleppo pine plants in Mediterranean climate face their first water stress potentially affecting plant survival. Water potential (ψ_w), net photosynthesis and stomatal conductance decreased during drought in both provenances and showed minimal values 28 days after beginning the treatment (DAT). Otricoli seedlings adjusted ψ_w gradually as the stress level increased and 21 DAT showed a lower ψ_w than North Euboea. In contrast, in North Euboea seedlings ψ_w that was not affected until 21 DAT rapidly dropped to a minimum of –3.81 MPa 28 DAT. At the onset of the stress the intercellular CO_2 concentration (C_i) was reduced, and the "instrinsic" water-use efficiency (WUE_i) was enhanced in both provenances, as stomatal conductance decreased more rapidly than photosynthesis. However, 28 DAT, C_i increased and WUE_i decreased as stomatal conductance and photosynthesis declined to minimum levels, revealing nonstomatal limitations of photosynthesis. A rapid decrease in PSII maximal photochemical efficiency estimated by chlorophyll (Chl) fluorescence (F_v/F_m) was also observed when the stress became severe. At the final stage of water stress, North Euboea seedlings maintained significantly higher values of F_v/F_m than Otricoli seedlings. Upon rewatering, photosynthesis did not fully recover in Otricoli seedlings (41 DAT), while all other parameters recovered to control levels in both provenances. No drought-induced physiological differences were consistent with the regional climatic features of these two provenances. Our results suggest that phenotypic plasticity in drought response may help Otricoli provenance cope with global warming, but that recurrent drought episode may slow down the primary productivity of this provenance.

Introduction

The global average temperature increased about 0.6°C during the last 100 years and is predicted to continue to rise at a rapid rate even under a conservative scenario (Root *et al.* 2003, Craig *et al.* 2010). Global warming will likely reduce water availability thus increasing frequency and severity of drought stress. In the Mediterranean area, summer droughts already negatively affect growth of natural vegetation and cultivated plants, and ongoing climate changes are likely to further exacerbate the negative effects of limited water resources on forests ecosystems.

Drought stress decreases the rate of photosynthesis as

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Abbreviations: C_i – intercellular concentration CO_2; Chl – chlorophyll; F_v/F_m – maximum photochemical efficiency of PSII; g_c – stomatal conductance to CO_2; g_s – stomatal conductance to H_2O; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; WUE_i – intrinsic water-use efficiency; ψ_w – predawn leaf water potential.

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a consequence of the increasing diffusive resistances and/or by directly damaging carbon metabolism (Johnson *et al.* 1987, Gimenez *et al.* 1992, Sharkey and Seemann 1989, Chaves 1991, Quick *et al.* 1992, Evans and Loreto 2000). These two limitations generally occur sequentially, with stomatal and mesophyll resistances limiting CO_2 acquisition at early stages of drought stress, and leading to biochemical limitations when the stress becomes more severe (Flexas *et al.* 2004). However, restricted CO_2 availability under drought stress may also lead to inefficient use of incident light and to increased susceptibility to photodamage (Powles 1984, Maxwell *et al.* 1995, Baroli and Melis 1998). Fluorescence measurements may provide useful information about photosynthetic performances of drought-stressed plants, and especially of photosystem II functionality (Maxwell and Johnson 2000). In particular, a sustained decrease of the variable to maximum fluorescence ratio (F_v/F_m), coupled to a reduced minimum fluorescence yield (F_o), as measured on dark-adapted leaves, indicate the occurrence of photoinhibitory damage. Such damage was indeed observed in response to high temperature, excess of absorbed light, water stress and other environmental stress factors (Gamon and Pearcy 1990, Maxwell and Johnson 2000).

Due to the above specified rapid changes in climatic conditions, the characterization of drought-tolerant plant species is receiving great attention for reforestation of the Mediterranean region (Semerci and Cobb 2010, Regato 2008, Prieto *et al.* 2009). The Aleppo pine *(Pinus halepensis* Mill.) is widely distributed around the Mediterranean area, and grows in very dry habitats (Nahal 1981). *P. halepensis* is a pioneer species and is considered a suitable pine species for forestation purposes, mainly because of its tolerance to drought (Schiller 2000).

