

Changes in morpho-physiological attributes of *Eucalyptus globulus* plants in response to different drought hardening treatments

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Abbreviations: e: volumetric module of elasticity

$\Psi\pi_{tlp}$: osmotic potential at the turgor loss point

$\Psi\pi_{sat}$: saturation osmotic potential

Ψ_{pd} : Predawn stem xylem water potential

Ψ_{stw} : stem xylem total water potential

ARL: the average length of the longest three new roots

INT: Ice nucleation temperature.

LT_(10, 50, 90): freezing damage indexes of leaves

NNR: new roots longer than 1 cm

RWC_{tlp}: relative water content

Morpho-physiological attributes exhibited in response to drought hardening at the end of the growing season of *Eucalyptus globulus* Labill under nursery conditions were studied to evaluate the effect of three drought hardening treatments in morpho-physiological traits used as suitable indicators of drought hardiness, such as, plant growth, root growth potential, plant water relationships and survival. Freezing resistance of drought hardened plants was also studied in order to evaluate cross hardening effects in cuttings of *Eucalyptus globulus* Labill. Drought hardening consisted in induced water stress by watering restriction, until plant stem xylem water potentials (Ψ_{pd}) reached to -0.2, -1.3 and -2.4 MPa. Two water stress-rewatering cycles were applied during 54 days of treatment. The hardening treatments caused a significant reduction in plant height, leaf area, specific leaf area, plant, leaf, stem and root biomass. However, stem diameter was not affected. Root growth potential increased with the exposure to moderate water stress (-1.3 MPa). Drought hardening treatments have not effect on water relationship parameters such as saturation osmotic potential ($\Psi\pi_{sat}$), volumetric module of elasticity (e), relative water content (RWC_{tlp}) and osmotic potential ($\Psi\pi_{tlp}$) at the turgor loss point. Only 1.7% and 6% of dehydrated dead plants were observed on treatments at -1.3 and -2.4 MPa respectively. Finally, the freezing damage index of leaves (LT₅₀) was not significantly affected by drought hardening treatments. Furthermore, a reduction of 1.1°C of supercooling capacity was observed at -2.4 MPa. As a conclusion, drought hardening is an important step of plants production programs during the final phase of nursery, because changes in morphological attributes caused by exposure to moderate drought, enable the plants to maintain the balance between transpiration and absorption areas and increase the capacity of plants to generate new roots.

The high pulp yield and fast growth of *Eucalyptus globulus* Labill have determined that about 436.706 ha have been planted in Chile representing approximately 21.3% of the

national planted territory (Avila et al. 2005). Due to the high rates of forestry plantation establishment during the past 15 years, availability of edaphoclimatically favourable land is scarce, therefore, it has been necessary the use of less favourable sites for plantation establishment like costal dry lands and above 650 m.a.s.l. in the foot hills of Andes Mountains. Both sites exhibited severe dryness during summer and low temperature in winter. The evident challenges to improve the *Eucalyptus globulus* capacity to withstand drought and freezing temperatures, and its clonal propagation have received major attention (Pita and Pardos, 2001; Ngugi et al. 2003). However, there is no substantial information about morpho-physiological variations induced by an acclimation phase under nursery (phenotypic plasticity), which is essential for water stress survival of plants in unfavourable sites (Guarnaschelli et al. 2006).

It is known that sub lethal water stress exposed plants exhibited less drought injury than no stressed plants (Villar-Salvador et al. 2004; Banon et al. 2006). Several studies have been conducted about drought and its associated effects on *Eucalyptus globulus* under several controlled and natural environments (White et al. 2000; Pita and Pardos, 2001; Gindaba et al. 2004). During the acclimation to water stress, reduction in growth rates and changes in the balance between aerial and root growth are usually seen (Hsiao and Xu, 2000). Plant morphological attributes like collar diameter, plant height have been consistently reported as good indicators for survival and growth potential after planting (Mason, 2001). Root growth potential is also a useful indicator of plant vitality, especially when plants are planted in stressful sites (Ritchie and Tanaka, 1990). Plants can respond to drought with a variety of mechanisms that allows them to avoid water stress or tolerate certain levels of desiccation (Larcher, 2003). Osmotic adjustment and the increment of cell wall elasticity have been shown that help to maintain cellular turgor at low tissue water potentials in *Eucalyptus* sp. (Guarnaschelli et al. 2003), which could be essential for plant survival and growth during drought. Plant water relations give useful information about the capacity of trees to maintain growth, gas exchange and water use during summer drought (White et al. 2000).

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Finally, there is evidence that cold acclimation and water stress induce cell dehydration which triggers a series of common physiological responses like decrease of protoplast osmotic potential and common proteins synthesis (Pérez et al. 1997; Chaves et al. 2003), therefore, a drought acclimated plant should be more freezing resistant than a non-acclimated one.

