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A greenhouse investigation of responses to different water stress regimes of *Laurus nobilis* trees from two climatic regions

S. Maatallah [a,b], M.E. Ghanem [b], A. Albouchi [c], E. Bizid [a], S. Lutts [b,*]

[a] Unité d'Ecophysiologie et Nutrition des Plantes, Faculté des Sciences de Tunis, Campus Universitaire, 1060 Tunis, Tunisia
[b] Groupe de Recherche en Physiologie végétale, Université catholique de Louvain, 5 (Bte 13) Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium
[c] Unité de Agrosylvopastoralisme, Institut National de Recherche en Génie Rural Eau et Fôrets, 2080 Ariana, Tunisia

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ABSTRACT

Plants from two populations of *Laurus nobilis* (Tunisia issued from a semi-arid inland site and Algeria originating from a coastal sub-humid area) were exposed during three months under similar controlled conditions to two stress intensities of permanent stress (60% (S1) and 20% (S2) of field capacity) or to cyclic water stress, plants being re-watered when the soil moisture dropped to 60% (S11) or 20% (S22) of field capacity. One-year old plants displayed contrasting physiological strategies to cope with water stress. Algeria exhibited a higher decrease in osmotic potential (Ψs) in relation to stress-induced proline accumulation. Glycinebetaine accumulated in response to drought in response to permanent stress (Algeria) or cyclic stress (Tunisia). The two populations had similar net photosynthesis (A) but Algeria exhibited higher water use efficiency (WUE) than Tunisia. A drought-induced increase in the apoplastic water content (AWC) was noticed in response to mild stress intensities (S1 and S11) in Tunisia and in response to higher stress intensities (S2 and S22) in Algeria in relation to a stress-induced accumulation of pectin and proportion of arabinose within the pectic fraction. Bulk modulus of elasticity (ε) increased in Tunisia in response to permanent drought and in Algeria in response to cyclic stress, as a result of a stress-induced increase in cellulose (Algeria) or hemicellulose (Tunisia). It is concluded that water stress tolerance could be achieved by both osmotic and elastic adjustment in the coastal population which did not exhibit a prodigal water use comparatively to the inland population. Differences between populations are strongly influenced by the kinetics of water stress application.

1. Introduction

Laurus nobilis (bay laurel) is a large shrub with aromatic evergreen leaves belonging to the family of Lauraceae and native to the southern Mediterranean region (Conforti et al., 2006). This plant species is frequently encountered under semi-arid conditions and thus has to cope with long periods of water shortage, although it is also well adapted to coastal humid environments and requires high amounts of water during the first year of plant establishment (Rhizopoulou and Mitrakos, 1990).

Plant respond to water deficit and adapt to drought through numerous physiological and biochemical changes. Water use efficiency (the amount of dry matter produced per unit amount of water transpired) is a multigene controlled trait which is highly modulated by the prevailing environmental conditions through various physiological properties (Impa et al., 2005). One of the earliest responses of plants to water shortage is to close stomata in order to prevent excessive loss of water but a negative consequence of stomatal closure is to limit CO_2 diffusion rate and thus photosynthesis (Niinemets et al., 2005). Osmotic adjustment consists in the accumulation of compatible organic solutes in order to decrease the osmotic potential of stressed tissues. It allows turgor maintenance and thus plant growth as well as stomatal opening (Ritte et al., 1999). Accumulation of proline and soluble sugars has been reported in a large set of taxa (Hare et al., 1998). Glycinebetaine was reported to act as an efficient osmocompatible solute in a limited number of stress-resistant taxa (Rhodes and Hanson, 1993) but its presence was never reported in *L. nobilis*. Beside organic compounds, ions (especially K^+) could also assume key functions in osmotic adjustment (Morgan, 1984).

Regulation of cell wall elasticity and apoplastic water content may also contribute to some extent to water stress resistance strategies, although conflicting data are available in the literature in this respect. Resistance to water stress has been associated with an increase (Patakas and Noitsakis, 1997; Ngugi et al., 2003) or with

* Corresponding author. Groupe de Recherche en Physiologie végétale (GRPV), Université catholique de Louvain, 5 (Bte 13) Place croix du Sud, 1348 Louvain-la-Neuve, Belgium. Tel.: +32 10 47 20 37/34 46; fax: +32 10 47 34 35.
E-mail address: stanley.lutts@uclouvain.be (S. Lutts).

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a decrease (Fan et al., 1994) in the bulk modulus of elasticity (ε) of mature stressed tissues. Cell wall extensibility of elongating cells was also reported to be quickly affected by drought and both hardening and loosening processes may occur in plants exposed to water stress depending on the considered species (Neumann, 1995 and references therein). Drought-induced modification of water distribution between apoplasm and symplasm has been, at least partly, due to the ability of leaf tissue to increase the apoplastic water content, thus leading to a decrease in osmotic potential without decreasing total leaf water content (Torrecillas et al., 1999; Ngugi et al., 2003). Biochemical composition of the cell wall may directly influence biophysical parameters such as bulk modulus of elasticity and extensibility but also the cell wall water content and the amounts of bound water which is considered to contribute to water stress resistance in numerous plant species (Vertucci and Leopold, 1987; Rascio et al., 1998; Thompson, 2005; Evered et al., 2007). Hemicellulose is an important determinant of cell wall response to water deficit since it constitutes a polymeric gel with important water holding capacities. A decrease in the metabolic turnover of β-glucan through the inhibition of β-glucanase activity may be involved in the alteration of the molecular mass of hemicellulosic polysaccharides (Sakurai et al., 1987). As far as *L. nobilis* is concerned, it has not yet been established whether osmotic and elastic adjustment may occur simultaneously in the same leaf organs and if the relative importance of those strategies differs between populations issued from contrasting environments.

Genotypic variation has been reported for osmotic adjustment within species (Tangpremsri et al., 1991). It was recently demonstrated that populations from a halophyte plant species issued from coastal and inland areas exhibited contrasting behaviours in terms of osmotic adjustment in salt conditions, even when seedlings issued from these populations were tested in an uniform controlled environment (Ben Hassine et al., 2008). It may therefore be postulated that local adaptation of populations from contrasting climatic regions should also occur in response to water stress. Both osmotic adjustment and stress-induced modification in cell wall properties may occur rapidly in response to water stress but are also considered as reversible processes after the stress relief (Chazen and Neumann, 1994). In regions with eratic rainfall, plants are frequently exposed to cyclic events of drought rather than to permanent water stress and there is no exhaustive proof that a given population will react in the same way in response to different kinetics of stress. The tested hypothesis are therefore i) that populations of *L. nobilis* issued from sites differing in annual precipitation display contrasting behaviours for osmotic adjustment and regulation of cell wall properties and ii) that the differences recorded between populations may vary depending on the kinetics and intensity of water stress.

2. Materials and methods

2.1. Plant materials and experimental design

Seeds of *L. nobilis* L. were collected in October 2003 on plants growing at the location of Bardo (36° 13′ N; 10° 23° W), near Tunis and on plants growing in the North–East of Algeria, near the city of Annaba (38° 31′ N; 7° 46′ W). Tunis is characterized by a semi-arid climate (mean annual precipitation: 340 mm; mean maximal temperature: 35.3 °C) while Annaba encounters a sub-humid climate (mean annual precipitation: 850 mm; mean maximal temperature: 27.9 °C). Distributions of precipitations throughout the year are presented in Fig. 1 for the two considered sites. Plant material issued from Bardo will be hereafter designated as "Tunisia" and plant material from Annaba as "Algeria".

