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Original article

Resistance to water stress in seedlings of eight European provenances of *Pinus halepensis* Mill.

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Abstract – In this study, pressure/volume curves were performed on 24-week-old seedlings of eight European provenances of *Pinus halepensis* Mill. subsp. *halepensis* after one week of water stress (–0.033, –0.4, –0.8, –1.2, –1.6 MPa). *P. halepensis* showed osmotic adjustments as a response to water stress, although the response varied between the provenances. Apoplastic water remained relatively constant. The elasticity module did not differ significantly. Water deficit at incipient plasmolysis and water content decreased as the stress increased. Water potential was markedly negative, even in seedlings not subjected to stress. The provenances from less xeric sites behaved similarly to species from non-arid sites, while those from more xeric sites displayed the strategy typical of drought-tolerant species. The impact on all provenances of one week of low-intensity water stress (–0.4 MPa) was slight. As the stress increased, the response varied between the provenances: N-Eubea > Kassandra > Litorale tarantino ≅ Guardiola > Otricoli ≅ E-Bouches du Rhône ≥ Hérault ≅ N-Vaucluse (in decreasing order).

Aleppo pine / drought resistance / geographic variation / pressure-volume curves / provenances

Résumé – Résistance au stress hydrique des plantules appartenant à huit provenances européennes de *Pinus halepensis* Mill. Sur des plantules âgées de 24 semaines, appartenant à 8 provenances de *Pinus halepensis* (Mill.) subsp. *halepensis*, des courbes pression-volume ont été effectuées après une semaine de stress hydrique (–0,033, –0,4, –0,8, –1,2, –1,6 MPa). *P. halepensis* a montré des ajustements osmotiques en réponse au stress hydrique, bien que de façon différenciée parmi les provenances. L'eau apoplastique a été relativement constante. Le module d'élasticité n'a pas différé significativement. Le déficit hydrique en début de plasmolyse et la teneur en eau ont baissé avec l'augmentation du stress. Le potentiel hydrique a été très négatif, même en absence de stress. Les semences provenant des milieux les moins secs se sont comporté de façon identique aux espèces des milieux non arides, tandis que les provenances originaires des milieux les plus secs ont révélé la stratégie caractéristique des espèces tolérant la sécheresse. En conclusion les provenances ont été insuffisamment influencées par une semaine de stress hydrique de faible intensité. En présence d'un accroissement du stress, les réponses ont permis de différencier les provenances : N-Eubea > Kassandra > Litorale tarantino ≅ Guardiola > Otricoli ≅ E-Bouches du Rhône ≥ Hérault ≅ N-Vaucluse (en ordre décroissant).

courbes pression-volume / Pin d'Alep / provenances / résistance à la sécheresse / variation géographique

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1. INTRODUCTION

Water stress influences the growth, survival and distribution of forest tree species [25]; it can affect the outcome of conifer seedling reafforestation programmes, especially in poor soil and dry zones, since it influences gas exchanges and root growth [5, 31]. In these cases, the seedlings' drought resistance becomes a decisive factor [2]. Measuring water potential and its components is one of the best tools to study plant response to drought [29] Pressure-volume curves method allow to measure such components together with other parameters of water condition [13].

Aleppo pine, *Pinus halepensis* Mill. subsp. *halepensis*, is a species noted for its ability to grow in difficult environmental conditions. In the Mediterranean environment, Aleppo pine's marked drought resistance is especially important. When Mediterranean vegetation belts are drawn up according to the intensity of summer droughts, this species is assigned to the semi-arid belts [15]. Considering the extension and the fragmentation of Aleppo pine's indigenous distribution [1], it appears legitimate to expect that different geographical provenances will behave differently in their response to external stress factors. Ecophysiological and morphological differences between various geographical provenances of this species have already been investigated [7, 8, 9, 10, 11, 14, 16, 33, 35, 39]. Previous researches have analysed water stress resistance during seed germination and in the early stages of root growth [10, 16], showing that different survival behaviours may be adopted by different provenances.