It is unknown how desiccation resistance and cold acclimation are modulated by the nursery environment and in some cases published information is contradictory (Colombo et al. 2003). There is limited information about how morphological traits usually used to assess growth potential are suitable indicators of drought hardiness and if water stress induce freezing hardiness in nursery plants of *Eucalyptus globulus*. Therefore, the objective of this study was to evaluate the effect of drought hardening in plant growth, root growth potential, plant water relations and freezing resistance of rooted cuttings of *Eucalyptus globulus* Labill at the nursery phase.

MATERIALS AND METHODS

Plant material and experimental setup

A total of 2,800 eight month old rooted cuttings of *Eucalyptus globulus* Labill were obtained from the Forestry Company Bosques Arauco S.A. Chile. Cuttings were rooted in trays of 88 containers of 130 ml each, using a mixture of turf: perlite 1:1 (v/v) with a total porosity, aeration and water storage capacity of 75, 32 and 42% respectively. Growth density was 393 plants m⁻², in a greenhouse with mist irrigation. After rooting, plants were growth outdoor for 5 months and then for drought hardening were kept under transparent roof in order to cover from rainfall. Local environmental conditions were monitored by a weather station installed in the place of experiment (Table 1).

Drought hardiness

Three different treatments were applied and consisted in watering restriction until plants reach a total predawn stem xylem water potential of (Ψ_{pd} ; -0.2, -1.3 and -2.4 MPa) and then were watered to reach substrate saturation and a new cycle of drought proceed. The treatment with $\Psi_{pd} = -0.2$ MPa was considered non-stressed control, the -1.3 MPa (moderate stress) value is in the center of the target range (-1.0 to -1.5 MPa) for drought hardening processes that has been commonly used in most USA forest nurseries (Landis, 1989) and is just below to the wilting point of the experiment's plants. Finally -2.4 MPa (severe stress) correspond to a permanent wilting point of experiment's plants. The hardening phase last 54 days (May-June 2004) and include two cycles of low water potential (Figure 1). Total stem xylem water potential were measured in a sample of 20 plants per treatment (n = 20) at predawn with the Scholander pressure chamber (Soilmoisture Equipment Corp, Santa Barbara, CA USA), approximately every two

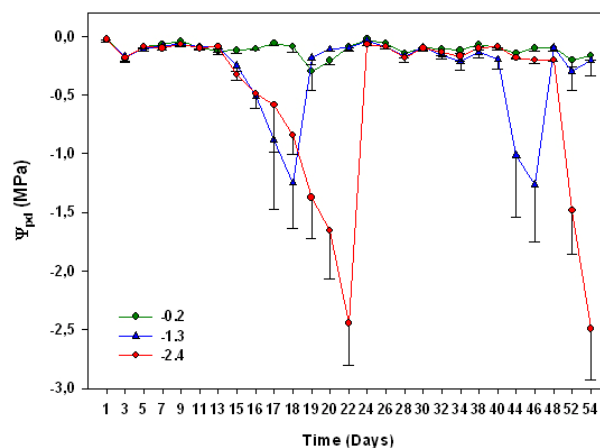


Figure 1. Variation of total stem xylem water potential (Ψ_{pd}) of *Eucalyptus globulus* during drought hardening time course. Three drought hardening treatments (-0.2, -1.3 and -2.4 MPa) were applied during 54 days. Error bars represent -1 SE, n = 20.

days. The treatment with $\Psi_{pd} = -0.2$ MPa was considered non-stressed control maintaining a watering frequency of about 7 days. The Ψ_{pd} did not change until day 13, then decreased at a rate of -0.27 MPa day⁻¹ reaching the objective water potentials at 18 and 22 days respectively, in the first cycle and after rewatering plant reached again the objective water potential after 46 and 54 days respectively (Figure 1). Before the beginning of the assay and immediately after the hardening treatment, all plants were profusely watered in order to uniform the water content of the substrate to an initial saturated condition.

Growth

Twenty plants from each treatment were taken approximately every 5 days to determine plant height (cm), stem diameter (mm), root, stem, leaf and total dry biomasses (g). Additionally, leaf area and specific leaf area were determined using a leaf area meter Li-3100 (Li-Cor Inc., Lincon, Ne USA).