In February 2004 (experiment 1) and February 2005 (experiment 2), fruit pericarp was mechanically removed and seeds were surface sterilized with calcium hypochloride 5%. Seeds were then individually placed into pots (0.5 L) filled with a loam–sand mixture (1:2) daily watered at field capacity which was determined according to Jury and Horton (2004). Pots were placed in a greenhouse located in the experimental station of INRGREF at Ariana (36° 50′ N; 10° 14′ W), Tunisia, under semi-controlled environment with a temperature ranging from 22 to 31 °C during the day and 14–18 °C during the night. A mean PAR of 480 μmol m^{-2} s^{-1} was maintained at the top of the canopy and relative humidity was fixed to 60 ± 4% during the day and 72 ± 3% during the night. At the 6 leaves stage, all plants were individually transferred to 10 L pots filled with the same substrate and maintained under the same environmental conditions. Water stress was applied after one year for duration of three months; two repetitions were performed (from 15th May to 15th August 2005 and 15th May to 15th August 2006). Five different treatments were considered: control (soil water content maintained at 100% of field capacity by daily irrigation), permanent stresses: S1 (soil water content maintained at 60% of field capacity) and S2 (20% of field capacity) or cyclic stresses: soil was irrigated to field capacity when its water content fell to 60% (S11) or 20% (S12) of the field capacity. Plants were disposed in a complete randomized block design and plants were randomly rearranged weekly in the greenhouse. The amounts of water required for adequate irrigation were estimated according to Van Genuchten (1980) model with slight modifications according to Martínez et al. (2003). The volumetric water content was measured by the method of responding to changes in apparent dielectric constant using the ThetaProbe soil moisture sensor type ML1 (Delta-T Devices Ltd–UK) and converted to gravimetric water content on the basis of calibration obtained for the considered substrate with samples of known gravimetric water contents. All physiological and biochemical parameters were quantified after three months of stress exposure.

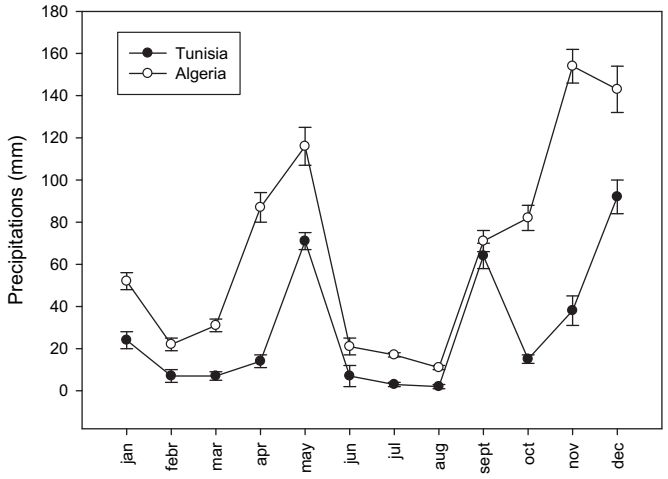

Fig. 1. Distribution of precipitations during the year at the semi-arid site of Tunis (Tunisia) and sub-humid site of Annaba (Algeria). Data presented are means for the years 2000, 2001 and 2003. Vertical bars are standard errors.

2.2. Growth measurements

During the stress period, plant height and diameter of the stem base were estimated monthly on all plants. At the end of the stress period, 6 plants per treatment were harvested: leaves, stems and roots were separated. Roots were carefully separated from the surrounding soil and gently washed to remove adhering soil particles (root water status was consequently not analysed in the present study). Fresh weight was estimated immediately after

harvest for each plant and the corresponding dry weight was estimated after drying samples in an oven at 70 °C for 48 h.

2.3. Plant water status

Predawn leaf water potential Ψ_w of *L. nobilis* was measured at 5:00 a.m. on three groups of four leaves per plant using a pressure bomb. As far as cyclic stress is concerned, Ψ_w was considered 4 days after the last irrigation. A sheet of wet filter paper was added inside the chamber to avoid water loss during measurements. For each population and treatment, 6 randomly-chosen plants were considered. Pressure–volume curves were established using lateral branches. Twig segments were harvested in the middle portion of the plants and their turgid weight (TW) was measured after leafty twigs had remained in the dark at 7 °C covered with plastic bags and their cut ends immersed in distilled water for 20 h. Samples were allowed to equilibrate for 2 h before measurements of RWC at different pressure (−0.2 MPa–−4.7 MPa; Scholander-type pressure chamber (PMS Instrument Co. Corvallis, OR, USA)). This enabled the calculation of the apoplastic water content (AWC), relative water content at zero turgor (RWC_0) and the maximum bulk modulus of elasticity (ε) according to Tyree and Hammel (1972) as well as measurements of the osmotic potential between the two reference points of full turgor ($\Psi^{100}{}_\pi$) and incipient plasmolysis ($\Psi^{0}{}_\pi$). All measurements were made at 20 °C in constant room. For actual leaf osmotic potential at the time of harvest (Ψs), leaves were quickly collected, cut into small segments, then placed in Eppendorf tubes perforated with four small holes and immediately frozen in liquid nitrogen. After being encased individually in a second intact Eppendorf tube, they were allowed to thaw for 30 min and centrifuged at 15,000 *g* for 15 min at 4 °C. The collected tissue sap was analysed for Ψs estimation and ion analysis (see below). Osmolarity (c) was assessed with a vapour pressure osmometer (Wescor 5500) and converted from mOsm kg^{-1} to MPa using the formula: $\Psi s\ (MPa) = -c\ (mOsm\ kg^{-1}) \times 2.58\ 10^{-3}$ according to the Van't Hoff equation.

Leaf stomatal conductance (g_s) was measured on the abaxial surface from 4 leaves of each plant per treatment, using an automatic porometer (MKIII; Delta-T Devices LTD, Cambridge, UK), each g_s measurement was completed within 30 s and the air humidity inside the chamber was kept near to ambient to reproduce the external conditions.

2.4. Net photosynthesis

Gas exchange parameters were measured on the most middle fully-expanded leaves on four plants in each treatment using an open portable system ADC model LCA-4 infrared gas analyser (Analytical Development Co., Hoddesdon, UK) operated at 200 μmol m^{-2} s^{-1} flow rate in conjunction with a portable temperature- and humidity-controlled leaf chamber with a surface area of 6.25 cm^2, under ambient temperature, relative humidity and full sunlight conditions (at 11:00–13:00). The CO_2 concentration inside the leaf chamber was fixed at 338 μmol mol^{-1}. Net assimilation (A; μmol CO_2 m^{-2} s^{-1}), E (transpiration rate, mmol H_2O m^{-2} s^{-1}) and instantaneous water use efficiency (WUEi, μmol CO_2 fixed $mmol^{-1}$ H_2O transpired; A/E) were calculated according to von Caemmerer and Farquhar (1981).

2.5. Analysis of proline, glycinebetaïne and total soluble sugars

For glycinebetaine quantification, 200 mg fresh matter were ground in a cold mortar in the presence of liquid nitrogen. The resulting powder was mixed with 5 mL distilled water and the crude extracts were applied to a small column (1.6 mL) containing an AG1 X8 resin (200–400 mesh, OH– form BioRad). The column was dried down by centrifugation (3 min, 4 °C, 300 *g*) and then washed with 875 μL of distilled water. Extracted glycinebetaine was quantified according to Bessieres et al. (1999) after HPLC separation on a Spherisorb 5 ODS2 column (250 × 4.6 mm) preceded by a precolumn (10 × 1 mm) packed with the same phase. The mobile phase contained 13 mM sodium heptane sulfonic acid and 5 mM Na_2SO_4 in deionised water (pH adjusted to 3.7 with 1 N H_2SO_4) at a flow rate of 0.8 mL min^{-1}. Detection was obtained by an UV detector (BioRad 1801 UV Monitor) and quantification was performed by the ValueChrom ® HPLC System (BioRad Chromatography Software version 4).

For free proline quantification, 1 g of tissue was extracted with 5 mL of salycilic acid 5%; after centrifugation at 5000 *g*, free proline was specifically quantified according to Bates et al. (1973). Soluble sugars were extracted in 80% ethanol from 1 g of leaf fresh tissue and quantified by the classical anthrone method (Yemm and Willis, 1954) using a spectrophotometer (Beckman DU® 640, USA). Standard curve was established using glucose and results were therefore expressed in μmol equivalent glucose g^{-1} FW.

2.6. Determination of ion concentrations

For soluble ion quantification, leaf tissues were frozen with liquid nitrogen. After thawing, the samples were centrifuged for 10 min at 10,000 *g* and then for 5 min at 20,000 *g* to obtain the bulk tissues sap. Necessary dilutions were performed in order to measure major cations concentration by using a Shimadzu AA-60 atomic absorption spectrophotometer (Shimadzu Ltd, Kyoto, Japan). NO_3^- and NH_4^+ were quantified by nitration of salicylic acid and phenol–hypochlorite reactions, respectively (Bajji et al., 2001). All measurements were performed in three replicates.