The aim of this study was to investigate differences in water relations in 24-week-old seedlings from eight provenances, which are representative of Aleppo pine European distribution area, in order to suggest criteria for early selection of provenances to be planted in areas exposed to drought risk. In the Mediterranean climate, seedlings germinating in early spring face their first hot and dry season at around 24 weeks, which is why in this experiment seedlings of this age were used.

2. MATERIALS AND METHODS

2.1. Plant material and treatment

The *P. halepensis* provenances studied, their location and some climate data are shown in *table I* and *figure 1*.

Seeds were placed to germinate in pots containing peat and agriperlite (1:1 v/v), watered daily and kept at 20 ± 0.5 °C, 60–65% relative humidity and 16-hour photoperiod. Lighting (550 µE m^{-2} s^{-1}) was provided by metal halide (OSRAM Powerstars) and incandescent lamps (Philips). When they reached 50% emergence, the seedlings were transferred to 23/17 °C (day/night), and watered with Hoagland solution (Basal Salt Misture, Sigma) [21, 22] every three days, till the age of 21 weeks. At the beginning of their 22nd week, the seedlings were transferred to pots containing half-strength Hoagland solution. The solution was oxygenated with air diffusors to prevent the occurrence of root asphyxia. After two weeks of acclimation, Polyethilenglycol (P.E.G. 8000, Fluka) was added to the nutrient solution, so as to reach water potentials of –0.4, –0.8, –1.2 and –1.6 MPa, according to the formula proposed by Michel [26]. The water potential of the control substrate was –0.033 MPa.

Table I. Provenances of *Pinus halepensis* investigated: geographical and climatic features of their zones of origin. Provenances are indicated by the codes of access used by FAO [17].

Provenance	Country	Latitude N	Longitude E	Altitude (m a.s.l.)	Annual precipitation (mm)	Mean annual temperature (°C)
Guardiola A26	Spain	41°49'	1°45'	420	363	11.4
Hérault	France	43°23'	3°07'	17	568	14.5
N-Vaucluse	France	44°30'	4°43'	57	846	13.5
E-Bouches du Rhône	France	43°25'	5°40'	150	546	14.2
Otricoli A23	Italy	42°26'	12°28'	400	830	13.0
Lit. tarantino A27	Italy	40°29'	16°61'	8	445	16.8
N-Eubea A3	Greece	38°56'	23°17'	125	432	17.9
Kassandra A4	Greece	40°02'	23°28'	80	486	15.9

Figure 1. Location of the provenances studied (1, Guardiola; 2, Hérault; 3, N-Vaucluse; 4, E-Bouches du Rhône; 5, Otricoli; 6, Litorale tarantino; 7, N-Eubea; 8, Kassandra).

Twelve seedlings from each stress level group, all the same size, were kept in the substrate for a week. During the stress period, the seedlings were kept at a constant temperature of 20 ± 0.5 °C, 60–65% relative humidity and 16-hour photoperiod. The substrates were replaced twice a week.

The 24-week-old seedlings had only primary needles along the axis. In fact, in the first year, *P. halepensis*, like other pine species, shows free or indeterminate growth, i.e. the extent of annual growth is not restricted by a limited complement of preformed primordia [9, 34] and the first long-shoot primordia appear after 6–7 weeks at the axillary region of the first 2–3 primary needles, just above the cotyledons [9].

2.2. Pressure-volume curves

At the end of 1 week of stress, pressure/volume curves were performed on six 24-week old seedlings from each stress level group. Sample gathering and pressure-volume measurement started at the same time in different days, i.e. five hours after light switching on in growth chambers. Seedlings were cut at the collar under water, but were not re-hydrated, as our aims were to simulate realistic field conditions and to test provenances' responses to drought and not to recovery. Preliminary investigations had shown that provenances may have different recovery abilities after re-hydration. After cutting, seedlings were placed through a split rubber bung. This assembly was immediately weighed and placed inside a pressure chamber (Tecnogas, Pisa, Italy). Initial balancing pressure causing xylem exudation was recorded. Then, in sequence: i) pressure was gradually increased (0.01 MPa s^{-1}) to a total increase of 0.3–0.4 MPa and maintained for 5 minutes with the exuded sap being blotted off; ii) pressure was released slowly (0.01 MPa s^{-1}); iii) the seedling+bung assembly was removed, rapidly weighed and iv) put back in the pressure chamber where the new balancing pressure was measured. This sequence, i-iv, was repeated 12–14 times. Dry weight was determined on seedlings kept at 70 °C for 48 hours. All findings were analysed in accordance with Wilson et al. [40]. As well as measuring initial water potential (Ψ) and actual percentage of water content (WC), the osmotic potential at full turgor ($\Psi_{\pi 100}$), the percentage of apoplastic water (B), the percentage of water deficit at incipient plasmolysis (WD_0) and the maximum elasticity module (ε) were also estimated.