Several studies have been performed on the response of Aleppo pine to drought. Aleppo pine trees occupying different geographic areas of the natural range of the species (hereby defined as provenances) showed significant variations in water relations and hydraulic architecture (Calamassi *et al.* 1980, Falusi *et al.* 1983, Tognetti *et al.* 1997, Borghetti *et al.* 1998, Calamassi *et al.* 2001). However, very little is known about how changing water conditions will impact on photosynthesis of Aleppo pine seedlings.

In Italy, native Aleppo pine forests grow along the coastal areas. However, one provenance from Umbria region in central Italy (Otricoli) grows in an unusual location, 300–1,000 m a.s.l. Sylvicultural and ecophysiological investigations of plant response to water stress showed differences between Otricoli and the other Italian provenances (Falusi *et al.* 1983). Eccher (1996) described Otricoli trees as the most interesting from a sylvicultural point of view since they are characterized by better shape, height, and cylindrical stem than all other Italian Aleppo pine provenances.

Previous investigations provided support for a Near-Eastern origin of Otricoli trees (Schiller and Brunori 1992). Studies using isoenzymes identified two groups within the range of *P. halepensis*: one in the western part (from Spain to Italy and Morocco to Libya) and the other in the eastern part of the Mediterranean basin. The latter also included Otricoli, showing similarities with native Israeli provenances (Schiller *et al.* 1986). Paleo-pollen studies and analysis of allele frequencies of Aleppo pine forests in Greece and Turkey suggest that Otricoli cannot be considered a remnant of an ancient East Mediterranean distribution and point to its artificial introduction in very ancient times (Schiller and Brunori 1992). Otricoli trees introduced into Umbria region could have developed specific functional adjustments under high water availability in the new environmental conditions, since phenotypic expression of single genotypes is largely conditioned by the environment (Sultan 2000).

In consideration of the predictions of a global temperature increase and consequently more extreme drought conditions which will threaten the distribution (persistence, mortality, genetic adaptation) of the Otricoli provenance as well as other provenances of importance for sylviculture, the main objective of the present study was (*1*) to investigate the responses of Otricoli plants during drought-recovery cycles. We focused in particular on gas exchanges and Chl fluorescence to determine *in vivo* transient or permanent limitation to photosynthesis that could impair plant growth. Moreover, the study also aims (*2*) to evaluate how the long-term adaptive adjustments under the environmental constraints in Umbria region could have altered the drought response in Otricoli trees of Near East origin; (*3*) to compare the ecophysiological response to drought of the Otricoli provenance and of a North Euboea (Greece) provenance, selected as a representative of a xeric area.

Materials and methods

Plants and growth conditions: The study was conducted on six-month-old seedlings from two provenances of Aleppo pine (*Pinus halepensis*): Otricoli (Italy) and North Euboea (Greece) representing different locations along a climate gradient in the natural range of this species (Table 1). Six-month-old seedlings were used in this experiment since at this age Aleppo pine plants first face drought stress that can affect their survival in a Mediterranean climate.

Sixty seedlings per provenance were selected for dimensional uniformity and transplanted to 2.5-liter plastic pots filled with a mixture of sandy-loam soil and peat. At the age of six months the seedlings were moved to a (2 × 2 × 2 m) walk-in growth chamber programmed with a 16-h photoperiod, a photosynthetic photon flux density (PPFD) of 500 μmol(photon) m^{-2} s^{-1} at the top of

Table 1. Geographical and climatic features of original zones of *Pinus halepensis* Mill. provenances investigated. Provenances are also indicated by the codes of access used by the FAO (1974).

Provenance	Country	Latitude N	Longitude E	Altitude [m a.s.l.]	Annual rainfall [mm]	Mean annual temperature [°C]
North Euboea (A3)	Greece	38°56’	23°17’	125	432	17.9
Otricoli (A26)	Italy	42°26’	12°28’	400	830	13.0

the foliage, 17–24°C night-day temperature, and 65% relative humidity. Plants were fertilized once a week with half strength Hoagland’s nutrient solution.