2.7. Collection and analysis of cell wall material

Collected leaf segments were fixed in boiling methanol for 10 min. The methanol extract was designated as a methanol-soluble fraction. Rehydrated leaf segments were homogenized in deionised water with a polytron homogenizer. The homogenate was centrifuged for 10 min at 1000 *g* and the residue was washed with deionised water, acetone and a methanol:chloroform mixture (1:1 v/v) and dried. The dried cell wall material was treated for 2 h with 2 units mL^{-1} porcine pancreatic α-amylase (type 1-A Sigma) in Na–acetate buffer (pH 6.5, 50 mM) to remove starch and then for 18 h with 200 mg mL^{-1} Pronase (Roche) in Na–phosphate buffer (pH 6.5; 50 mM) containing 5% ethanol to remove proteins. The pectic substance was extracted from cell wall material by three treatments with 50 mM ethylenediaminetetraacetic acid at 95 °C for 15 min. The hemicellulosic polysaccharides were extracted for 18 h with 17.5% NaOH containing 0.02% $NaBH_4$. The hemicellulosic fraction was neutralized with glacial acetic acid. The pectic and neutralized hemicellulosic fraction were dialyzed against deionised water for 36 h and lyophilized with freeze drier. The alkali-insoluble residue was first hydrolyzed with trifluoracetic acid (2 M, 121 °C, 1 h) to release remaining part of hemicellulosic substances (Yeo et al., 1995), and then washed with ethanol, ethyl acetate (1:1, v/v), dried in air under a fume hood and designated as cellulose.

Total sugar content of pectic and hemicellulosic fraction was quantified by the phenol–sulfuric acid method (Dubois et al., 1956) using glucose as standard. Uronic acids were measured by modified carbazole–sulfuric acid method (Dische, 1962) using galacturonic acid as standard. The neutral sugars of pectin and hemicellulose were analysed by gas–liquid chromatography (GLC). Acetylating of the sugars after conversion to alditol acetate was performed according to Blakeney et al. (1983). GLC was carried out on a Shimadzu GC-18A apparatus equipped with a flame ionization detector. A capillary

column (25 m, 0.22 mm, Hicap CPB10) was used and operated at 220 °C with gas flow rate of 60 mL min^{-1} of nitrogen. Peak areas were measured with a Shimadzu Chromatocorder-21.

2.8. β-glucanase assay

Frozen leaf samples (ca. 500 mg fresh weight) were homogenized with cold 10 mM sodium phosphate buffer (pH 7.0). The homogenate was filtered through propylene mesh (32 μm). The fraction was washed with the same buffer and then suspended in 10 mM sodium phosphate buffer (pH 6.0) containing 1 M NaCl. The suspension was kept for 24 h at 4 °C and filtered through propylene mesh. The filtrate was used as enzyme extract for the measurement of β-glucanase activity. The reaction mixture (total 100 μL) contained 50 μg of β-glucans (β-1,3; 1,4-D-glucans) and 10 μg of cell wall protein in 10 mM sodium phosphate buffer (pH 6.0). The mixture of solution was incubated for 6 h at 37 °C and the reaction was then terminated by boiling. Enzyme activity (β endo-1-4 glucanases) was determined by monitoring the increase in reducing sugars liberated from β-glucans by the Somogyi–Nelson (Somogyi, 1952) method and expressed as glucose equivalent.

2.9. Statistical analysis

For each stress treatment and each population, 24 plants were considered for physiological characterization. The experimental layout was a complete randomized block design with 10 blocks. Data were analysed using a two-way analysis of variance (ANOVA) at a significance level of $P \leq 0.05$ (*) or $P \leq 0.01$ (**). The model is defined on the basis of fixed main effects (treatment and population). When the ANOVA was significant at $P \leq 0.05$, Duncan Multiple Range Test was used for mean comparison. Data were analysed using the SAS software (SAS Institutes, Tervueren, Belgium). Pearson correlation coefficient between parameters was determined when required. Whatever the considered parameter, similar results were obtained in 2005 on the one hand and 2006 on the other hand. As a consequence, only results obtained in 2006 are presented in the Results section.

3. Results

3.1. Plant growth and water status

Young plants issued from the two considered populations displayed contrasting behaviours in terms of growing habit, even in control conditions since Tunisia exhibited a stunted growth with a lower leaf number than Algeria. Dry weights of leaves, stems and roots of stressed plants were always lower for Tunisia than for Algeria (Table 1). Drought treatments induced a decrease in the plant dry weight for all organs although leaves and stem growth were more sensitive to water stress than root growth for both populations. The shoot/root ratio consequently decreased in response to water stress, and to a higher extent in Tunisia than in Algeria. As expected, permanent water stress (S1 and S2) induced a higher growth inhibition than cyclic stress treatments (S11 and S22). The two considered stress intensities (S1 and S2 on the one hand, S11 and S22 on the other hand) induced almost similar growth inhibition at the leaf level, therefore suggesting that plants issued from both populations were able to cope with different levels of soil moisture deficit. Permanent stresses (S1 and S2) reduced root growth in Algeria but only the highest stress intensity had such an impact in Tunisia. Cyclic stress did not affect root growth in Algeria. When growth was analysed on a relative (in terms of percentage of unstressed control) rather than absolute terms, no significant difference was recorded for the impact of moderate stress (S1 and S11) for the two populations. Tunisia appeared slightly more sensitive than Algeria in response to higher water deficit (S2 and S22; interaction treatment × population being significant ($P < 0.05$) for all considered organs).

Leaf water potentials (Ψw; Fig. 2) were similar for control plants of the two considered populations but decreased to a higher extent in Algeria than in Tunisia in response to a permanent water stress. In contrast, leaf water potential remained similar for both populations in response to cyclic water stress (S11 and S22) and no difference was recorded for the two stress intensities. Leaf osmotic potential (Ψs; Fig. 2) of Tunisia remained almost constant whatever the treatment (except for S1) while it decreased in Algeria, showing a minimal value in response to S2 treatment.

The kinetics of stress application has a strong impact on the relative water content at zero turgor (RWC_0; Table 2) and on the bulk

Table 1
Dry weight (in g) of leaves, stems and roots, shoot/root ratios, height of the plant and diameter of the stem base in plants of *L. nobilis* issued from a population of Algeria (A) or Tunisia (T) and exposed during three months to permanent water stress (S1: 60% of field capacity; S2: 20% of field capacity) or to cyclic water stress (plants being irrigated when the soil moisture dropped to 60% (S11) or 20% (S22) of the field capacity).

Treatment	Population	Leaves (g)	Stems (g)	Roots (g)	Shoot/Root	Plant height (cm)	Diameter (mm)
Control	A	40.9 ± 2.9 a	51.7 ± 4.1 a	33.3 ± 3.6 a	2.78 ± 0.15 a	175.2 ± 2.7 a	15.6 ± 0.5 a
	T	23.9 ± 1.7 c	28.6 ± 2.1 c	22.4 ± 1.4 b	2.34 ± 0.07 b	103.0 ± 3.0 b	14.5 ± 1.0 a
S1	A	23.3 ± 3.8 c	25.2 ± 1.1 c	25.0 ± 3.5 b	1.94 ± 0.07 c	104.2 ± 4.4 b	11.9 ± 0.4 bc
	T	13.8 ± 2.0 d	16.6 ± 2.4 d	19.4 ± 2.4 b	1.56 ± 0.12 d	83.2 ± 7.8 bc	10.9 ± 0.3 c
S2	A	25.8 ± 0.7 c	19.9 ± 1.4 c,d	21.8 ± 1.8 b	2.09 ± 0.10 bc	67.4 ± 4.0 c	9.9 ± 0.8 c
	T	11.2 ± 0.8 d	8.4 ± 0.9 e	15.1 ± 1.6 c	1.29 ± 0.15 e	53.2 ± 3.2 d	9.9 ± 0.4 c
S11	A	32.9 ± 2.9 b	34.7 ± 2.8 b	39.4 ± 3.4 a	1.72 ± 0.08 cd	97.4 ± 2.5 b	14.8 ± 0.3 a
	T	20.4 ± 1.9 c	17.6 ± 2.9 d	18.9 ± 2.2 bc	2.01 ± 0.11 bc	94.8 ± 4.9 b	12.8 ± 0.3 b
S22	A	35.2 ± 2.0 b	35.8 ± 2.6 b	32.8 ± 1.6 a	2.16 ± 0.05 b	106.6 ± 2.2 b	13.6 ± 0.4 ab
	T	18.9 ± 1.9 cd	16.8 ± 1.6 d	16.7 ± 1.5 c	2.14 ± 0.12 b	64.6 ± 6.8 c	10.8 ± 0.8 c
F values	*df*						
Treatment	4	14.0**	26.3**	12.1**	5.2*	24.9**	3.4*
Population	1	10.9**	17.8**	15.9**	6.1*	4.3*	4.8*
Interaction	4	3.2*	1.2 NS	3.4*	3.1*	1.0 NS	2.1 NS