2.3. Data analysis

All findings were then put through 2-way variance analysis (and the mean values were compared by LSD, with P = 0.05) and a Multivariate Discriminant Analysis using the programme STATISTICA 6.0®.

3. RESULTS

Water potential (Ψ) was highly negative even in the controls and it was not correlated with the above-ground biomass expressed as dry weight (*r* = –0.1233). In all provenances its negativity increased as the substrate water potential decreased, but it differed significantly from controls only at a stress level of –0.8 MPa or greater (*table II*). The provenances can be subdivided into two groups: N-Eubea, Guardiola, Kassandra and Litorale tarantino presenting very negative control Ψ values (< –1.0 MPa), and Hérault, E-Bouches du Rhône, Otricoli and N-Vaucluse presenting less negative control values (> –0.9 MPa) (*table II*). In the first group, in Litorale tarantino and N-Eubea Ψ did not vary significantly until the highest stress level was reached, whereas in Kassandra and Guardiola the difference was already significant at –1.2 MPa and was followed by a further reduction at –1.6 MPa. N-Eubea presented the lowest Ψ percentage increase, from the control to –1.6 MPa. The seedlings in the second group always maintained a Ψ slightly above the substrate potential (*table II*) and differed from controls at medium-moderate stress levels: –0.8 MPa in Otricoli, E-Bouches du Rhône and N-Vaucluse; and already at –0.4 MPa in Hérault. Although starting from a scarcely negative control Ψ (–0.7 MPa), at the highest stress level Hérault reached the most markedly negative potential (–2.1 MPa).

N-Vaucluse presented the highest WC (> 75%); E-Bouches du Rhône and Hérault the lowest (< 70%) (*table III*). In all provenances, WC decreased as stress level increased, differing significantly from controls already at –0.4 MPa (*table III*). In fact, almost all provenances displayed a significant WC reduction already at –0.4 MPa. The Greek provenances, however, significantly decreased their WC only at –1.2 MPa and more (in the case of Kassandra) or even not changing at all (in the case of N-Eubea) (*table III*).

In all provenances, osmotic potential at full turgor changes ($\Psi_{\pi 100}$) reflected the trend of Ψ, with a significant reduction at –0.8 MPa (*table IV*). N-Eubea and Litorale tarantino seedlings presented a $\Psi_{\pi 100}$ that differed from controls only at the highest stress level (–1.6 MPa), whereas Kassandra, Otricoli and N-Vaucluse displayed a significant difference already at –1.2 MPa (*table IV*). But Kassandra differed from the other two provenances, since it presented a further reduction at –1.6 MPa. E-Bouches du Rhône and Hérault were very similar also in relation to this parameter, behaving differently from controls already at a moderate stress level, i.e. at –0.8 MPa; Hérault presented a further significant decrease at –1.6 MPa, registering the most negative $\Psi_{\pi 100}$ value of all (–2.4 MPa) (*table IV*). The Guardiola provenance seedlings presented constant $\Psi_{\pi 100}$ values (*table IV*).

As far as maximum elasticity module is concerned (ε), no significant differences were recorded between the provenances or the different stress levels. The mean value of all provenances and all treatments was 6.3 MPa.

The mean value for all provenances of the percentage of water deficit at incipient plasmolysis (WD_0) decreased gradually in response to the reduction of the substrate

Table II. Water potential (MPa) in 24-week-old Aleppo pines from 8 provenances and 5 water stress levels. Different letters indicate significant differences (LSD, *P* = 0.05) between the single values (*n* = 6), the means in column (*n* = 38), and the means in row (*n* = 30).