After a three-week acclimation to the climatic conditions of the chamber, measurements were initiated under such a control condition (control or prestressed plants). This was day 0 of the treatment, The irrigation was then stopped for 28 days after beginning the treatment (DAT), after which the seedlings were reirrigated to avoid irreversible damage. Twig water potential, leaf stomatal conductance, photosynthesis, transpiration, and Chl fluorescence measurements were performed in the growth chamber during drought development (7, 21, and 28 DAT) and during recovery from drought-stress (35 and 41 DAT).

Water potential and gas-exchange measurements: Predawn water potential (ψ_w) was measured on five plants per provenance using a Scholander pressure chamber (*PMS*, Corvallis, OR). Gas-exchange properties were measured at midday, on 15 plants per provenance using a portable infrared gas analysis system (*Ciras-1, PP-System*, UK) equipped with a conifer cuvette with a light unit; ambient CO_2 was set at 360 ppm, air humidity at 60–70%, temperature at 25°C and PPFD at 800 μmol m^{-2} s^{-1}. These conditions were selected as they yielded maximal photosynthetic rates under preliminary trials in control conditions (data not shown). After each measurement, the needles were cut and put in plastic bags on ice until analysis of leaf area, which was performed with a *Licor-3000* (*LiCor*, NE, USA) planimeter. Net photosynthetic rate (P_N) and stomatal conductance to CO_2 (g_c) were expressed on a needle area basis. The intercellular CO_2 concentration (C_i) was calculated according to von Caemmerer and Farquhar (1981). WUE_i was defined as μmol of net CO_2 uptake per mmol of lost H_2O ($WUE_i = P_N/g_s$) where g_s is the stomatal conductance to water. The difference between g_c and g_s was calculated using the diffusion coefficient of the two gases in air, under a turbulent flux of air.

Chl fluorescence measurements were performed on intact needles, with a portable *PAM-2000* fluorimeter (*Walz*, Effeltrich, Germany), on four plants per provenance. Needles of plants darkened for 8 h during the night course of the growth climate chamber cycle were used. Minimal (dark) fluorescence (F_0) yield was obtained upon excitation with a weak measuring beam from a pulse light-emitting diode, while maximal fluorescence yield (F_M) was determined after exposure to a 0.8-s saturating pulse [> 10,000 μmol(photon) m^{-2} s^{-1}] of white light. The maximal PSII photochemical efficiency (F_v/F_m) was then estimated as the ratio of variable ($F_V = F_m - F_0$) to maximal fluorescence (Schreiber *et al.* 1994). Measurements were performed during the morning hours between 08:00 and 09:00 h.

Statistical analysis: Data were not normally distributed (*Kolmogorov-Smirnov* one-sample test) and were analysed by the *Friedman* test, the nonparametric analogue of repeated measures analyses of variance, and by the nonparametric *Mann-Whitney U*-test for samples from the two Aleppo pine provenances collected on the same day. Differences were accepted when significant at the 5% level. Statistical analyses were performed by using *SYSTAT 12.0* software (*Systat Software Inc.*, USA).

Results

All quantitative ecophysiological traits were negatively affected by drought, as indicated by the *Friedmann* test (Table 2).

When comparing the response to drought of the two provenances, 21 DAT ψ_w dropped significantly more in Otricoli than in North Euboea seedlings, but 28 DAT ψ_w of North Euboea seedlings was the lowest (–3.81 MPa) while ψ reached –2.60 MPa in Otricoli plants (Fig. 1). *Mann-Whitney U*-test between the two provenances (d.f. = 1, $n = 99$) indeed showed significant differences in ψ_w on days 21 ($n = 10$, $\chi^2 = 3.9$, $p<0.05$) and 28 ($n = 10$, $\chi^2 = 4.8$, $p<0.05$). However, after rewatering both provenances restored ψ_w similar to those found in control conditions, before drought occurrence.

Differences of g_c between provenances were not significant in control conditions and during the drought stress. In both provenances g_c dropped immediately after starting the stress, and reached a minimum, corresponding to totally closed stomata, 28 DAT (Fig. 2). The stomatal conductance recovered to only slightly more than half of the initial rate after rewatering (41 DAT), in both provenances.