Root growth potential

Twenty plants from each treatment were taken every 13 days of treatment, the substrate was carefully removed and roots extensively washed. New roots (white roots) were removed and then plants were placed in an aeroponical chamber (Forcap Ltda., Concepción, Chile). The chamber was programmed to provide a pulse of 6 sec of water mist at 22°C directly to the root system every 10 min. Photoperiod was 16 hrs light at a PFD of 50 $\mu\text{molm}^{-2}\text{s}^{-1}$ air temperatures for the aerial part of the plant oscillate from 16-20°C day and 8-12°C night. After 28 days, the number of new roots longer that 1 cm (NNR) and the average length of the longest three new roots (ARL) were recorded.

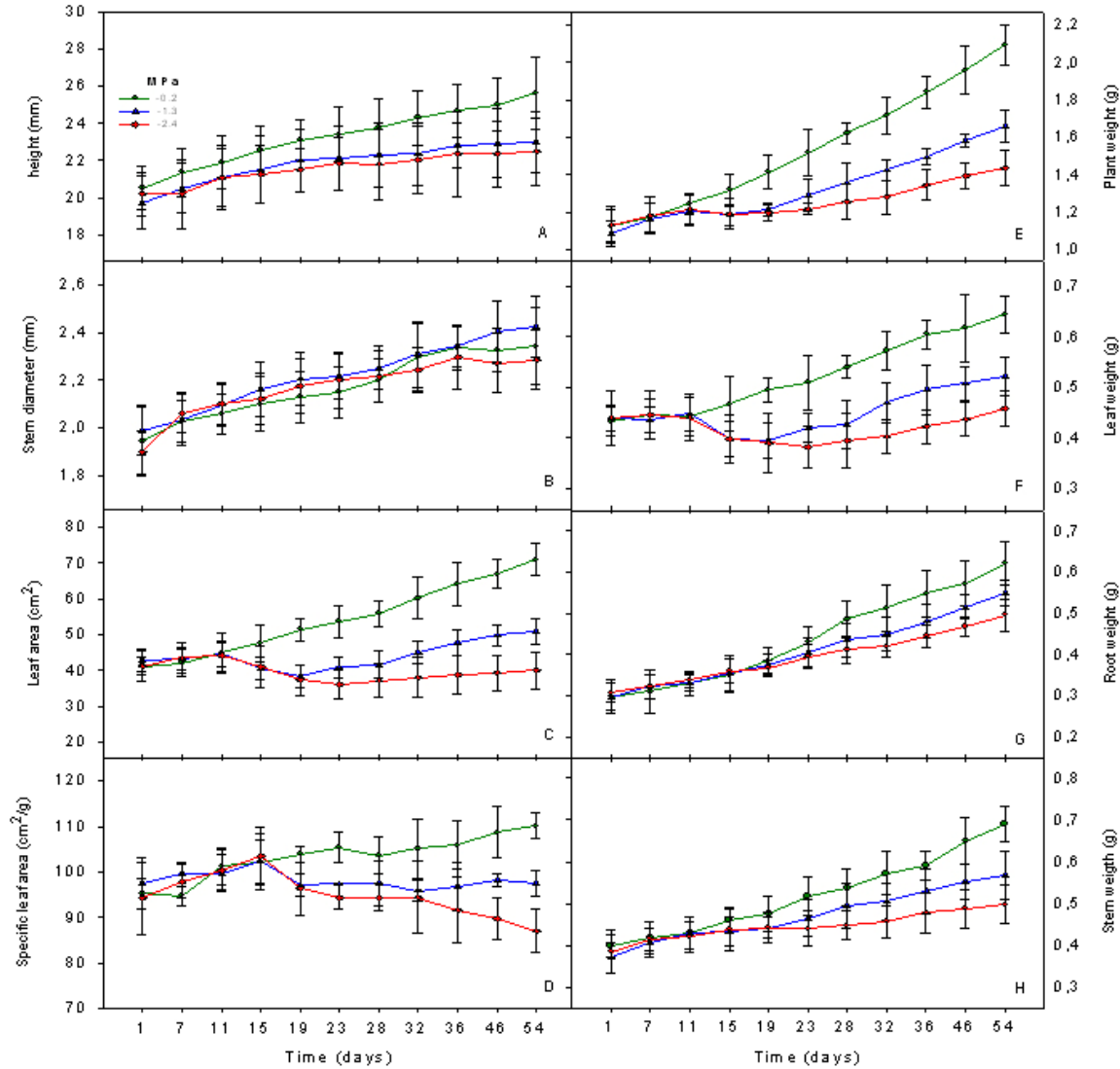


Figure 2. Variation in morphological traits during the time course of drought hardening. The evolution of height (A), stem diameter (B), leaf area (C) specific leaf area (D), plant (E), leaf (F), root (G) and stem biomass (H) of *E. globulus* plants in response to three drought hardening treatment (-0.2, -1.3 and -2.4 MPa) were evaluated. Error bars represent ± 1 SE, $n = 20$.