Stress	Guardiola	Hérault	N-Vaucluse	E-Bouches du Rhône	Otricoli	Lit. tarantino	N-Eubea	Kassandra	Mean
Control	–1.12 efghil	–0.70 a	–0.88 abcd	–0.84 abc	–0.75 ab	–1.07 defghi	–1.26 ilmn	–1.08 defghi	–0.96 a
–0.4 MPa	–0.90 abcde	–1.07 defghi	–1.03 cdefgh	–1.06 cdefghi	–0.92 abcde	–0.93 bcde	–1.02 cdefgh	–0.97 bcdef	–0.99 a
–0.8 MPa	–1.24 hilmn	–1.41 nop	–1.18 fghilm	–1.22 ghilmn	–1.06 cdefghi	–1.19 fghilmn	–1.23 ghilmn	–1.01 cdefg	–1.19 b
–1.2 MPa	–1.37 mno	–1.41 nop	–1.32 lmno	–1.66 qr	–1.77 rs	–1.21 ghilmn	–1.18 fghilm	–1.35 mno	–1.41 c
–1.6 MPa	–1.65 qr	–2.10 t	–1.53 opq	–1.83 rs	–1.76 qrs	–1.70 qrs	–1.63 pqr	–1.90 st	–1.76 d
Mean	–1.26 a	–1.34 a	–1.19 a	–1.32 a	–1.25 a	–1.22 a	–1.26 a	–1.26 a	

Table III. Water content (%) in 24-week-old Aleppo pines from 8 provenances and 5 water stress levels. Different letters indicate significant differences (LSD, $P = 0.05$) between the single values ($n = 6$), the means in column ($n = 48$), and the means in row ($n = 30$).

Stress	Guardiola	Hérault	N-Vaucluse	E-Bouches du Rhône	Otricoli	Lit. tarantino	N-Eubea	Kassandra	Mean
Control	73.87 pq	69.28 efghi	75.62 r	69.73 fghil	73.76 pq	74.24 qr	71.42 lmno	72.04 no	72.49 d
–0.4 MPa	70.38 ghilmn	66.89 abc	72.86 opq	68.79 defg	70.26 fghilm	70.82 ilmn	72.52 op	72.13 opq	70.58 c
–0.8 MPa	69.39 efghi	67.74 bcde	73.89 pq	67.39 abcd	68.87 defgh	67.92 cde	71.36 lmno	71.39 mno	69.74 b
–1.2 MPa	69.06 defgh	66.08 ab	71.69 mno	67.38 abcd	69.43 efghi	70.20 fghilm	70.60 hilmn	70.30 ghilm	69.34 b
–1.6 MPa	67.74 bcde	65.87 a	70.85 ilmn	66.30 abc	68.50 cdef	69.46 hilmn	70.28 ghilm	67.88 defgh	68.36 a
Mean	70.09 b	67.17 a	72.98 d	67.92 a	70.16 b	70.53 bc	71.24 c	70.75 bc	

Table IV. Osmotic potential at full turgor (MPa) in 24-week-old Aleppo pines from 8 provenances and 5 water stress levels. Different letters indicate significant differences (LSD, $P = 0.05$) between the single values ($n = 6$), the means in column ($n = 48$), and the means in row ($n = 30$).

Stress	Guardiola	Hérault	N-Vaucluse	E-Bouches du Rhône	Otricoli	Lit. tarantino	N-Eubea	Kassandra	Mean
Control	–1.49 bcdefghi	–1.20 a	–1.31 abcd	–1.26 abc	–1.49 bcdefghi	–1.62 efghilmn	–1.55 defghil	–1.27 abc	–1.40 a
–0.4 MPa	–1.37 abcde	–1.39 abcdef	–1.30 abcd	–1.39 abcdef	–1.52 cdefghi	–1.30 abcd	–1.40 abcdefg	–1.24 ab	–1.36 a
–0.8 MPa	–1.68 hilmnop	–1.59 efghilm	–1.53 cdefghi	–1.72 ilmnop	–1.45 abcdefgh	–1.66 ghilmnop	–1.80 lmnop	–1.27 abc	–1.59 b
–1.2 MPa	–1.62 efghilmn	–1.83 mnopq	–1.72 ilmnop	–1.93 pqrs	–2.08 qrst	–1.52 cdefghi	–1.62 efghilmn	–1.65 fghilmno	–1.75 c
–1.6 MPa	–1.68 hilmnop	–2.38 t	–1.81 lmnop	–2.11 rst	–2.23 st	–1.90 opqr	–1.88 nopqr	–2.17 rst	–2.02 d
Mean	–1.57 abc	–1.68 cd	–1.53 ab	–1.68 cd	–1.75 d	–1.60 abc	–1.65 bcd	–1.52 a	