Each value is the mean of 6 replicates ± S.E.
Means followed by different letters are significantly different at $P < 0.05$.
F values are given at the bottom of the Table for the main analysed factors (Treatment and Population) as well as their interaction.
* and ** refer to $P < 0.5$ and $P < 0.01$, respectively.

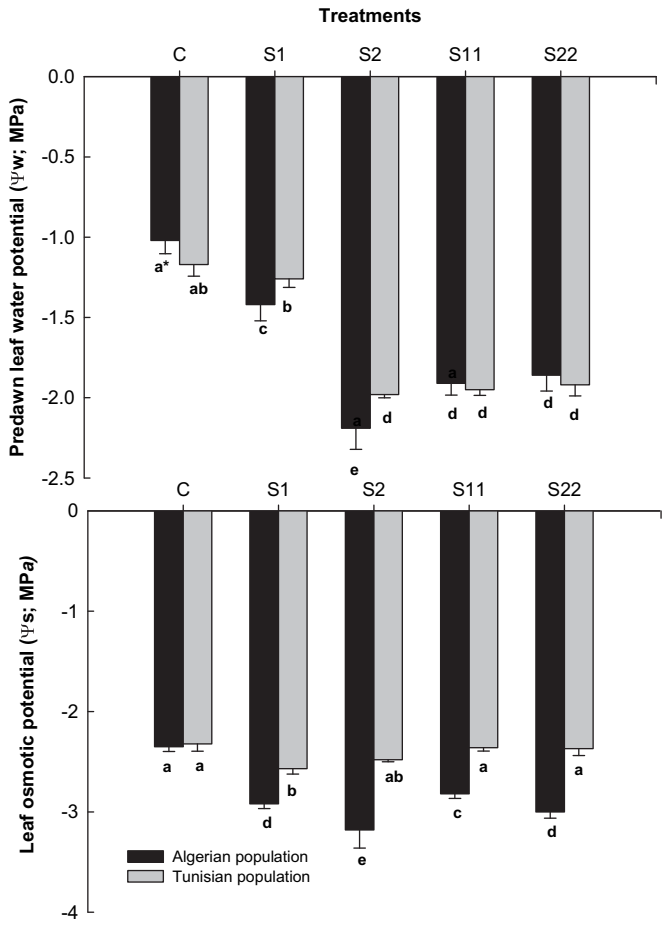

Fig. 2. Leaf water potential (Ψw) and leaf actual osmotic potential (Ψs) in plants of *L. nobilis* issued from a population of Algeria or Tunisia and exposed during three months to permanent water stress (S1: 60% of field capacity; S2: 20% of field capacity) or to cyclic water stress (plants being irrigated when the soil moisture dropped to 60% (S11) or 20% (S22) of the field capacity). Each value is the mean of 6 replicates and the vertical bars are standard errors of the mean. Values sharing a common letter are not significantly different at $P < 0.05$.

modulus of elasticity (ε; Table 2). Leaf RWC_0 was lower in control plants for Tunisia comparatively to Algeria; it remained unaffected in Algeria exposed to permanent stress (S1 and S2) while it significantly increased in Tunisia. An opposite trend was noticed in response to cyclic water stress since RWC_0 increased in Algeria exposed to S11 and S22 but remained constant in Tunisia. In response to permanent stress, the bulk modulus of elasticity was higher in Tunisia than in Algeria while an inverse relationship was observed for plants exposed to cyclic stresses. In contrast, the apoplastic water content (AWC) appeared to be influenced by stress intensity rather than by stress frequency: although it increased in all stressed leaves comparatively to controls, Algeria exhibited a higher AWC value than Tunisia for moderate stress intensities (S1 and S11) while an inverse trend was recorded for highest stress intensities (S2 and S22). There was no significant correlation between AWC and ε, suggesting that the biochemical changes conditioning cell wall elasticity did not influence water retention by apoplasm.

In contrast to Ψs of tissue sap collected after freeze–thawing treatment (Fig. 2), both $\Psi^{100}{}_{\Pi}$ and $\Psi^{0}{}_{\Pi}$ of stressed plants from Tunisia estimated by pressure–volume curve decreased, comparatively to control (Table 2). Nevertheless, whatever the considered treatment, $\Psi^{100}{}_{\Pi}$ still remained lower for Algeria than for Tunisia. This was not true anymore for $\Psi^{0}{}_{\Pi}$ which remained similar in plants from both populations exposed to S2, S11 and S22 treatments. However, when data are pooled for all treated plants, we found a slight (although significant $P = 0.032$) unexpected negative correlation between RWC_0 and $\Psi^{0}{}_{\Pi}$.

3.2. Net photosynthesis and stomatal conductance

The two considered populations exhibited similar rates of net photosynthesis (Fig. 3), whatever the kinetics or the intensity of drought treatment: while net photosynthesis was clearly lower in S2 comparatively to S1 treatment ($P < 0.01$), no differences were recorded between plants exposed to S11 and S22 treatment. Drought-induced inhibition of photosynthesis could be due to both stomatal and non-stomatal causes. As far as stomatal conductance is concerned, a drastic decrease was observed in S1- and S2-treated plants but no differences were recorded between populations. In response to cyclic water stress (S11 and S22), g_s was clearly higher for Tunisia comparatively to Algeria. In all cases except in control

Table 2
Leaf relative water content at zero turgor (RWC_0, in %), apoplasmic water content (AWC, in %), maximum bulk modulus of elasticity (ε) in plants of *L. nobilis* issued from a population of Algeria (A) or Tunisia (T) and exposed during three months to permanent water stress (S1 : 60% of field capacity; S2: 20% of field capacity) or to cyclic water stress (plants being irrigated when the soil moisture dropped to 60% (S11) or 20% (S22) of the field capacity).

Treatment	Population	RWC_0 (%)	AWC (%)	ε (MPa)	$\Psi^{100}{}_{\Pi}$	$\Psi^{0}{}_{\Pi}$
Control	A	85.3 ± 1.1 b	19.6 ± 2.3 ab	9.87 ± 0.19 b	−2.41 ± 0.04 bc	−3.18 ± 0.07 b
	T	82.3 ± 1.3 a	15.6 ± 1.5 a	8.65 ± 0.65 a	−2.18 ± 0.05 a	−2.77 ± 0.12 a
S1	A	88.1 ± 0.5 bc	31.9 ± 1.4 c	10.85 ± 0.69 b	−2.89 ± 0.21 d	−4.04 ± 0.04 d
	T	85.7 ± 0.5 b	20.9 ± 1.5 b	12.11 ± 0.51 c	−2.63 ± 0.05 c	−3.33 ± 0.01 b
S2	A	86.5 ± 1.1 b	25.3 ± 2.8 b	9.70 ± 0.41 ab	−2.91 ± 0.12 d	−3.39 ± 0.06 bc
	T	88.8 ± 0.3 c	35.2 ± 2.6 d	12.06 ± 0.51 c	−2.54 ± 0.06 bc	−3.57 ± 0.08 c
S11	A	89.0 ± 0.2 c	36.2 ± 1.9 d	11.15 ± 0.28 bc	−2.62 ± 0.14 c	−3.54 ± 0.03 c
	T	82.3 ± 0.8 a	23.3 ± 1.8 b	7.88 ± 0.64 a	−2.36 ± 0.08 b	−3.44 ± 0.08 bc
S22	A	88.6 ± 0.3 c	25.0 ± 1.1 b	13.47 ± 0.17 d	−2.63 ± 0.07 c	−3.27 ± 0.09 b
	T	82.2 ± 0.3 a	29.8 ± 0.7 c	8.30 ± 0.14 a	−2.38 ± 0.02 b	−3.33 ± 0.04 b
F values	*df*					
Treatment	4	3.5*	19.4**	3.3*	3.7*	7.5**
Population	1	6.3*	33.7**	10.8**	5.2*	5.9*
Interaction	4	8.1**	3.4*	14.6**	1.7 NS	2.0 NS

Each value is the mean of 6 replicates ± S.E.
Means followed by different letters are significantly different at $P < 0.05$.
* and ** refer to $P < 0.5$ and $P < 0.01$, respectively.

