

Subcellular Effects of Drought Stress in *Rosmarinus officinalis*

E. Olmos¹, M. J. Sánchez-Blanco², T. Ferrández², and J. J. Alarcón²

¹ Departamento de Nutrición, CEBAS-CSIC, P.O. Box 164, 30100 Espinardo-Murcia, Spain

² Departamento de Riegos, CEBAS-CSIC, P.O. Box 164, 30100 Espinardo-Murcia, Spain

Received: March 16, 2006; Accepted: July 11, 2006

Abstract: The use of *Rosmarinus officinalis*, and other wild plant species, in the Mediterranean area is an interesting solution in order to avoid the desertification and rapid soil erosion, because of their good resistance to environmental conditions. Previous articles have described experiments designed to determine the impact of water stress at the plant level in this species, but more knowledge is required at the subcellular and ultrastructural levels. An anatomic and ultrastructural study of the leaves was conducted on *Rosmarinus officinalis* plants growing under different water treatments. In the leaves of water-stressed plants, the leaf water potential and turgor decreased, and leaf osmotic potential became more negative with respect to control plants. The anatomic investigations showed that both the mesophyll intercellular spaces and the epidermal cell size were reduced significantly under the more intense drought stress conditions. At the subcellular level, chloroplasts accumulated plastoglobuli and lipid bodies, and cuticle thickness was increased under water stress. In our experiment, the anatomic and ultrastructural modifications of *Rosmarinus officinalis* could be considered an additional adaptation to drought stress together with physiological and biochemical modifications as antioxidant accumulation.

Key words: *Rosmarinus officinalis*, chloroplasts, leaf anatomy, water stress, water relations, ultrastructure, plastoglobuli.

Introduction

Desertification is a serious problem in the Mediterranean area and generates a progressive reduction in vegetation cover, coupled with rapid soil erosion (Naveh, 1987). To combat this, the use of wild Mediterranean plant species such as *Rosmarinus officinalis* may be an interesting solution because of their resistance to environmental conditions (Savé et al., 1993; Sánchez-Blanco et al., 1998; Franco et al., 2000).

Under severe drought stress, electron transport to O₂ and increased quenching of excitation energy in the PSII antennae may be unable to dissipate the excess excitation energy in the

PSII antennae, and photodamage to PSII will result. However, the accumulation of antioxidant compounds and/or the increased activity of antioxidant enzymes can prevent the subcellular damage induced by free radical formation in this process (Foyer et al., 1994; Munné-Bosch et al., 2001). In previous papers, some authors have demonstrated that *Rosmarinus officinalis* accumulates the lipid-soluble antioxidant α -tocopherol by about 15-fold, and carotenoids by 25% under water stress without photochemical damage to PS II (Munné-Bosch et al., 1999).

Drought stress is characterised by specific changes in plant anatomy and cell ultrastructure, including chromatin condensation, swelling of chloroplasts, a decreased amount of starch grains, accumulation of plastoglobuli in the stroma, and distortion of thylakoids (Ristic and Cass, 1991; Pääkkönen et al., 1998; Eymery and Rey, 1999; Munné-Bosch et al., 2001). Many of the subcellular modifications observed under drought stress are similar to those observed in developmental leaf senescence (Inada et al., 1998) and have been related to leaf senescence induced by drought stress (Munné-Bosch and Alegre, 2004).

The aim of this work was to investigate the water relations in *Rosmarinus officinalis* and anatomic and ultrastructural modifications which could be correlated with the capacity of this species to survive under drought stress.

Materials and Methods

Plants of *Rosmarinus officinalis*, obtained from cuttings in October 1999, were planted in 12 cm diameter pots with peat as substrate and fert-irrigated with nutrient solution (NO₃⁻ 9.12 mM; H₂PO₄⁻ 0.96 mM; SO₄²⁻ 16.04 mM; K⁺ 5.44 mM; Ca²⁺ 17.0 mM; Mg²⁺ 6.74 mM; Fe²⁺ 35.2 mM; Mn²⁺ 19.8 mM; Zn²⁺ 3.64 mM; Cu²⁺ 15.74 mM) daily. The plants were transferred to a greenhouse 3 months before the experiments took place. The experiments were conducted in a polyethylene-covered greenhouse equipped with a cooling system, and a drip irrigation system with one emitter per plant provided 2 l h⁻¹. The environmental conditions during the experimental period were 33/18°C maximum/minimum average temperatures and the relative humidity ranged between 30 and 90%. The average maximum photosynthetically active radiation (PAR) was 1250 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

From 1 May to 30 October 2000, groups of 51 plants were submitted to one of three different treatments: 400 ml irrigation water per pot supplied daily (control); or on 4 days per week (WD1 treatment); or on 2 days per week (WD2 treatment).