potential (*table V*), presenting a significant difference compared to controls at –1.2 MPa. The stress-provenance interaction shows that only two provenances differed from the others, Otricoli and Guardiola; the former differed from controls only at the highest stress level (–1.6 MPa), whereas the latter differed already at –0.4 MPa (*table V*). The seedlings of all the other provenances presented fairly constant values, unrelated to the variations in the substrate's water potential.

The mean value for all provenances of the percentage of apoplastic water (B), became significantly reduced only at the highest stress level (*table VI*). This trend reflected the behaviour under stress of only three provenances: Hérault, which presented a significant reduction at –1.6 MPa, Guardiola at –0.4 MPa and Kassandra, the only provenance displaying a gradual reduction of this parameter in relation to the reduction of the substrate's water potential, with two significant thresholds, one at –0.4 and the other at –1.6 MPa (*table VI*).

Multivariate discriminant analysis shows that the first four functions accounted for 92% of discriminating power. The discriminating power of the first function

Table V. Water deficit at incipient plasmolysis (%) in 24-week-old Aleppo pines from 8 provenances and 5 water stress levels. Different letters indicate significant differences (LSD, $P = 0.05$) between the single values ($n = 6$), the means in column ($n = 48$), and the means in row ($n = 30$).

Stress	Guardiola	Hérault	N-Vaucluse	E-Bouches du Rhône	Otricoli	Lit. taranti-no	N-Eubea	Kassandra	Mean
Control	10.34 mnop	4.51 abcde	8.18 fghilmno	4.23 abcde	8.77 hilmnop	9.24 ilmnop	8.55 ghilmnop	5.62 abcdefgh	7.43 b
–0.4 MPa	5.00 abcdef	3.76 ab	7.24 cdefghilm	7.18 bcdefghilm	8.48 ghilmnop	11.01 nop	6.32 abcdefghil	11.80 p	7.60 b
–0.8 MPa	6.27 abcdefghil	4.05 abcd	5.90 abcdefghi	3.82 abc	6.24 abcdefghil	10.47 mnop	7.46 defghilm	11.56 op	6.97 ab
–1.2 MPa	4.53 abcde	5.59 abcdefgh	5.00 abcdef	5.68 abcdefgh	5.66 abcdefgh	9.49 lmnop	8.12 fghilmno	4.92 abcdef	6.12 a
–1.6 MPa	7.67 efghilmn	4.73 abcdef	5.43 abcdefgh	3.72 a	5.23 abcdefg	9.24 ilmnop	7.30 defghilm	5.21 abcdefg	6.07 a
Mean	6.76 c	4.53 a	6.35 bc	4.93 ab	6.88 c	9.89 d	7.55 c	7.82 c	

Table VI. Apoplastic water (%) in 24-week-old Aleppo pines from 8 provenances and 5 water stress levels. Different letters indicate significant differences (LSD, $P = 0.05$) between the single values ($n = 6$), the means in column ($n = 48$), and the means in row ($n = 30$).