Plant water relations

Pressure-volume curves were developed combining air dehydration of the sample with measurements of xylem stem water potential (Tyree and Hammel, 1972). The procedure consisted in the application of pressure with a Scholander pressure chamber (Soilmoisture Equipment Corp, Santa Barbara, CA USA) to the terminal end of the plant cut at 10 cm from the apex, until the internal tension of the xylem equilibrate with the pressure of the chamber and a humid film was seen at the cut surface ($\Psi_{sxtw} =$ Stem xylem total water potential). Immediately after, the apex was weighed (W_f). Three plants were used at each step ($n = 3$), and then they were left to dry on top of a bench and the procedure was repeated again during 8 hrs. After the last measurement plant tissue was completely dry at 85°C for 48

hrs and dry weight was determined (W_d) Weight at maximum turgor (W_t) was obtained by extrapolation to $\Psi_{sxtw} = 0$ in the curve Ψ_{sxtw} vs fresh weight (W_f). Relative water content (RWC) was calculated as $(W_f - W_d)/(W_t - W_d)$. Parameters like, osmotic potential at saturation point ($\Psi\pi_{sat}$), RWC at turgor loss (RWC_{tlp}) and osmotic potential at turgor loss ($\Psi\pi_{tlp}$) were obtained from the pressure-volume curve (P-V) (Tyree and Hammel, 1972). The pressure potential (Ψ_p) was calculated from the difference between Ψ_{sxtw} and the osmotic water potential derived from P-V curve. The tissue volumetric module of elasticity (ϵ) was calculated as:

$$\epsilon = \frac{\Delta\Psi_p}{\Delta RWC} RWC$$

Where $\Delta\Psi_p/\Delta RWC$ is the slope of a curve obtained when Ψ_p is expressed as a function of RWC within the range of positive turgor (White et al. 2000; Ngugi et al. 2003). Osmotic adjustment was defined as the difference between $\Psi\pi_{sat}$ from the drought hardiness treatments (-1.3, -2.4 MPa) and Ψp_{sat} from the control treatment (-0.2 MPa) (Ngugi et al. 2003; Villar-Salvador et al. 2004).

Freezing resistance

Three weeks after the hardening phase, 12 plants from each hardening treatment were collected and 2 leaf disks (8 mm diameter) were obtained from each third pair of leaves which are the first totally expanded. The leaf disks were put into a polycarbonate test tubes and exposed to freezing temperatures -3.0, -4.5, -6.0, -7.5 and -9.0°C, without ice nucleating agents in a chamber cooled by forced cooling air. The above zero rate of cooling was 30°C h⁻¹ and below zero 2.1°C h⁻¹. Time of exposure to each objective temperature was 1.5 hrs and then plant were thawed at 4°C in darkness for 24 hrs and the leaf injury indexes (LT₁₀, LT₅₀, LT₉₀) were evaluated in leaf disks by ion leakage measuring relative electrolyte conductivity (RC) according to Moraga et al. (2006). Thermal analyses were conducted in the same leaf material in order to determine ice nucleation temperature and freezing point of the leaf (Larcher, 2003). Essentially the procedure was the same as used by Reyes-Díaz et al. (2005), briefly, a piece of leaf was attached to a fine type T thermocouple copper-constantan, gauge 30, junction diameter about 0.5 mm (Cole Palmer, Vernon Hills, IL USA), immediately enclosed in a cryotube to avoid tissue dehydration. Cryotubes were immerse in a cryostat (C25P, ThermoHaake, Karlsruhe, Germany) and a linear descend was programmed from 0 to -15°C at a rate of 2°C h⁻¹. The temperature was continuously monitored and registered every 1 sec with a USB data acquisition system (Personal Daq/56, IOtech. Inc, Cleveland, Ohio USA) connected to a personal computer.

Data analyses

Data were analyzed by one-way repeated-measures ANOVA, considering the effect of drought hardening treatment (-0.2, -1.3 and -2.4 MPa) as a factor measured at several times of exposition (Piepho et al. 2003). All variables were subjected to normality and variance homogeneity test prior to ANOVA. A post-hoc Tukey test

was applied when ANOVAS give significant differences, considering $P \leq 0.05$. All these analyses were performed using the PROC Mixed procedure of SAS software version 9.1, 2001 (SAS Institute Inc. Cary, NC, USA).