Volumetric water content of the soil was measured every few days at midday using time-domain reflectometry (TDR) equipment (Tektroix 1502C cable tester, Redmon, Ore.). A 10-cm TDR probe was inserted into the wet root zone of 5 pots per treatment.

The volume of water applied to control plants (400 ml a day) was enough to recover the daily loss of water, and thus in well-watered plants the soil water content was maintained around 60% during the experiments. In the other treatments, the amount of water in the soil decreased (30% and 20% for WD1 and WD2, respectively).

On three occasions (15 May, 20 July and 25 October), 4 plants per treatment were harvested and plant biomass was measured. Plant growth was expressed as dry weight (DW).

Leaf water potential (Ψ_l), leaf osmotic potential (Ψ_s), leaf turgor potential (Ψ_p), and leaf osmotic potential at full turgor (Ψ_{os}) were measured at midday on 5 plants per treatment, at three different times (May, July and October). The leaf water potential was estimated according to the method described by Scholander et al. (1965), using a pressure chamber (Soil Moisture Equipment Co., Santa Barbara, CA, USA). For these measurements, leaves were enclosed in a plastic bag and sealed in the chamber within 20 s of collection and pressurised at a rate of 0.02 MPa s⁻¹ (Turner, 1988). Leaves from the Ψ_l measurements were frozen in liquid nitrogen. After thawing, the osmotic potential (Ψ_s) was measured in the extracted sap using a Wescor 5500 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci et al. (1991). Estimates of leaf turgor potential were based on the difference between Ψ_l and Ψ_s . Leaf osmotic potential at full turgor (Ψ_{os}) was estimated as indicated above for Ψ_s , using leaves excised, placed in plastic bags with their petioles in distilled water and allowed to reach full turgor overnight.

Transmission electron microscopy

Leaf samples, 10 per treatment, were collected at the end of the experiments and from the same position and age (fully expanded leaves from the apical shoots). Leaf samples were fixed for 2.5 h at 4°C in a 0.1 M Na phosphate-buffered (pH 7.2) mixture of 2.5% glutaraldehyde and 4% paraformaldehyde (Morales et al. 2001). Tissue was post-fixed with 1% osmium tetroxide for 2 h. The samples were then dehydrated in a graded alcohol series and embedded in Spurr's (Spurr, 1969) resin. Blocks were sectioned on a Leica ultracut microsystem (Leica Mikrosysteme, Hernalser Hauptstraße, Vienna, Austria). Ultra-thin sections (60–70 nm) for electron microscopy were placed on copper grids and stained with uranyl acetate followed by lead citrate (Reynolds, 1963). The ultrastructure of the tissue was observed with Zeiss EM10 and Zeiss EM109 electron microscopes (Oberkochen, Germany).

Semi-thin sections (0.5 μ m) from the same blocks were stained with 0.5% toluidine blue in borate buffer and examined with a Leica DMR light microscope (Leica, Wetzlar, Germany) as de-

Table 1 Leaf water potential, leaf osmotic potential, leaf turgor potential and total dry weight of *Rosmarinus officinalis* during the experimental period, for the control and two water stress treatments, WD1 and WD2