Stress	Guardiola	Hérault	N-Vaucluse	E-Bouches du Rhône	Otricoli	Lit. tarantino	N-Eubea	Kassandra	Mean
Control	52.37 abc	85.90 rs	61.84 cdefgh	82.30 pqrs	57.36 bcdef	52.31 abc	64.92 efghil	76.67 lmnopqr	66.71 b
–0.4 MPa	77.50 mnopqr	90.55 s	68.47 fghilmno	76.58 lmnopqr	61.18 cdefg	55.35 abcde	72.97 ghilmnop	61.97 cdefgh	70.57 b
–0.8 MPa	68.17 fghilmno	85.86 qrs	59.37 cdef	77.94 nopqr	73.29 hilmnop	57.75 cdef	52.95 abcd	57.29 bcdef	66.58 b
–1.2 MPa	76.05 lmnopqr	85.39 qrs	60.02 cdef	74.36 ilmnopq	61.89 cdefgh	55.94 abcde	66.57 efghilmn	65.68 efghil	68.24 b
–1.6 MPa	66.40 efghilmn	73.12 hilmnop	65.85 efghilm	78.69 opqr	59.92 cdef	45.70 ab	64.18 defghi	45.35 a	62.40 a
Mean	68.10 c	84.16 e	63.11 bc	77.97 d	62.73 b	53.41 a	64.32 bc	61.39 b	

(46.6%) is determined mainly by B (–0.4 MPa), followed by $\Psi_{\pi100}$ (–0.4 MPa), WC (–0.8 MPa) and WD_0 (–1.2 MPa); whereas the discriminating power of the second function (30.3%) was determined primarily by WC (Contr.), followed by $\Psi_{\pi100}$ (Contr.), Ψ(–0.4 MPa), Ψ (–0.8 MPa), and $\Psi_{\pi100}$ (–1.2 MPa). The first function discriminated primarily Guardiola, but also N-Vaucluse (*figure 2*). The second function distinguished E-Bouches du Rhône and Hérault. The percentage of correctly classified cases is 100%. In *figure 2* one can observe how, within the central group made up of the four closest provenances, Otricoli and N-Eubea were positioned very near to each other, while Litorale tarantino and Kassandra are located on either side of them.

4. DISCUSSION

The drought resistance of *P. halepensis* is well-known in the literature; according to Oppenheimer [27], this species is the most resistant of all *Pinus* species.

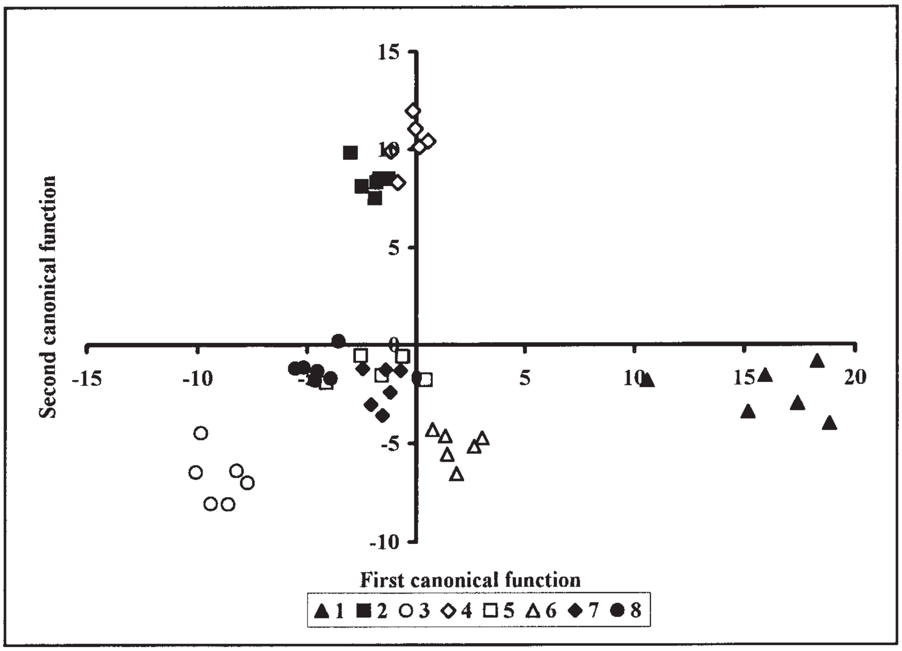

Figure 2. Discriminant scores for 8 Aleppo pine provenances in the plane of the 1st two canonical functions (1, Guardiola; 2, Hérault; 3, N-Vaucluse; 4, E-Bouches du Rhône; 5, Otricoli; 6, Litorale tarantino; 7, N-Eubea; 8, Kassandra).

Variations in response to stress, as observed in this study, confirm Aleppo pine's high degree of drought resistance.