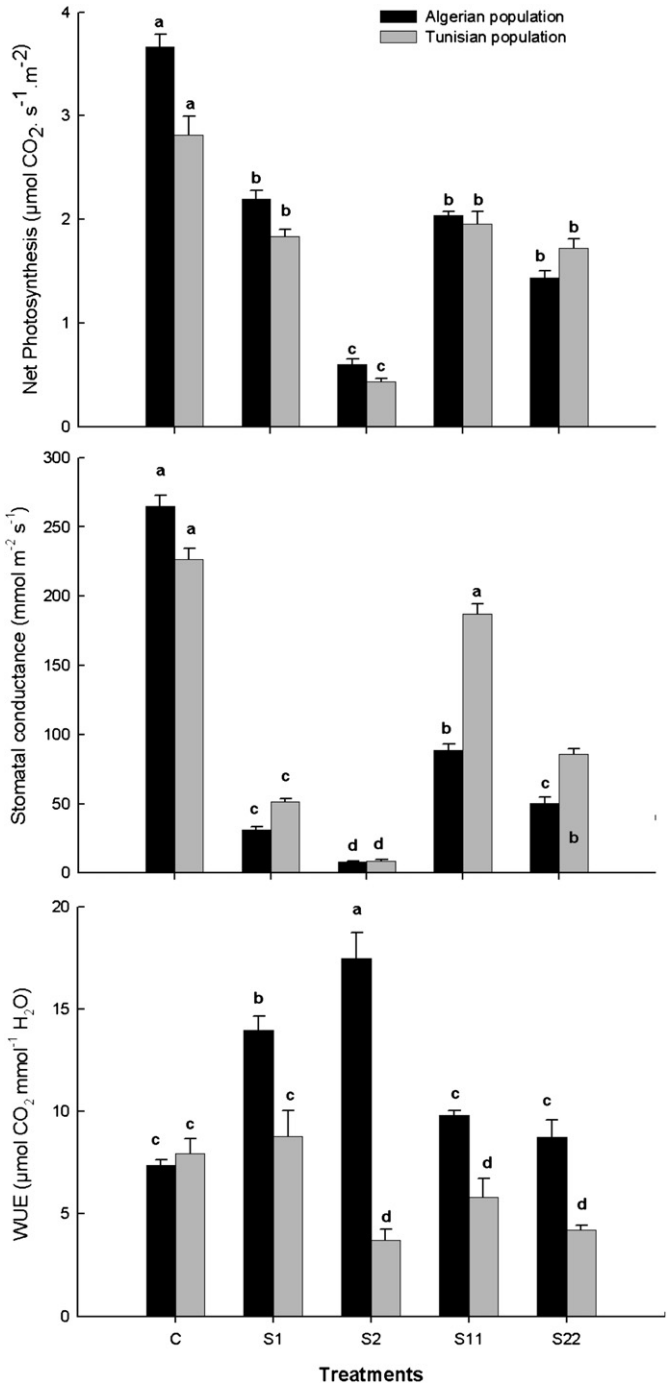

Fig. 3. Net photosynthesis (*A*), stomatal conductance (g_s) and instantaneous water use efficiency (WUE) in plants of *L. nobilis* issued from a population of Algeria or Tunisia and exposed during three months to permanent water stress (S1: 60% of field capacity; S2: 20% of field capacity) or to cyclic water stress (plants being irrigated when the soil moisture dropped to 60% (S11) or 20% (S22) of the field capacity). Each value is the mean of 6 replicates and the vertical bars are standard errors of the mean. Values sharing a common letter are not significantly different at $P < 0.05$.

plants, transpiration rate was lower for Algeria than for Tunisia (detailed data not shown) and the instantaneous WUE was thus higher for the former than for the latter (Fig. 3). Permanent water stress (S1 and S2) induced a strong increase in WUE of Algeria while cyclic water stress had no impact on WUE comparatively to control for this population. In contrast, all treatments (except S1) induced a decrease in the WUE of Tunisia.

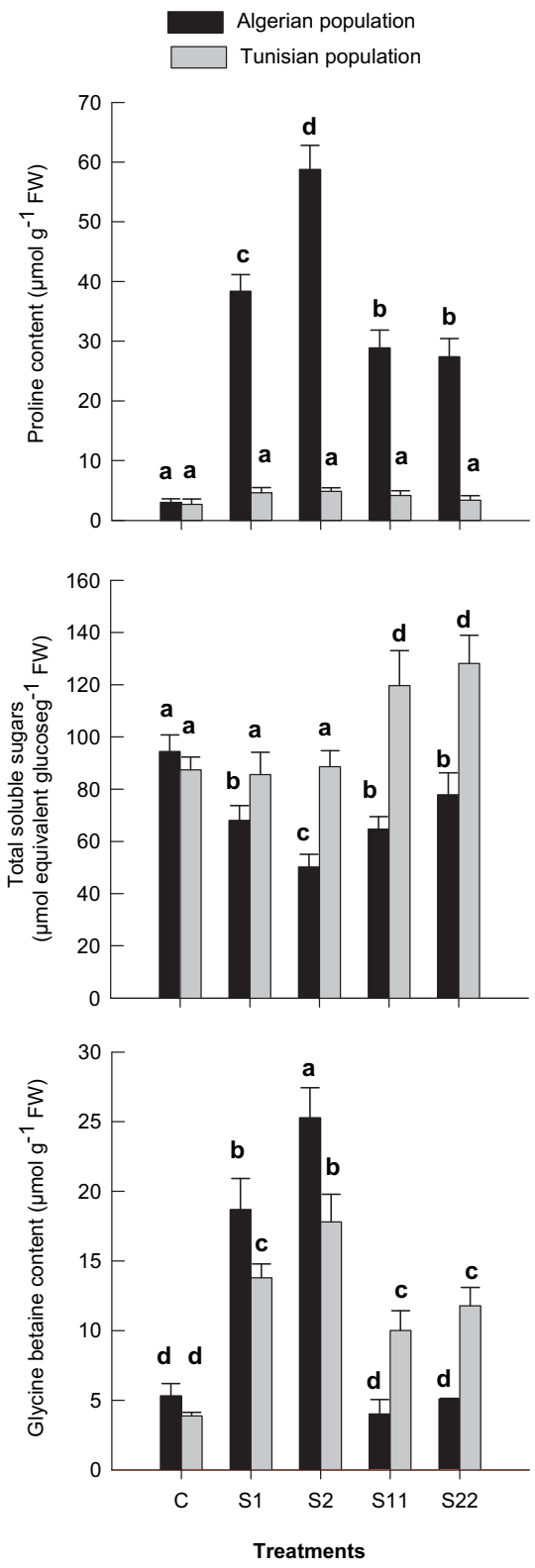

Fig. 4. Leaf proline, total soluble sugars and glycinebetaine concentration of *L. nobilis* issued from a population of Algeria or Tunisia and exposed during three months to permanent water stress (S1: 60% of field capacity; S2: 20% of field capacity) or to cyclic water stress (plants being irrigated when the soil moisture dropped to 60% (S11) or 20% (S22) of the field capacity). Each value is the mean of 6 replicates and the vertical bars are standard errors of the mean. Values sharing a common letter are not significantly different at $P < 0.05$.