Table 2. Statistical results of the *Friedman* test examining the variations of predawn water potential (ψ_w), stomatal conductance (g_c), net photosynthetic rate (P_N), relative fluorescence (F_v/F_m), intercellular CO_2 concentration (C_i), and instantaneous water-use efficiency (WUE_i) in seedlings of two Aleppo pine provenances during the course of a drought stress-recovery cycle: d.f. – degrees of freedom; χ^2 value; *p*-value where * $p<0.05$, ** $p<0.01$, and *** $p<0.001$.

Variable	Provenance	N° samples	d.f.	$\chi^2(p)$
ψ_w [MPa]	North Euboea	27	5	13.8^{*}
	Otricoli	26	5	12.1^{*}
g_c [mmol (CO_2) m^{-2} s^{-1}]	North Euboea	92	5	43.8^{***}
	Otricoli	92	5	38.9^{***}
P_N [µmol(CO_2) m^{-2} s^{-1}]	North Euboea	91	5	35.8^{***}
	Otricoli	94	5	46.2^{***}
F_v/F_m	North Euboea	18	5	10.8^{*}
	Otricoli	18	5	13.6^{*}
C_i [ppm(CO_2)]	North Euboea	92	5	16.9^{**}
	Otricoli	94	5	39.8^{***}
WUE_i [µmol(CO_2) mmol(H_2O)$^{-1}$]	North Euboea	92	5	16.9^{*}
	Otricoli	92	5	28.1^{*}

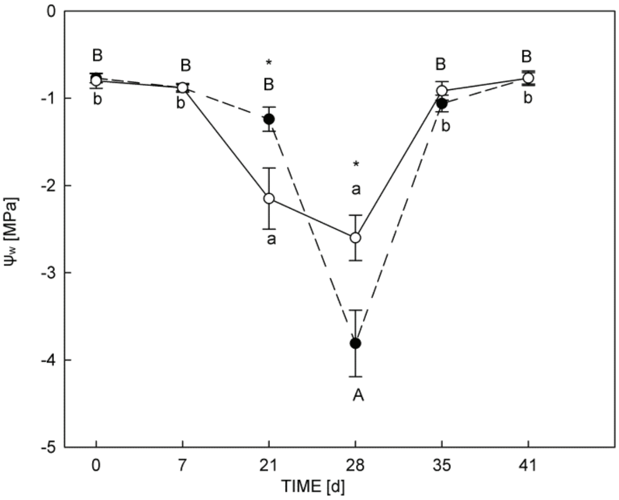

Fig. 1. Predawn water potential (ψ_w), during the course of a drought stress-recovery cycle in seedlings of *Pinus halepensis* Mill. from Otricoli (*open circles*) and North Euboea (*filled circles*) provenances. *Bars* represent means ± SE of 5 measurements except for Otricoli ($n = 4$) on day 0, Otricoli ($n = 4$) and North Eoboea ($n = 4$) on day 35; bars not visible indicate SE smaller than the symbol. *Different letters* indicate significant differences within each population (*Friedman* test, $p<0.05$), while an asterisk indicates significant differences between provenances (*Mann-Whitney U*-test, $p<0.05$); *lowercase letters* refer to measurements made for Otricoli seedlings; *uppercase letters* refer to measurements made for North Euboea seedlings.

As also observed for ψ_w, P_N did not decrease after 7 d of drought, but then dropped strongly and reached the minimum values 28 DAT, on both provenances (Fig. 2). *Mann-Whitney U*-test between the two provenances showed significant differences in P_N of the two provenances on day 21 ($n = 40$, $\chi^2 = 3.8$, $p<0.05$) when photosynthesis was less inhibited in the North Euboea seedlings. Two weeks after rewatering, P_N did not completely recover in Otricoli seedlings while it recovered to control level in North Euboea plants. The difference in photosynthesis between the two genotypes at 41 DAT was statistically very significant ($n = 23$, $\chi^2 = 7.0, p<0.01$).