The reduction of $\Psi_{\pi100}$ as the water potential of the substrate decreases has already been observed and has been considered a clear response to drought [18, 35, 36]. Five and a half month old control seedlings of three North American conifers (*Picea mariana* Mill., *Picea glauca* Moench, *Pinus banksiana* Lamb.) [6] presented $\Psi_{\pi100}$ values similar to those observed in this study in Aleppo pine, but reached their lowest negative values at low levels of stress. In these three conifers significant changes in $\Psi_{\pi100}$ values occurred even after moderate stress (–0.4 MPa) [6], suggesting that, since these species are not particularly drought resistant, they immediately resort to osmotic adjustments as soon as the substrate potential starts becoming more negative. In our study, in the mean values relating to all provenances, this threshold is reached at a higher stress level (–0.8 MPa), confirming that *P. halepensis* can tolerate moderate or medium stress and resorts to osmotic adjustments only in the most critical conditions of water stress. Some provenances of *P. halepensis* examined in this study differentiate this parameter only at very high stress levels (–1.6 MPa). An osmotic adjustment potential as a response to water stress, that varies from provenance to provenance, is of the same type as that observed in 2-year-old Aleppo pine seedlings from Italian provenances [35]. Conversely, a study performed on only one provenance of Aleppo pine at the beginning of autumn has shown that drought did not induce any osmotic adjustment in 1-year old plants [38].

Water deficit at incipient plasmolysis (WD_0), in the seedlings included in this study, displayed an overall decreasing trend as stress increased, contrary to the observations reported by Boucher et al. on *Pinus strobus* L. [3] and Fernàndez et al. on *Pinus pinaster* Ait. [18], in agreement with Tognetti et al. on *P. halepensis* [35], although these last authors recorded higher values than were observed in our study. This difference may be due to the fact that we did not re-hydrate our samples or to the fact that the seedlings we examined were younger. Conversely, Villar-Salvador et al. [38] did not observe any WD_0 variations in Aleppo pines subjected to water stress.

Apoplastic water content (B) was high, if compared to values observed in mesophilic plants, and remained relatively constant even as the substrate's water potential changed, except at the highest stress level. Apoplastic water is considered a sort of reservoir that plants turn to in cases of excessive dehydration [12], or as something fixed and irremovable except in cases of extremely high tensions [36]. In any case, a high content of apoplastic water is a feature shared by all plants that have adapted to dry climates [12].

The elasticity module (ε), whose fluctuations are dependant on the structural properties of the tissue and the walls of individual cells, as well as on their pressure and volume, showed no significant differences between the various provenances or at different stress levels, perhaps because the test only lasted 1 week, during which period it is legitimate not to expect to observe structural variations in the tissues. Even in stress tests lasting much longer [35, 38], no significant variations of ε were recorded in drought-stressed Aleppo pines and provenances.

Water content (WC) decreased progressively as the substrate's water potential decreased. A stress-induced reduction of water content was observed also in 2-year-old *Pinus strobus* [3]. Plants that respond to a reduced water content by decreasing water potential display a reaction to the stress condition, and therefore have a greater chance of survival [20, 23]. But this conclusion is the result of studies carried out on plants that are not especially drought-resistant. In our study, a comparable behaviour was recorded only in some provenances (Otricoli, Hérault, E-Bouches du Rhône, N-Vaucluse), but not in Kassandra and N-Eubea in which the behaviour remains fairly constant.

Aleppo pine's marked adaptation to drought was further confirmed by the Ψ values, which were always very negative, even when the stress was absent as already reported by [28]. Such a low water potential in well-watered seedlings may indicate an intrinsic ability to face adverse water conditions and is a common trait in Mediterranean species [19, 30]. The provenances from less xeric sites (Otricoli, N-Vaucluse, E-Bouches du Rhône, Hérault) behaved in a manner similar to species typical of non-dry ambients. The seedlings did not have a particularly negative Ψ value in controls and adjusted gradually as the stress level increased, starting already at moderate levels of stress, and always maintaining this value at a slightly more negative level than the substrate's water potential. This tendency to a reduction in Ψ as the stress level increases is similar to the trend observed in 5-and-a-half months old seedlings of *Picea mariana, Picea glauca* and *Pinus banksiana*, subjected to stress for one week with PEG 8000 [6] and on adult plants of *Pinus taeda* L. and *Pinus strobus* [24]. These species, originally from the West Coast of North America, had control Ψ values typical of not especially drought-resistant species. But, as a confirmation of *P. halepensis*'s marked xerotolerance, one must point out that even in the less drought-resistant provenances there was never a marked difference between control values of Ψ and stress-induced values (except at very high levels of stress); whereas in *Pinus taeda* and *Pinus strobus* the absolute values of Ψ doubled even at low or medium levels of stress [24]. The most typical example of this gradual adaptation is offered by the Hérault seedlings.