3.3. Organic compatible solutes and inorganic ions concentrations

Proline, total soluble sugar and glycinebetaine concentrations expressed on a fresh weight basis were similar in control plants from both populations (Fig. 4). Plants issued from Tunisia appeared unable to accumulate proline in response to drought treatment while plants originating from Algeria accumulated proline to a higher extent in response to permanent comparatively to cyclic water stress. An opposite trend was recorded for total soluble sugars which decreased in response to drought in Algeria but remained stable (permanent stress) or even increased (cyclic stress) in Tunisia. High concentrations of glycinebetaine were detected in *L. nobilis*: this quaternary ammonium compound accumulated in response to S1 and S2 treatment and to higher amounts in Algeria than in Tunisia. As far as cyclic stresses are concerned, however, glycinebetaine accumulated in Tunisia but not in Algeria.

Water stress had no impact on K^+, Mg^{2+} and NO_3^- concentration and no significant difference was recorded between the two considered populations in this respect (Table 3). Water stress slightly reduced Ca^{2+} concentrations in Algeria but in response to the highest stress intensities only (S2 and S22). Water stress also induced a significant increase in NH_4^+ concentration in Tunisia.

3.4. Cell wall composition

The mean cellulose concentration in leaves of plants from Tunisia significantly increased in response to permanent stresses only ($P < 0.01$) although it remained at similar values for S1 and S2 treatments (Table 4). Cellulose concentration was not affected by water stress in Algeria, whatever the kinetics of stress imposition or the stress intensity. In contrast, hemicellulose concentration in leaves collected on plants from Algeria decreased in response to permanent stress (S1 and S2) but increased in response to cyclic stress (S11 and S22). Hemicellulose decreased in response to all treatments for leaves from Tunisia. Pectin concentration increased in response to moderate stress intensities (S1 and S11) in Algeria but only in response to the highest stress intensity (S2 and S22) in Tunisia.

The kinetics of stress imposition had an impact on the sugar composition of hemicellulosic fraction (Fig. 5), although no difference could be recorded in this respect between the two studied populations. As shown in Fig. 5 for plants exposed to moderate stress intensity, both arabinose and xylose proportions in hemicellulosic fraction increased in response to cyclic (S11) comparatively to permanent (S1) water stress while galactose and glucose exhibited an inverse trend. Uronic acid (quantified by the carbazole–sulfuric acid method) remained unaffected in permanent stress comparatively to controls (17.3% versus 16.8%) but increased in response to cyclic stress (25.1%). A similar increase, mainly in arabinose and, to a lesser extent in xylose, associated with a decrease in galactose and glucose was noticed in pectin fraction of plants exposed to S1 and S11 (Fig. 5B and 5D). In the pectin fraction, uronic acid increased in response to both type of stress (45.2%, 55.1% and 53.9% in control, S1 and S11-treated plants, respectively).

The β-glucanase activities exhibited similar trends when expressed on a fresh weight basis (Fig. 6) or on a protein basis (detailed data not shown). Enzyme activities were similar in control

Table 4
Cellulose, hemicellulose and pectin content (in $\mu g\,g^{-1}$ FW) in leaves of *L. nobilis* issued from a population of Algeria (A) or Tunisia (T) and exposed during three months to permanent water stress (S1: 60% of field capacity; S2: 20% of field capacity) or to cyclic water stress (plants being irrigated when the soil moisture dropped to 60% (S11) or 20% (S22) of the field capacity).

Treatment	Population	Cellulose ($\mu g\,g^{-1}$ FW)	Hemicellulose ($\mu g\,g^{-1}$ FW)	Pectin ($\mu g\,g^{-1}$ FW)
Control	A	20.3 ± 2.3 a	17.1 ± 0.5 a	5.7 ± 0.2 a
	T	18.1 ± 2.0 a	18.1 ± 1.9 a	6.0 ± 0.5 a
S1	A	23.1 ± 1.4 a	14.2 ± 1.2 b	13.9 ± 1.0 b
	T	28.9 ± 0.9 b	16.5 ± 0.4 ab	7.1 ± 0.9 a
S2	A	21.2 ± 1.8 a	13.9 ± 1.0 b	6.4 ± 0.2 a
	T	29.3 ± 1.4 b	14.6 ± 0.7 b	16.3 ± 0.8 b
S11	A	19.5 ± 0.9 a	26.5 ± 1.8 c	15.9 ± 1.4 b
	T	17.3 ± 0.8 c	11.3 ± 0.6 d	8.7 ± 0.6 a
S22	A	21.2 ± 1.0 a	28.7 ± 2.5 c	8.3 ± 1.1 a
	T	16.1 ± 1.9 c	12.5 ± 0.8 db	14.1 ± 0.3 b
F values	*df*			
Treatment	4	2.7*	3.4*	6.3**
Population	1	11.8**	6.2*	19.4**
Interaction	4	13.1**	33.4**	27.2**

Each value is the mean of 6 replicates ± S.E.
Means followed by different letters are significantly different at $P < 0.05$.
* and ** refer to $P < 0.5$ and $P < 0.01$, respectively.

Table 3
Ion concentrations (K^+, Ca^{2+}, Mg^{2+}, NO_3^-, NH_4^+) in leaves of *L. nobilis* issued from a population of Algeria (A) or Tunisia (T) and exposed during three months to permanent water stress (S1: 60% of field capacity; S2: 20% of field capacity) or to cyclic water stress (plants being irrigated when the soil moisture dropped to 60% (S11) or 20% (S22) of the field capacity).

Treatment	Population	K^+	Ca^{2+}	Mg^{2+}	NO_3^-	NH_4^+
Control	A	75.3 ± 6.1 a	14.2 ± 1.1 a	6.9 ± 0.4 a	22.8 ± 3.1 a	2.1 ± 0.2 a
	T	74.2 ± 2.3 a	12.8 ± 1.0 a	5.5 ± 0.2 a	17.3 ± 2.2 ab	1.9 ± 0.2 a
S1	A	70.4 ± 4.8 a	14.5 ± 0.8 a	4.8 ± 1.0 a	20.1 ± 1.8 a	2.2 ± 0.3 a
	T	71.6 ± 4.9 a	13.6 ± 2.3 a	5.6 ± 0.4 a	15.7 ± 0.4 b	4.5 ± 0.2 b
S2	A	68.9 ± 7.5 a	7.5 ± 0.6 b	6.1 ± 0.5 a	16.9 ± 1.8 ab	2.4 ± 0.2 a
	T	73.4 ± 4.8 a	12.4 ± 2.4 a	4.4 ± 0.7 a	18.3 ± 1.1 a	6.3 ± 0.8 c
S11	A	69.6 ± 4.0 a	13.5 ± 1.7 a	5.2 ± 0.7 a	19.7 ± 2.1 a	1.8 ± 0.3 a
	T	73.6 ± 8.1 a	14.4 ± 2.1 a	4.9 ± 0.4 a	20.5 ± 1.9 a	4.4 ± 0.9 b
S22	A	77.4 ± 8.2 a	8.3 ± 0.7 b	5.1 ± 0.4 a	15.2 ± 0.7 b	1.9 ± 0.6 a
	T	75.1 ± 5.4 a	14.2 ± 1.2 a	5.3 ± 0.3 a	16.7 ± 0.5 ab	5.8 ± 0.8 bc
F values	*df*					
Treatment	4	1.4 NS	2.1 NS	1.7 NS	2.5 NS	3.4*
Population	1	2.7 NS	2.2*	1.3 NS	3.5 NS	9.7**
Interaction	4	2.1 NS	3.2*	3.0 NS	2.8 NS	12.3**

Each value is the mean of 6 replicates ± S.E.
Means followed by different letters are significantly different at $P < 0.05$.
* and ** refer to $P < 0.5$ and $P < 0.01$, respectively. Ion concentrations were expressed in mM in the extracted tissue sap.