	May	July	October
Total dry weight			
Control	4.17 ± 0.88	25.64 ± 4.13 a	147.4 ± 9.4 a
WD1	–	12.64 ± 1.80 b	49.36 ± 6.78 b
WD2	–	6.80 ± 1.30 c	19.62 ± 1.69 c
Leaf water potential			
Control	–0.95 ± 0.01	–0.93 ± 0.05 a	–0.93 ± 0.06 a
WD1	–	–1.18 ± 0.08 b	–1.15 ± 0.08 b
WD2	–	–1.51 ± 0.04 c	–1.50 ± 0.06 c
Leaf osmotic potential			
Control	–1.23 ± 0.10	–1.17 ± 0.03 a	–1.16 ± 0.04 a
WD1	–	–1.23 ± 0.01 b	–1.28 ± 0.01 b
WD2	–	–1.53 ± 0.08 c	–1.53 ± 0.08 c
Leaf turgor potential			
Control	0.28 ± 0.01	0.24 ± 0.04 a	0.23 ± 0.05 a
WD1	–	0.05 ± 0.02 b	0.13 ± 0.03 b
WD2	–	0.02 ± 0.01 c	0.03 ± 0.02 c
Leaf osmotic potential at full saturation			
Control	–0.74 ± 0.05	–0.91 ± 0.04 a	–0.90 ± 0.08 a
WD1	–	–0.92 ± 0.04 a	–0.93 ± 0.04 a
WD2	–	–0.91 ± 0.03 a	–1.00 ± 0.06 b

Mean values ± SD (n = 5). The means were compared by analysis of variance and using the Duncan multiple range test at $p < 0.05$. Significant differences between treatments are indicated by different letters.

scribed by Olmos and Hellin (1998). For morphometric analysis, a minimum of 10 different leaves per treatment were studied. Parameters were measured using a Leica QM500 imaging analysis system (Q500MC; Leica, Wetzlar, Germany), as described previously by Olmos and Hellin (1998).

Statistical analysis

The means were compared by analysis of variance and using the Duncan multiple range test at $p < 0.05$.

Results

Plants submitted to drought stress showed a reduction in growth during the experiment, this being more discernible in plants receiving the lowest irrigation doses (Table 1). Drought stress treatments promoted significant differences in stressed plants versus control plants in relation to midday leaf water potential (Ψ_l). Leaf osmotic potential (Ψ_s) became more negative and leaf turgor potential (Ψ_p) decreased under water deficit, particularly in WD2 plants (Table 1). However, no important changes were observed in leaf osmotic potential at full turgor (Ψ_{os}).

The leaf anatomy of control *Rosmarinus officinalis* plants is shown in Fig. 1A. The anatomic modifications were more evident in WD2 than in WD1 plants (Figs. 1B–G). We observed