PSII maximal photochemical efficiency, estimated by the fluorescence parameter F_v/F_m, was not affected by the early stages of drought, but dropped in both provenances 21 DAT, reaching minimal values 28 DAT (Fig. 3). The reduction of F_v/F_m was more rapid in Otricoli seedlings, as 21 DAT a lower value than in North Euboea seedlings was observed. After rewatering, both provenances showed a similar recovery of PSII efficiency, attaining 41 DAT F_v/F_m values similar to those recorded in control leaves.

C_i and WUE_i did not vary significantly between the two provenances, but these two parameters were significantly influenced by drought stress. Under increasing drought stress, C_i slightly declined in the two provenances, but 21 DAT the decline was more remarkable in North Euboea seedlings (Fig. 2). Under heavy stress conditions (28 DAT), C_i increased in both provenances and reached values significantly higher than in control leaves. Upon rewatering, C_i decreased again in both provenances and values detected 41 DAT were significantly lower than in control plants.

WUE_i significantly increased as water stress was induced, mirroring the earlier reduction of stomatal conductance with respect to P_N (Fig. 2). WUE_i dropped when the stress was severe, with no significant differences between provenances. During rewatering, WUE_i was restored in both provenances with similar trends.

Discussion

Six-month-old pine seedlings only have primary needles along the stem axis. In fact, in the first years of development, *P. halepensis* shows free or indeterminate growth (Calamassi *et al.* 1988). The fine structure of the primary needles of the North Euboea provenance was similar to that observed in other Aleppo pine provenances including Otricoli pine trees, including a thin cuticle, living epidermal cells with relatively thin walls and unoccluded stomata, wide intercellular spaces in the mesophyll, and unmodified endodermal cell walls (Boddi