RESULTS

Growth

The interaction between drought hardening treatment and its duration was not statistically significant for variables plant height and stem diameter. Nonetheless, plants from the control treatment (-0.2 MPa) exhibited higher plant height than -1.3 and -2.4 MPa treatments, which did not differ from each other. Plant height showed a significant increase at day 19 of treatment respect to the beginning of the experiment. However plant height did not differ from day 15 to the end of treatments (day 54) (Figure 2a) Stem diameter was not affected by drought hardiness treatments but showed a significant increase with time exposure from day 15 (Figure 2b). Interaction between hardening treatment and its duration significantly affected leaf area and specific leaf area (Figure 2c and Figure 2d). Leaf area of plant exposed to -0.2 MPa increased at a constant rate exhibiting a significant higher leaf area than the other treatments from day 19, while other treatments showed a decrease in leaf area without differing from each other until day 19. Plant from treatment -1.3 MPa exhibited an increase in leaf area toward the end of the experiment, which was not observed in plant from treatment -2.4 MPa. Nevertheless, both treatments no reached a significant difference during the hardening phase (Figure 2c). The SLA increased without significant difference within treatments until day 15. SLA was significantly higher in plants exposed to -0.2 MPa from day 36, however SLA decrease in the other treatments toward day 19, and plants of treatment -1.3 MPa maintains the same value of SLA until the end of the experiment, while plants from treatment -2.4 MPa significantly decreased their SLA (Figure 2d).

Weight of plants exposed to -0.2 MPa increased significantly from 28 to 54 days, respect to the beginning of the experiment. Plant weight did not differ within drought hardiness treatments until day 36. Plants exposed to -1.3 and -2.4 MPa did not increase their weight until day 28, from this time ahead weights of plants from treatment -1.3 MPa increased at a higher rate than those from treatment at -2.4 MPa, nevertheless, not significantly different were

Table 1. Average climatic variables during drought hardening treatments. Substrate (ST_{max}, ST_{min}) and air (AT_{max}, AT_{min}) maximum and minimum temperatures, maximum solar radiation (SR), maximum and minimum relative humidity (RH_{max}, RH_{min}) and wind velocity (WV) were registered. Values are the average of 54 daily measurements \pm 1 SE.

ST _{max} (°C)	ST _{min} (°C)	AT _{max} (°C)	AT _{min} (°C)	SR _{max} (w m ⁻²)	RH _{max} (%)	RH _{min} (%)	WV (m s ⁻¹)
15.7 \pm 0.3	6.8 \pm 0.4	16.8 \pm 0.4	6.3 \pm 0.4	339 \pm 22	94.9 \pm 0.2	65 \pm 2.1	0.4 \pm 0.4

detected until the end of treatments (Figure 2e). Leaf weight of plants exposed to -0.2 MPa increased reaching significantly higher values than leaves of -1.3 and 2.4 MPa from day 23. Plants exposed to -1.3 and -2.4 MPa showed no significant differences in leaf weight between each other during the experiment (Figure 2f). Root weight increased significantly since day 23, drought hardening treatment did not affect root weight until day 36, from this day ahead differences in root weight were observed only between treatments at -0.2 and -2.4 MPa (Figure 2g). Stem weight of plants exposed to -0.2 and -1.3 MPa increase from day 28 having significant differences only at day 54. Plants treated at -2.4 MPa did not increase their stem weight, showing no significant differences from treatment at -1.3 MPa during the experiment (Figure 2h). The shoot/root biomass ratio was significantly reduced by the drought hardening regime (-1.3 and -2.4 MPa) from day 15 to 23 and in the final 8 days of hardening. However the control plants (-0.2 MPa) also reduced its shoot/root biomass ratio since day 19 to 28 (Figure 3).

Root growth potential

There was a highly significant interaction between intensity and duration of drought hardening for number of new roots, longer than 1 cm (NNR) and for the average length of longest three roots (ARL). The NNR of the extreme treatments (-0.2 and -2.4 MPa) significantly increased until day 12, while the NNR in the moderate drought hardening treatment (-1.3 MPa) raised until day 28. All treatments then decreased reaching similar values to the beginning of the experiment. There was no significant difference in the NNR within drought hardening treatments except in the day 28, where plants exposed to -1.3 MPa reached the highest value (Figure 4a). The ARL tended to increase until days 12 or 28 depending on the treatment, and then decrease significantly in all treated plants during the time course. There were significant differences within treatments only at day 28 (Figure 4b).