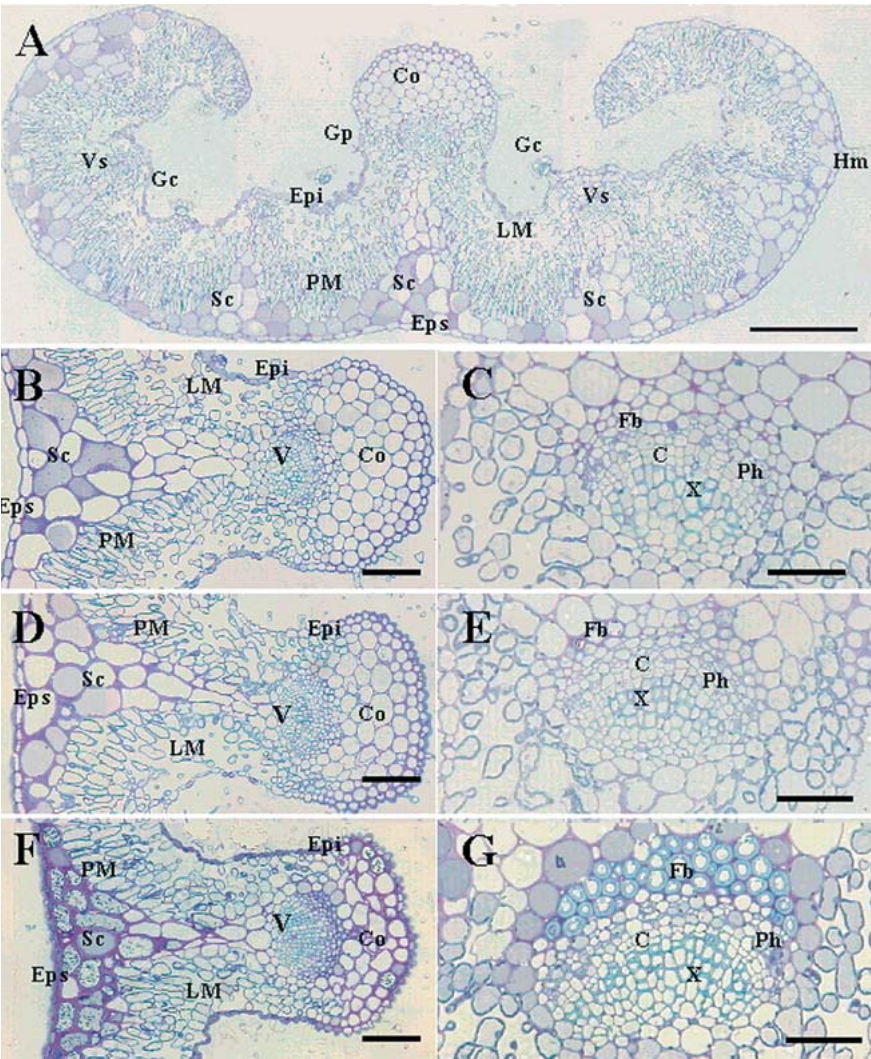


Fig. 1 (A) Transverse section of control leaf from *Rosmarinus officinalis*. Transverse sections from *Rosmarinus officinalis* control (B), WD1 treatment (D), and WD2 treatment (F), showing magnification of the main vein. Transverse sections from *Rosmarinus officinalis* control (C), WD1 treatment (E), and WD2 treatment (G), showing details of the main vascular bundles. C = Cambium; Epi = abaxial epidermis; Eps = adaxial epidermis; Fb = phloem fibres; Gc = capitate gland; Gp = peltate gland; Co = collenchyma; LM = lacunar mesophyll; Ph = phloem; PM = parenchyma mesophyll; Sc = sclerenchyma; V = main vascular bundle; Vs = secondary vascular bundle; X = xylem. Bars: A = 250 μ m, B, D, F = 100 μ m, C, E, G = 50 μ m.

Table 2 Quantitative analysis of morphometric data from control, WD1 and WD2 treated plants of *Rosmarinus officinalis*

	Lagunar paren. inter space (%) (n = 10)	Lipid bodies area (μ m ²) (n = 40)	Cuticle (μ m) (n = 15)	Epidermal cell (ab) area (μ m ²) (n = 40)	Phloem fibre cell wall (μ m) (n = 15)
Control	36.9 \pm 1.9 a	2.5 \pm 0.5 c	2.2 \pm 0.4 b	230.8 \pm 26.2 a	0.9 \pm 0.1 c
WD1	33.5 \pm 3.2 a	21.1 \pm 1.4 b	5.7 \pm 0.3 a	221.3 \pm 35.5 a	1.7 \pm 0.3 b
WD2	24.5 \pm 2.2 b	26.4 \pm 1.6 a	5.9 \pm 0.3 a	108.7 \pm 7.5 b	3.1 \pm 0.4 a

Mean values \pm SD. The means were compared by analysis of variance and using the Duncan multiple range test at $p < 0.05$. Significant differences between treatments are indicated by different letters.

an incremental lignification and thickening in the collenchyma and sclerenchyma tissues (Fig. 1F, Table 2, phloem fibre cell wall). Moreover, this thickening was greater in the vascular fibres from the main vein (Figs. 1C,E,G, Table 2). We also recorded a significant reduction in the size of epidermal cells from the adaxial and abaxial sides of the leaf (Figs. 1B,D,F, Table 2). Similarly, we observed a significant reduction in the intercellular spaces in the spongy mesophyll only in treatment WD2.

Peltate glandular hairs are composed of one secretory head and eight cells distributed in a circle around a central tube connected with the secretory head; the gland is joined to the epidermis by one basal cell and is covered by a thick cuticle (Figs. 2A,B). These glands were highly affected in both the WD1 and WD2 treatments. We noticed that the secretory cells collapsed in both WD1 and WD2 plants, the basal cell was flattened, and, in many cases, the cuticle was disrupted (Figs. 2C,D). Capitate glandular hairs are composed of only 1 secretory cell covered by a thick cuticle and two basal cells