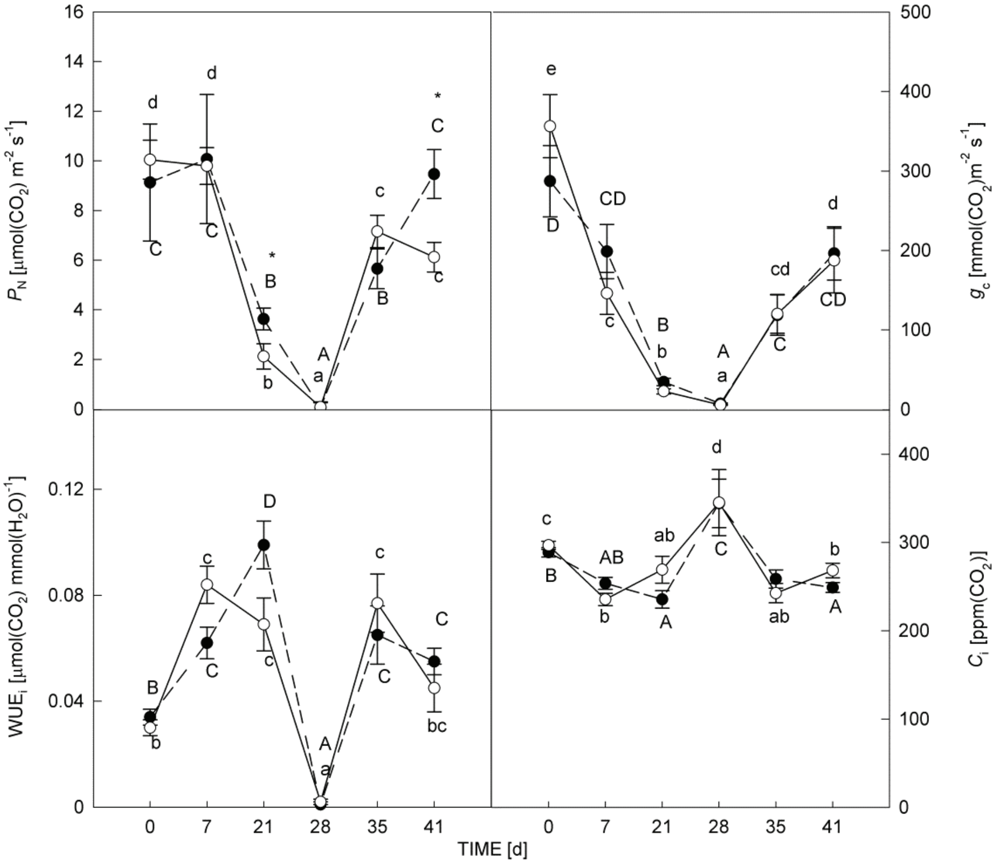

Fig. 2. Net photosynthetic rate (P_N), stomatal conductance (g_c), instantaneous water-use efficiency (WUE$_i$), and intercellular CO$_2$ concentration (C_i) during the course of a drought stress-recovery cycle in seedlings of *Pinus halepensis* Mill. from Otricoli (*open circles*) and North Euboea (*filled circles*) provenances. *Bars* represent means ± SE of 15 measurements except for Otricoli ($n = 12$) on day 0, Otricoli ($n = 14$) and North Euboea ($n = 14$) on day 35, Otricoli ($n = 11$) and North Euboea ($n = 12$) on day 41; bars not visible indicate SE smaller than the symbol. *Different letters* indicate significant differences within each population (*Friedman* test, $p<0.05$), while an asterisk indicates significant differences between provenances (*Mann-Whitney U*-test, $p<0.05$); *lowercase letters* refer to measurements made for Otricoli seedlings; *uppercase letters* refer to measurements made for North Euboea seedlings.

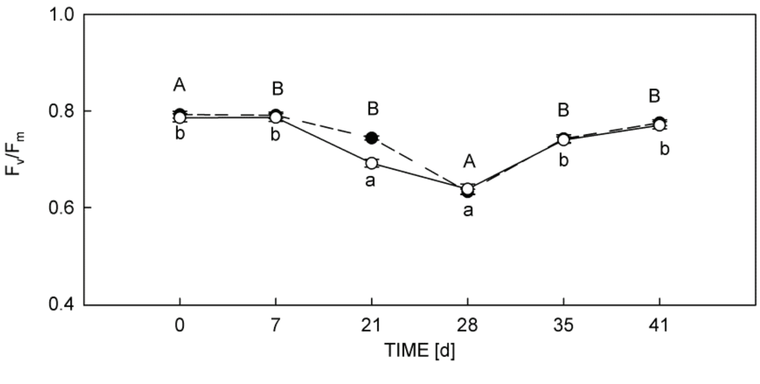

Fig. 3. Relative fluorescence (F_v/F_m) during the course of a drought stress-recovery cycle in seedlings of *Pinus halepensis* Mill. from Otricoli (*open circles*) and North Euboea (*filled circles*) provenances. *Bars* represent means ± SE of 3 measurements; bars not visible indicate SE smaller than the symbol. *Different letters* indicate significant differences within each population (*Friedman* test, $p<0.05$); *lowercase letters* refer to measurements made for Otricoli seedlings; *uppercase letters* refer to measurements made for North Euboea seedlings.

et al. 2002; data not shown). This indicates that the typical morpho-anatomic characteristics of secondary pine needles, that are generally associated with increased resistance to aridity (Calamassi 1986), were not yet present in all young provenances and did not affect comparison between provenances.

The response to drought of the seedlings of the two provenances coming from habitats with different climate was overall very similar. However, few differences were noticed. The reduction of ψ_w during drought development occurred clearly earlier in Otricoli than in North Euboea seedlings, although in the latter ψ_w attained more negative values when the stress was at peak severity and stomata were totally shut. This difference in the kinetics and extent of ψ_w reduction could be ecologically explained when considering the climatic features of the provenances' original sites: Otricoli zone of origin presents a moderate meso Mediterranean climate, with 40–75 biologically dry days according to Gaussen's xerothermal index, a yearly rainfall of 830 mm, and a mean annual temperature of 13°C; whereas the climate of North Euboea is markedly thermo-Mediterranean, with 125–150 biologically dry days, a yearly rainfall of 432 mm and a mean annual temperature of 17.9°C (UNESCO-FAO, 1963). North Euboea seedlings are therefore more adapted to drought conditions, but this seems to involve a "water-saving" strategy only when the stress in not severe. Under severe drought conditions, the very negative ψ_w value of North Euboea seedlings is related to an extremely low water content (less than 60% of fresh mass, data not shown); this is common in Aleppo pine seedlings (Calamassi, personal communication) and suggests that a different mechanism allows the plants to resist dehydration and to recover promptly after rewatering.