Water relations

Twenty days after drought hardening, plants did not exhibited significant differences in the P-V derived

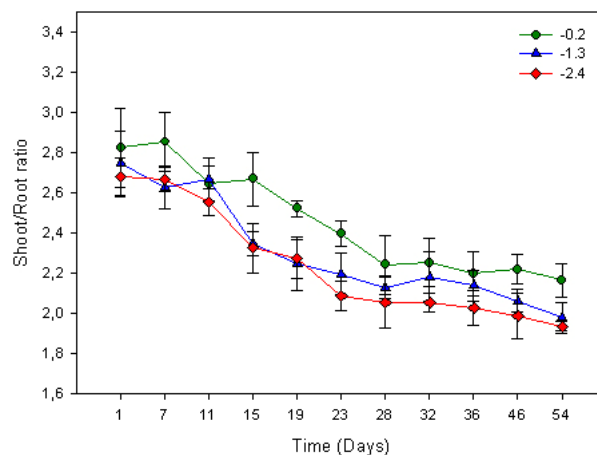


Figure 3. Shoot/Root biomass ratio of *E. globulus* in response to drought hardening treatment. Variation of shoot/root biomass was evaluated during the time course of three different drought treatments (-0.2, -1.3 and -2.4 MPa). Error bars represent SE, (n = 20).

parameters $\Psi\pi_{sat}$, $CRA_{t_{lp}}$, $\Psi\pi_{t_{lp}}$ and e (Table 2). Indicating that even the most severe treatment, -2.4 MPa, allows the plant to restore their water balance.

Cold injury

Drought hardening treatment did not affect freezing resistance of *E. globulus* plants measured three weeks after treatments. All treatments exhibited similar freezing injury, from incipient (LT_{10}) to severe (LT_{90}) damage (Figure 5). Ice nucleation temperature (INT) was significantly increased in most severe treatment reaching $-5.5^{\circ}C$, while the other two treatments exhibited INT around $-6.5^{\circ}C$ which indicates that supercooling capability was decreased after severe drought treatment.

DISCUSSION

Growth and morphology

Drought acclimation treatments significantly reduced

Table 2. Plant water relations obtained from P-V curves. Osmotic potential at saturation ($\Psi\pi_{sat}$), relative water content at turgor loss point ($RWC_{t_{lp}}$), Osmotic potential at turgor loss point ($\Psi\pi_{t_{lp}}$) and modulus of elasticity (e) of *Eucalyptus globulus*, after drought hardening regimes (-0.2, -1.3 and -2.4 MPa). Values are means \pm 1 SE, (n = 3). Means with different letters are significantly different ($P \leq 0.05$).

Hardening regime (MPa)	$\Psi\pi_{sat}$ (MPa)	$RWC_{t_{lp}}$ (%)	$\Psi\pi_{t_{lp}}$ (MPa)	e (MPa)
-0.2	-1.631 \pm 0.020 a	0.780 \pm 0.010 a	-2.228 \pm 0.039 a	11.49 \pm 0.75 a
-1.3	-1.719 \pm 0.130 a	0.776 \pm 0.018 a	-2.375 \pm 0.022 a	12.76 \pm 2.28 a
-2.4	-1.820 \pm 0.110 a	0.807 \pm 0.020 a	-2.375 \pm 0.119 a	13.05 \pm 0.57 a

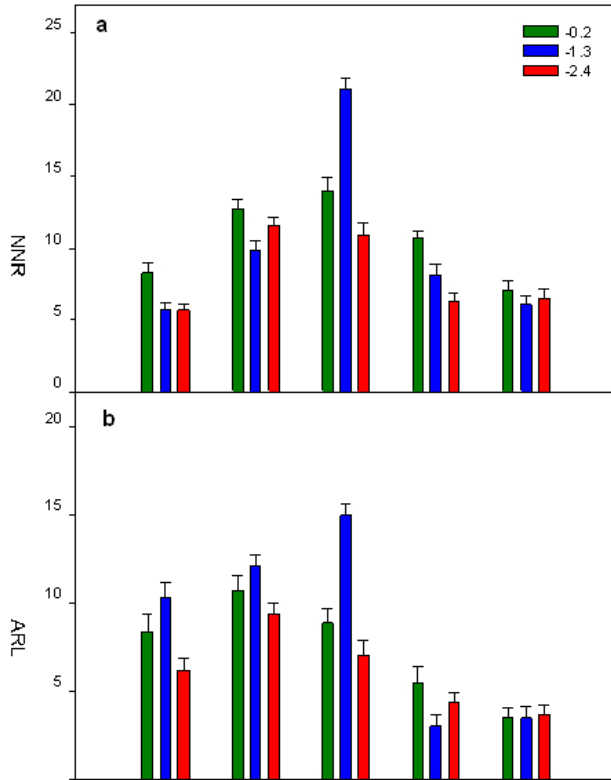


Figure 4. Root growth potential of *E. globulus* in response to drought hardening treatment. Variation of (a) Number of new roots longer than 1 cm (NNR) and (b) the average length of longest three roots (ARL) were evaluated during the time course of three different drought treatments (-0.2, -1.3 and -2.4 MPa). Error bars represent SE, (n = 20). Different letters between times indicate significant differences within each drought hardening treatment and asterisk show significant differences between treatments at each measuring time (Tukey test, $p \leq 0.005$).