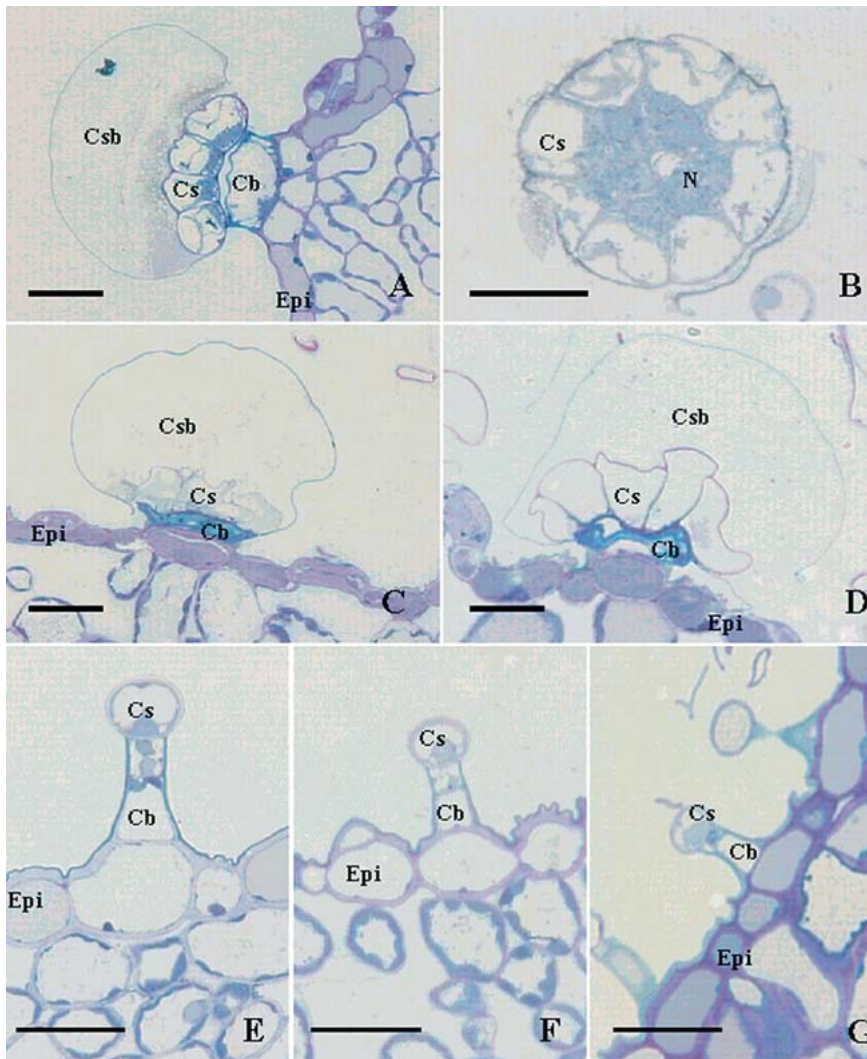


Fig. 2 Optical sections of capitate and peltate glands from *Rosmarinus officinalis* control, WD1 and WD2 treatments. (A) Longitudinal section of a peltate gland from a control plant. (B) Transverse sections of the head of a peltate gland from a control plant. Longitudinal sections of a peltate gland from WD1 (C) and WD2 (D) treated plants. Longitudinal sections of a capitate gland from control (E), WD1 (F), and WD2 (G) treated plants. Cb = basal cell; Cs = secretory cell; Csb = secretory head; Epi = abaxial epidermis; N = nucleus. Bars = 25 µm.

(Fig. 2E). In these glands, the effects of treatments WD1 and WD2 on the structure of the gland were not evident.

Ultrastructure

The palisade mesophyll cell ultrastructure shows a central vacuole surrounded by a thin layer of cytoplasm rich in chloroplasts, mitochondria and peroxisomes (Fig. 3A). Treatment WD1 slightly affected this general ultrastructure (Fig. 3B), but the WD2 treatment significantly modified the ultrastructure of these cells (Fig. 3C): the vacuole filled with dense material and we observed frequently the presence of dense bodies. The cytoplasm of the palisade mesophyll cells frequently contained a small lipid body (Fig. 4A, Table 2). However, we observed a large lipid body in the cytoplasm of many cells from WD1 and WD2 plants (Table 2), normally protruding into the vacuole but always surrounded by the tonoplast (Figs. 4B,C). This lipid body had a highly developed membrane system on its cytoplasmic side (Fig. 4C); this membrane system could be smooth endoplasmic reticulum. Interestingly, this lipid body was also seen in the basal cells of capitate gland hairs from WD2 plants (Figs. 3H,I).