The North Euboea seedlings also showed a somehow lower g_c in nonstressed leaves, but g_c declined as rapidly as in Otricoli seedlings upon stress occurrence. This indicates that any putative adaptation to drier environments does not involve a different stomatal control of water loss through transpiration. The more rapid decline in g_c as opposed to ψ_w in the two seedlings under progressive drought could be explained by a stomatal sensitivity to nonhydraulic signals during the initial stages of soil drying. It has been suggested that biochemical and stomatal responses might be mediated by plant hormones, primarily ABA, originated in dehydrating roots and translocated to the shoots by the transpiration stream (for a review see Umezawa *et al.* 2010). As this signalling action seemed to occur with equal intensity and timing in both provenances, it is unlikely to have set mechanisms of adaptation to drought.

Interestingly, photosynthesis was not affected by the reduction of stomatal conductance a week after drought-stress imposition. This is not in agreement with the general idea that the photosynthetic limitation in plants exposed to moderate soil and/or atmospheric water deficits is primarily due to stomatal closure and to reduced CO_2 diffusion in the mesophyll (Lawlor 1976, Ni and Pallardy 1991, Parker and Pallardy 1991, Ellsworth and Reich 1992, Ort *et al.* 1994, Chaves *et al.* 2002, 2003; Flexas *et al.* 2004). Except than in few cases (Sharkey and Seeman 1989, Tezara *et al.* 1999) photosynthesis does not seem to be limited by decreased activity of photosynthetic enzymes or other biochemical causes when the stress is mild. However, it is still debated whether stomatal and nonstomatal limitations of photosynthesis occur independently or in sequence (Lawlor and Cornic 2002, Flexas *et al.* 2004). In fact, the photosynthetic apparatus of Aleppo pine seems to be resistant to mild drought stress, and our results demonstrate that in these plants, independently of their adaptation to dry environments, photosynthesis is not affected even under sustained reductions of stomatal conductance and intercellular CO_2 concentration.

When a nonstomatal limitation of photosynthesis is effective, it causes an increase of C_i because in the mesophyll stomata allow enough CO_2 that cannot be used by photosynthesis. Indeed, such a nonstomatal effect was observed when the drought stress became severe. Such a biphasic response to increasing drought seems common in conifers as nine species also showed a reduction of C_i as g_c decreased under mild stress, and then a sudden increase in C_i as g_c reached a minimum in severely stressed needles (Brodribb 1996). The nonstomatal limitation of photosynthesis can be due to impairment of photochemistry or biochemistry of photosynthesis. Some authors have suggested that under mild water stress PSII photochemistry is not affected (Genty *et al.* 1987), while under severe stress both photosystems are damaged (Genty *et al.* 1987, Meyer and De Kouchkovsky 1993). Alterations of PSII activity might not indicate direct photochemical damage by water stress; they may rather be a consequence of transient photoinhibition occurring because of inefficient light use by photosynthesis, especially when photosynthesis is previously quenched by a diffusional limitation (Baker and Bowyer 1994, Flexas *et al.* 2004).

The efficiency of photochemical reactions driving photosynthesis was assessed *in vivo* by Chl fluorescence measurements (Genty *et al.* 1989, Maxwell and Johnson 2000). The fluorescence parameter F_v/F_m, in particular, indicates the maximal efficiency of PSII in dark-adapted leaves (Björkman and Demmig 1987), and can be used to detect the occurrence of photoinhibitory damage, in response to many environmental stresses (Maxwell and Johnson 2000). The F_v/F_m ratio was not affected by mild drought stress, but progressively declined as ψ_w and g_c reached the minimum. Thus, the photochemical reactions might indeed limit photosynthesis in severely drought-stressed Aleppo pine needles. Interestingly, the North Euboea provenance showed a slower decrease of F_v/F_m in response to the increasing level of drought, which was associated to a significantly higher photosynthesis than in the Otricoli seedlings after 21 days of drought (Fig. 2). This suggests that North Euboea seedlings have a more efficient photoinhibition defence mechanism during the first stages of water stress development.