growth rates, however some variables like stem diameter, root weight and stem weight were less sensitive to water stress (Figure 2 and Figure 3). Acclimation to substrate low water availability resulted in morpho-physiological adjustments that improve plant water balance (Chaves et al. 2003; Shvaleva et al. 2005). Both, time of exposure and intensity of water stress significantly reduced shoot/root biomass ratio (Figure 3). The effect of time of exposure on the decrease of shoot/root ratio could be due to a decrease in temperature and photoperiod shortage toward autumn. Leaf area was not affected by any drought hardening treatment until day 11, when plants from treatments at -1.3 and -2.4 MPa decreased leaf area mainly due to a defoliation that reach about 26 and 33% respect to the control at -0.2 MPa at days 19 and 23 of treatment respectively. A particularly efficient mechanism to reduce transpiratory surface is the partial leaf abscission (Larcher, 2003). The abscission of an important part of old and basal leaves observed during our experiments is a well known strategy to affront drought of *E. globulus* (Osorio et al. 1998). Plants under drought hardening reactivate leaf expansion but at

lower rates than control plants, exhibiting significant leaf area reduction, 30% at -1.3 MPa and 44% at -2.4 MPa, at the end of the experiment (Figure 2c). Drought hardened plants significantly reduced leaf area, being this variable one of the most sensitive to water stress as reported by several studies in *E. globulus* (Pita and Pardos, 2001; Costa E Silva et al. 2004).

There is evidence that root growth is less affected by drought than stem or shoots (Hsiao and Xu, 2000). Consistently, plants of *E. globulus* exhibited only an 11 and 19% reduction of root biomass in plants treated at -1.3 and -2.4 MPa respectively, while reductions in total plant biomass reached 23 and 34% in corresponding treatments, being leaf biomass the main determinant of this biomass reduction. Similar reductions in *E. globulus* leaf area and plant biomass were found by Pita and Pardos (2001).

Drought hardening treatments of -1.3 MPa and -2.4 MPa significantly reduced SLA, 8 and 14% respectively. Similar results have been reported by Pita and Pardos, (2001); Costa E Silva et al. (2004) and Marron et al. (2003). The maintenance of the balance between transpiration and absorption areas when water availability is reduced seems to be a determinant key of *E. globulus* plant performance (Marron et al. 2003; Costa E Silva et al. 2004).

Drought hardening treatments, -1.3 MPa and -2.4 MPa applied under nursery, significantly reduced plant height, leaf area and specific leaf area, stem and leaf biomass. Plant height, which is considered as a predictor of growth potential in the field, was reduced 3% and 7% in treatments -1.3 MPa and -2.4 MPa respectively. Nevertheless, excessive plant height is a disadvantage in water stressed sites (South and Zwolinski, 1997). A good quality plant is the biggest possible which maintains acceptable levels of survival in a designated site. Stem diameter, is considered the best predictor of survival and initial growth in a plantation (South and Zwolinski, 1997) which did not differ within our drought hardening treatments. Mortality of plants by dehydration was low but significantly different within treatments ($P \leq 0.01$). Plants exposed to -1.3 and -2.4 MPa only exhibited 1.7 and 6% mortality respectively.

Root growth potential

Root growth potential has become one of the most commonly used measures to report forest nurseries quality (Simpson and Ritchie, 1997). Formation of new roots is critical for establishment after transplanting (Kaushal and Aussenac, 1989), especially in sites with water limitations. Both, the number of new roots and the average length of the three longest roots from treatment -1.3 MPa increased significantly from the beginning until day 28 having values 41 and 47% higher than treatments at -0.2 and -2.4 MPa respectively (Figure 4). These results indicates that exposure to a single cycle of moderate (-1.3 MPa) water stress stimulate formation of new roots. These results are inconsistent with those reported by Tinus (1996) for

Pseudotsuga menziesii, who found that NNR decreased with the exposure to a previous event of water stress. However, moderate water stress during drought hardening phase at nursery increase drought resistance, but plants need some time to develop it (Guarnaschelli et al. 2003). Costa E Silva et al. (2004) reported higher root length in water stressed clones of *E. globulus* compares to well irrigated plants. The reductions of NNR and ARL in the severe treatment, -2.4 MPa, indicate a pour plant functional integrity according to Simpson and Ritchie (1997).