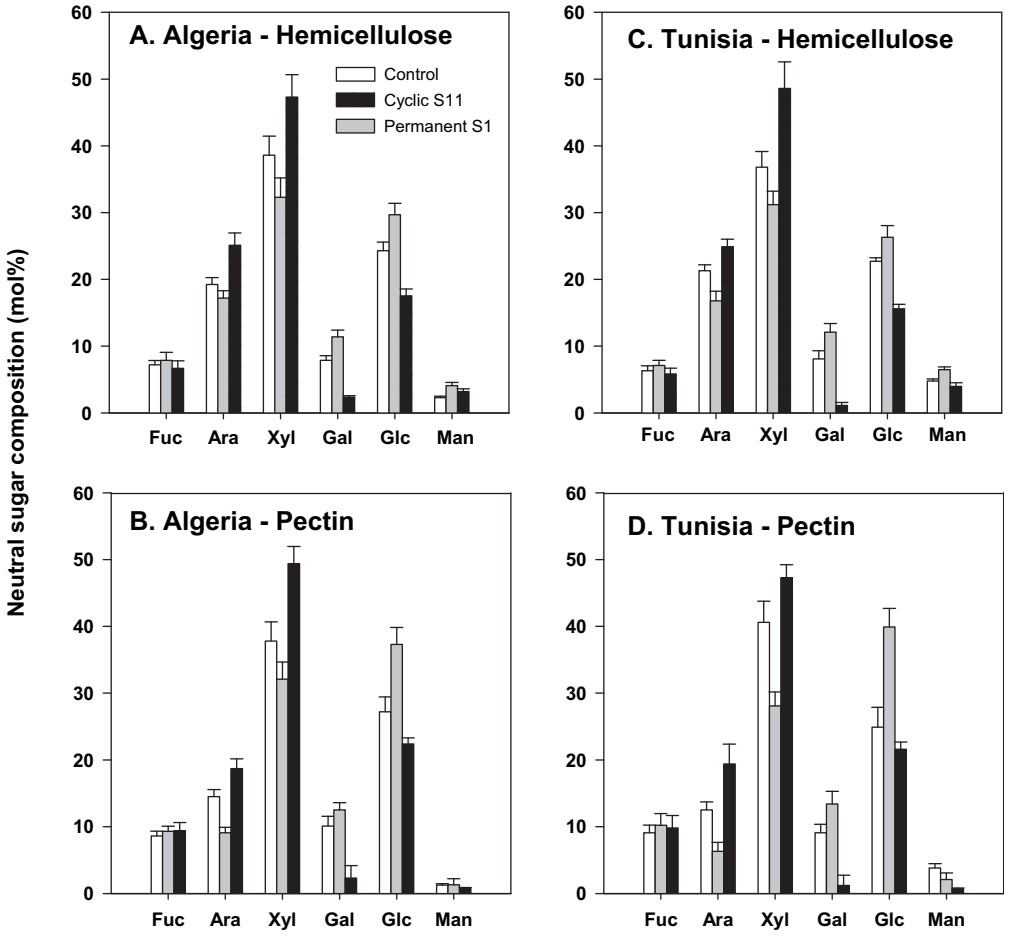

Fig. 5. Impact of the kinetics of stress imposition on the neutral sugar composition in hemicellulosic (A; C) and pectin (B; D) fraction in leaves of *L. nobilis* issued from a population of Algeria (A, B) and Tunisia (C, D) and exposed during three months to control conditions, to a permanent water stress (S1: 60% of field capacity) or to cyclic water stress (plants being irrigated when the soil moisture dropped to 60% (S11)). Each value is the mean of 10 replicates. Fuc, fucose; Ara, arabinose; Xyl, xylose; Gal, galactose; Glc, glucose; Rha, rhamnosre; Man, mannose.

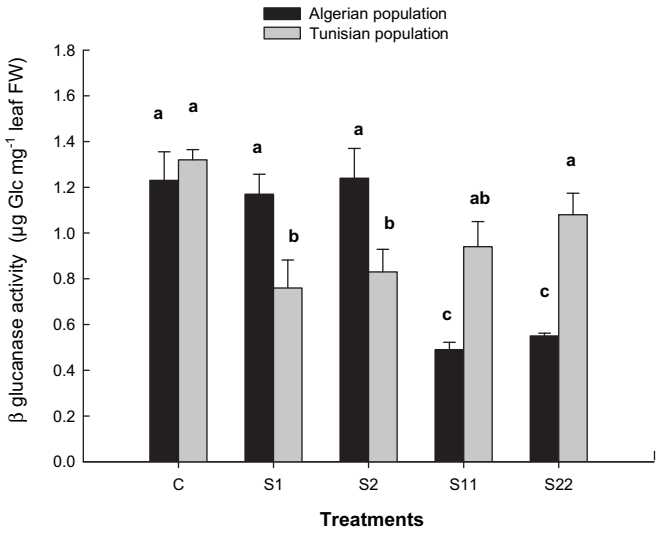

Fig. 6. β-glucanase activities in leaves of *L. nobilis* issued from a population of Algeria or Tunisia and exposed during three months to permanent water stress (S1: 60% of field capacity; S2: 20% of field capacity) or to cyclic water stress (plants being irrigated when the soil moisture dropped to 60% (S11) or 20% (S22) of the field capacity). Each value is the mean of 6 replicates and the vertical bars are standard errors of the mean. Values sharing a common letter are not significantly different at $P < 0.05$.

leaves of the two considered populations. Enzyme activities decreased in Tunisia in response to permanent stress only, and in Algeria in response to cyclic stress only.

4. Discussion

Plants growing in the Mediterranean basin region overcome long drought periods. Tolerance to extended water shortage usually results from a complex network of interacting physiological and biochemical properties. The sequence of metabolic and morphological modifications, as well as their ultimate consequence in terms of ability of the plants to cope with water stress depends on the considered species on the one hand, and on the duration and intensity of water stress on the other hand.

4.1. Plants from different populations differ for some but not all physiological parameters

In the present study, laurel plants issued from contrasting populations were maintained under similar environmental conditions and recorded differences should therefore reflect, at least partly, genetically controlled adaptative strategies resulting from natural selection of populations in their original habitat. Surprisingly, the two considered populations issued from contrasting habitats did not strongly differ in terms of relative impact of water stress on plant growth, thus suggesting that the population issued

from a sub-humid area (Algeria) was not more sensitive to water stress than a population issues from a semi-desertic area (Tunisia). Dry climate populations were reported to partition more biomass to the root system (Lei et al., 2006): this strategy should allow water absorption at higher depth and was adopted by Tunisia, although the resulting benefit remained limited.

The two considered populations did not differ for net photosynthesis but the population originating from Algeria clearly displayed a higher WUE than Tunisia, suggesting that in contrast to *Populus davidiana* (Zhang et al., 2004) and *Atriplex halimus* (Ben Hassine et al., 2008), populations of *L. nobilis* issued from costal areas do not necessarily display a prodigal water use strategy. Drought-induced decreases in stomatal conductance of plants exposed to permanent stresses (S1 and S2) were similar for both populations and the lower instantaneous transpiration rate of Algeria leading to higher WUE is therefore puzzling. It has been reported that xylem in *L. nobilis* could be exposed to embolism in relation to intrinsic vulnerability to cavitation of xylem conduits and that specific adaptations should have developed some repair mechanisms to cope with xylem dysfunctions (Salleo et al., 2004; Gascó et al., 2006). It could therefore not be excluded that distinct populations differ in terms of embolism avoidance and that Algeria, encountering cavitation processes at the time of measurements, thus displayed lower transpiration rates than Tunisia at a given stomatal conductance.

4.2. Studied populations differ for osmotic adjustment

Algeria and Tunisia also exhibited contrasting behaviour in terms of osmotic adjustment both from a quantitative and from a qualitative point of view. Data obtained from osmotic pressure measurements of extracted tissue sap after freeze and thawing (Fig. 2) and determination of $\Psi^{100}{}_{\Pi}$ from pressure–volume curves (Table 2) demonstrated that Algeria exhibited a higher ability than Tunisia to reduce its internal osmotic potential. Drought-induced proline accumulation is a well known response which has been reported in a huge number of plant species (Hare et al., 1998 and references therein), including *L. nobilis* (Dimantoglou and Rhizopoulou, 1992). We demonstrate, however, that one of the considered populations of *L. nobilis* (Tunisia; Fig. 4) is completely unable to overproduce and accumulate proline while another population (Algeria) is able to accumulate it in very high concentration, exhibiting a relative increase of 1200% of proline concentration at the leaf level in response to S2 comparatively to unstressed controls. In contrast to proline, glycinebetaine accumulation has been reported in a limited number of plant species, especially those which are frequently exposed to drought or highly saline environments (Rhodes and Hanson, 1993). To the best of our knowledge, the presence of this quaternary ammonium compound was never checked in *L. nobilis*. The present study demonstrates that it is present in this species and that it accumulated in response to water shortage (Fig. 4). Beside its direct involvement in osmotic adjustment, glycinebetaine was also reported to act as a protecting molecule helping to maintain membrane integrity and protein conformation, but also as a free radical scavenger protecting tissue from oxidative stress (Rhodes and Hanson, 1993; Martínez et al., 2003; Ben Hassine et al., 2008). In the specific case of *L. nobilis*, such antioxidant properties may also be assumed by several terpenes and sterols (Conforti et al., 2006).