PSII functionality of both provenances was restored when water was supplied again. If the proteins of PSII were denatured by the stress, a rather long recovery period would be expected to resynthesize the photochemical apparatus, affecting both oxygen-evolving centers and reaction centers (Havaux *et al.* 1987). On the other hand, if the functionality of PSII is affected by a down-regulation of the electron transport, due *e.g.* to inefficient electron use by Rubisco, then the recovery may occur more rapidly, generally in a few days (Bukhov and Carpentier 2004). We did not perform analysis of fluorescence in illuminated leaves that could provide further indication on whether the decay and fast recovery of F_v/F_m was associated to a similar kinetics of the photochemical quenching (q_P) and to a substantially unaffected nonphotochemical quenching coefficient (q_N) as it would be expected if the photosystem structure is not damaged. However, based on our results in darkened leaves, and particularly on the fast recovery time-course, we speculate that the drought stress was not sufficient to denature PSII in both provenances of Aleppo pine.

The question remains why photosynthesis did not fully recover in Otricoli seedlings after a 13-day rewatering. A plausible explanation could be that in this provenance drought induces a change in hydraulic conductivity that consequently limits gas exchange (Sperry *et al.* 1988) and, in general, the ability of plants to cope with water stress (Pockman and Sperry 2000, Sperry 2000, Terashima 1992, Borghetti *et al.* 1998). Alterations of xylem hydraulic conductivity in response to the drought treatment have been reported in two-year-old seedlings of Otricoli and other Italian provenances (Tognetti *et al.* 1997).

Our data did not show a relationship between drought-stress response and the geographical distribution of these provenances, while Schiller and Atzmon (2009) found that Otricoli and Mt Carmel (Israel) provenances showed a significantly lower WUE_i than provenances from Telagh (Algeria) and Elea (Greece) growing in a trial at Bet Dagan (Israel). Our data rather support earlier reported similarities between Otricoli and Aleppo pine provenance from more xeric habitats. Pressure/volume curves showed that Otricoli behaved similarly to provenances like Kassandra, North-Euboea and Litorale Tarantino that are located in areas with a far greater number of dry days (Calamassi *et al.* 2001). No significant variations in transpiration and photosynthesis were observed when these physiological parameters were compared in Otricoli and in other three provenances from drier areas: Mt Carmel (Israel), Telagh (Algeria) and Elea (Greece) (Schiller and Atzmon 2009). However, long-term selection under ecological constraints of Umbria region could have influenced seed germination of Otricoli pine trees (Calamassi *et al.* 1984) that showed higher frost resistance (Falusi *et al.* 1984) and a lower number of stomata per linear cm of leaf than the other Italian provenances (Calamassi 1986).

Conclusions: Ultimately, our study suggests that the photosynthetic apparatus of six-month-old seedlings of the two Aleppo pine provenances that grow in areas with different water availability throughout the year is resistant to mild drought stress, whereas more severe drought induces nonstomatal limitations of photosynthesis, impairing the photochemistry of photosynthesis. Otricoli provenance seemed to be unable to recover full photosynthetic activity after a severe drought as rapidly as provenances from more arid stations. However, this provenance showed physiological plasticity in response to drought, as also observed in other studies. This is in agreement with findings that Aleppo pine is considered a pioneer species (Schiller 2000) and widespread colonizing species are often characterized by high phenotypic plasticity (Sultan 2000).

Further investigations should specifically address the potential adaptive value of these ecophysiological mechanisms that underline short-term plastic response to drought. Differences between taxa in adaptive phenotypic plasticity might be an important factor when considering intraspecific competition and species-specific structure in natural ecosystems, as well as provenance resistance to ongoing and future climate warming and associated longer dry periods.

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