Plant water relations

Drought hardening treatments did not affect P-V derived parameters $\Psi\pi_{\text{sat}}$, CRA_{tip} , $\Psi\pi_{\text{tip}}$ and e , evaluated 20 days after drought hardening, which agreed with data reported by Villar-Salvador et al. (2004) in plants of *Quercus ilex*. These results demonstrate that physiological modifications induced by water stress are transient, what agreed with White et al. (2000) who states that compatible solutes accumulated in plants of *E. leucoxydon* and *E. platypus* exposed to drought in the field are lost or converted to osmotically inactive compounds during a rehydration period of only 12 to 14 hrs. On the other hand, it could be that this particular genotype of *E. globulus* did not modify its stem water relations, as it has been reported to some genotypes of *E. globulus* subjected to water stress, indicating intraspecific differences in tissue water relations (Pita and Pardos, 2001). A decrease in osmotic water potential results in higher pressure potentials at a particular water potential of leaves, while and increased in cell wall elasticity results in lower changes in pressure potential for a given change in RWC (Lemcoff et al. 2002). Although no significant differences were observed in the magnitude of osmotic adjustment in our drought hardening treatments -1.3 MPa and -2.4 MPa which exhibit -0.09 and -0.19 MPa respectively. These values are in the range reported for *E. globulus* growing in sites under water stress (Correia et al. 1989; Ngugi et al. 2003). The stem e changed from 11.5 to 13.1 MPa, given the limited adjustment of cell wall elasticity related to the level of osmotic adjustment observed in this *E. globulus* genotype. It seems that the last mechanism is an important determinant of drought tolerance in this genotype.

Freezing resistance

Thermal analyses combined with a freezing injury index (LTs) allow us to predict that the studied genotype is not able to tolerate ice formation within its leaf tissues, registering incipient damage at higher temperatures than INT. This behavior allows us to classify this *E. globulus* genotype as freezing sensitive. On the other hand, drought hardening did not significantly modify damage indexes of leaves, which is likely due to the long time after treatment (three weeks) that freezing resistance was evaluated. Hence, no significant relationship was found between the measured water-status and freezing tolerance parameters. This result is consistent with those found in plant water

relations where no major differences between plants exposed to drought hardening were found after 20 days of normal irrigation. It is known that after short time of normal irrigation most of compatible solutes that also may cryoprotect leaf cells are completely lost after few hours of rehydration (White et al. 2000). The higher INT exhibited after two weeks of normal irrigation in plants previously subjected to the most severe drought hardening treatment (-2.4 MPa) even higher than incipient damage (LT_{10}) allows us to state that plants slightly decrease their limited supercooling capacity after this treatment and were able to withstand ice formation within their tissues without suffering damage in a small range of freezing temperatures. The observed increased INT in plants that reached the lowest water potential (-2.4 MPa) during drought hardening may be related to the slightly higher cell wall elasticity exhibited by this plants (Table 2). It is recognized that cold acclimation increase cell wall rigidity decreasing extracellular ice nucleation and increasing cold tolerance (Fujikawa and Kuroda, 2000). Alternatively, it may be speculated that the extensive hydration state reached after rehydration due to the higher water potential gradient between substrate and plant tissues generated immediately after watering may influence ice nucleation temperatures, because higher hydration state increase the probability of ice formation at low temperatures (Pearce, 2001). It is important to notice that minimum average air temperature at the experimental site was 6.3°C which is out of the temperature range that *E. globulus* may express its maximum potential freezing resistance (Almeida et al. 1994).

In consequence, moderate drought hardening under the final phase of nursery is an important part of plant management programs for plant production to establish plantations in the coastal dry lands and above 650 m.a.s.l. in the Andes Mountains in Chile since drought hardened

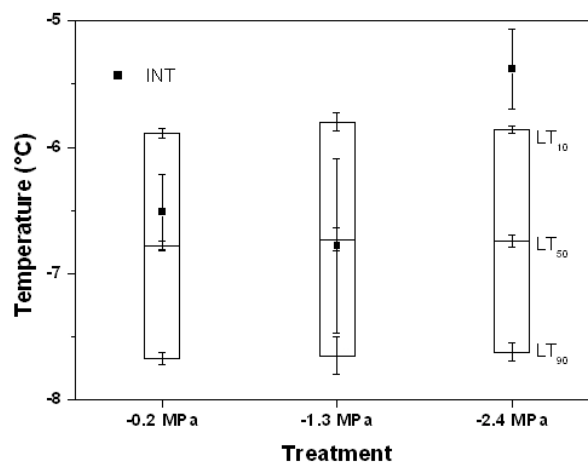


Figure 5. Freezing resistance in drought hardened plants. After drought treatments (-0.2, -1.3 and -2.4 MPa), lethal temperatures for 10, 50 and 90% of leaf damage ($n = 8$) and ice nucleation ($n = 6$) temperatures were determined. Vertical error bars represent standard error.

plants are best prepared to affront severe dryness during the first days of plantation and subsequently will restart growing after drought.

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