On the other hand, the other group of seedlings (Guardiola, Kassandra, N-Eubea, Litorale tarantino), from much more xeric sites, displayed the typical strategy of drought-tolerant species: very negative control Ψ values which did not change, at least not until medium or high stress levels were reached.

The correlation between the climatic features of the seedlings' original sites and the responses to drought enacted by the two groups of provenances becomes clear if we examine in detail the climate of the original sites of the two provenances representing the extremes of the group: the French provenance of N-Vaucluse (in the first group) can be considered a borderline between *sub-Mediterranean* climate (less than 40 days considered biologically dry, according to Gaussen's xerothermal index) and *temperate* climate, with a sub-dry period and an absence of biologically dry days [37], with a mean annual rainfall of 846 mm and a mean annual temperature of 13.5 °C. On the other hand, the climate of the Greek island of Eubea (second group) is considered a *markedly thermo-Mediterranean* climate, with 125–150 biologically dry days, a mean annual rainfall of 432 mm and a mean annual temperature of 17.9 °C.

To conclude, our findings show that 24-week-old *P. halepensis* seedlings were scarcely influenced by 1 week of low-intensity water stress, of the sort that can occur quite frequently in the natural environment of this species. At higher stress levels, the responses of the seedlings varied according to the provenances. The results obtained with the multivariate discriminant analysis confirmed a differentiated behaviour between the various provenances; in fact, these findings distribute the seedlings into groups that correlate quite satisfactorily to the geographical macro-zones of the distribution area. The Guardiola provenance (from the north-western part of the distribution area) differed from all the others. And N-Vaucluse (near the northernmost boundary of the distribution area) was also different from the others, although in a less marked manner, especially because of the high water content in the controls and of the climate of the site of origin. The two other French provenances (E-Bouches du Rhône and Hérault), from the more specifically Mediterranean region of France, displayed a similar behaviour. The remaining 4 provenances – the Greek

Kassandra and N-Eubea and the Italian Litorale tarantino and Otricoli, all from the central zone of the distribution area – did not present a marked differentiation. A similar picture has been already reported for Kassandra, N-Eubea and another Southern Italian provenance (Gargano) by detecting the haplotypic variation [4]. One observation is fairly surprising: Otricoli, whose zone of origin (borderline between *moderate meso-Mediterranean* with 40–75 biologically dry days and *sub-Mediterranean*) is markedly different, in terms of climate, from the other 3 provenances, displayed a behaviour similar to Kassandra, N-Eubea and Litorale tarantino, all originating from a *thermo-Mediterranean* or *marked meso-Mediterranean* climate, with a far greater number of biologically dry days. Yet, the behaviour displayed by Otricoli supports the theory that it was introduced by man, in ancient times, from the eastern shores of the Mediterranean (Israel) [32].

From our findings, the parameters better explaining drought resistance are Ψ, WC and $\Psi_{\pi100}$ both in stressed and well-watered seedlings. On such a basis we can thus suggest the following order, in terms of drought resistance, between the provenances studied: N-Eubea > Kassandra > Litorale tarantino ≅ Guardiola > Otricoli ≅ E-Bouches du Rhône ≥ Hérault ≅ N-Vaucluse. Such a scale nearly reflects the East to West distribution of Aleppo pine as already reported for Aleppo pine needle anatomical features [8] and seed germination [10, 15], so that the proposed classification appears as a combination of ecophysiological, genetic and geographical parameters. All these parameters can be suggested to select seedlings from drought resistant provenances.

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