Inorganic solutes may exhibit a high proportion in total solutes in some plant species (Bajji et al., 2001). Nevertheless, for both populations of *L. nobilis*, contribution of inorganic ions to osmotic potential never exceeded 0.31 MPa. Water stress had no impact on this contribution of inorganic ions to Ψs values, mainly because it did not modify the mineral nutrition (Table 3). In fact, only endogenous NH_4^+ concentration increased in response to water stress but its contribution to osmotic adjustment remained negligible considering its very low concentrations in absolute terms and it should thus reflect a stress-induced metabolic disorder in relation to N assimilation rather than an attempt to contribute to osmotic adjustment.

4.3. Studied populations differ for cell wall properties

Cell wall properties also widely differed between the two studied populations since both AWC and ε (Table 2) were differently affected by drought. Water redistribution between symplasm and apoplasm was reported to be involved in water stress resistance in different species (Joly and Zaerr, 1987; White et al., 2001; Wardlaw, 2005) and may contribute to lower the symplasm osmotic potential without affecting the leaf water content. In *L. nobilis*, we showed that AWC was a direct function of mean stress intensity and was not influenced by modalities of stress application; indeed, under both permanent and cyclic stress, Algeria increased AWC in response to mild-stress intensities (S1 and S11) while Tunisia increased it in response to high stress intensity (S2 and S22). Those modifications were directly paralleled by the pectin concentration, thus confirming that this cell wall matrix play a key role in apoplasm water content. An increased arabinose content of the pectic fraction in response to water stress (Fig. 5) could be directly related to cell wall water holding capacities, as previously suggested by Femenia et al. (2000). An increased apoplastic water content could lead to an overestimation of osmotic potential quantified after freeze/thawing procedure since the obtained tissue sap is in fact a mixture of symplasmic and apoplastic component while it is commonly considered that solute level of apoplastic fluid is very low (Wardlaw, 2005). In the present study, however, we found no significant correlation between AWC on the one hand and differences between Ψs and $\Psi^{100}{}_{\Pi}$. This suggests that apoplastic water in *L. nobilis* could be tightly bound to water components, such a strong binding contributing to water stress resistance as demonstrated by Vertucci and Leopold (1987) and Rascio et al. (1992, 1998). The observed negative correlation between RWC_0 and $\Psi^0{}_{\Pi}$ also supports this hypothesis.

Although the cell wall water content may have a direct influence on cell wall rheological properties in some species (Evered et al., 2007), such a trend was not observed in *L. nobilis*. It has been reported in other plant pecies that the bulk modulus of elasticity could be affected by water stress independently of AWC (Chimenti and Hall, 1994; Torrecillas et al., 1999). In contrast to AWC, ε in the two considered populations was a direct function of the kinetics of stress application rather than a function of mean stress intensities: cell wall elasticity decreased in Tunisia in response to permanent water stress while it only decreased in Algeria in response to cyclic stress (Table 2).

According to Rascio et al. (1992) and Neumann (1995), the relationship between cell wall elasticity and cell wall composition may be complex. As far as water-stressed *L. nobilis* is concerned, such a relationship may vary according to populations since an increase in ε (Table 2) occurred concomitantly with an increase in cellulose concentration in Tunisia and with an increase in hemicellulose concentration in Algeria (Table 4). Those recorded changes could be, at least partly, related to the impact of water stress on the β-glucanase activities (Fig. 6) since the registered inhibition of those enzymes may increase the molecular mass of cell wall polysaccharides through an inhibition of the metabolic turnover of β glucan. Cell wall proteins, which were not analysed in the study, could also directly influence rheological properties of cell walls. Several hydroxyproline-rich proteins could be overproduced in response to water stress (Zhu et al., 2007) and, beside its implication in osmotic adjustment, the stress-induced accumulation of proline in Algeria could also be related to drought-induced modification of cell wall proteome.

Physiological significance of drought-induced modification in ε (Table 2) remains unclear and several conflicting data are available

in this respect in the literature (Joly and Zaerr, 1987; Fan et al., 1994; Patakas and Noitsakis, 1997; White et al., 2001; Ngugi et al., 2003). Nevertheless, cell wall rigidification was frequently reported to contribute to turgor maintenance and thus water stress resistance (Neumann, 1995; Ngugi et al., 2003). According to Patakas and Noitsakis (1997), a decrease in the cell wall elasticity could result in a rapid change of osmotic potential in response to small water losses and may thus confer an efficient physiological mechanism to face short-term water stress conditions. Cell wall rigidification could also offer protection in response to transient stress, especially if osmotic adjustment has occurred during the drought period since it contributes to avoid cell wall disruption which may occur as a result of brutal rehydration (Chimenti and Hall, 1994; White et al., 2001). Conversely, according to Fan et al. (1994), the maintenance of cell wall elasticity in stressed tissues is an efficient strategy of resistance only in the absence of osmotic adjustment. In the present study, however, we found no clear relationship between osmotic adjustment and drought-induced decrease of cell wall elasticity in *L. nobilis*, thus suggesting that the two processes may occur concomitantly, at least in plants originating from Algeria.

4.4. Differences between populations differ according to the kinetics of stress application

It is noteworthy that, for several considered parameters (shoot growth, predawn leaf water potential, bulk modulus of elasticity,…), the kinetics of stress imposition had a more drastic influence than the mean stress intensity, suggesting that permanent stress on the one hand, and cyclic stress on the other hand, induced on the plant physiology two distinct types of constraint. Numerous events could be induced during rehydration which may influence the plant's ability to cope with a subsequent episode of water shortage (Gallé and Feller, 2007). The present data suggest that different kinetics of stress application may have contrasting effects on distinct populations of *L. nobilis* and are in line with the view of Turner et al. (2008) who recently demonstrated that annual rainfall does not directly determine the water use strategy in perennial plant species. Rainfall distribution undoubtedly also has a strong influence on the plant response and coastal populations may differ from inland populations in this respect. In the present case, however, sites from Tunisia and Algeria exhibited more or less the same distribution for annual precipitation with a maximal value in May and in November–December (Fig. 1) and differences in population behaviour should not be regarded as the consequence of different rainfall distribution in their sites of origin. Nevertheless, for numerous parameters (Ψ_w, A, WUE and concentration in some osmolytes), significant differences were reported between plants exposed to S1 and S2 treatment while no differences were detected for the same parameters between plants exposed to S11 and S22 treatments, suggesting that a progressive dehydration of the substrate between 60 and 20% of field capacity had a limited impact on the final behaviour of plants exposed to cyclic stress if soil is re-watered immediately to field capacity. Analysis of physiological parameters conditioning drought tolerance in *L. nobilis* should therefore not only consider the stress intensity at the time of measurements but also the kinetics of stress imposition.

4.5. Concluding remarks

The present work demonstrates that the physiological strategies adopted by *L. nobilis* in order to cope with water stress vary in relation to the considered population and that the differences between populations, strongly differ depending on the kinetics of stress application. Both osmotic and elastic adjustments may occur concomitantly in response to water stress. Distinct populations differ in terms of osmotic adjustment from both a qualitative (nature of accumulated organic solute) and quantitative point of view. Water stress induced an increase in apoplastic water content in relation to a stress-induced modification of pectin concentration and composition, as well as a decrease in cell wall elasticity in relation to stress-induced inhibition of β-glucanase activities.

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