The ultrastructure of chloroplasts was slightly affected in both WD1 and WD2 plants; the thylakoid ultrastructure was unaltered and we only recorded a significant increase in the number and size of the plastoglobuli in WD2 plants (Figs. 3D–F). The sclerenchyma cells had a very low number of plastids, normally one or two per cell section, with typical ultrastructure, having a small, dense body, while the dense body in WD2 plants was larger, occupying a high percentage of the stroma (Fig. 3G).

The cuticle thickness and ultrastructure were highly affected in both drought treatments. WD1 and WD2 plants had thicker cuticles (Table 2) and two different zones in control and drought treatments were noted, but in WD1 and WD2 plants, these zones showed different electron density and thickness compared with the control (Figs. 4D–F). An interesting result was that the abaxial epidermal cells had a well-developed cuticle in the internal epidermal cell wall in contact with the substomatal cavity in both drought treatments (Figs. 4G–I). Moreover, the cell area of these abaxial epidermal cells was reduced greatly in WD2, but no differences were observed between the control and WD1 plants.

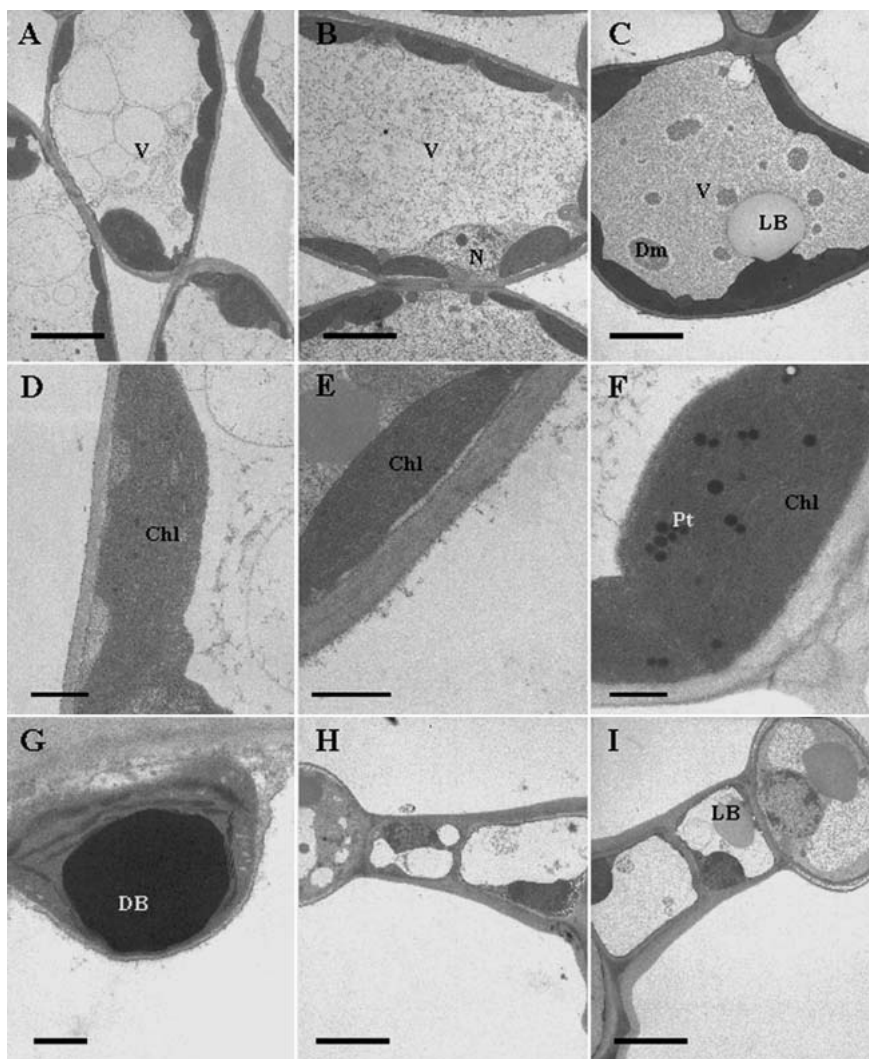


Fig. 3 Electron micrographs from mesophyll cells of control (A), WD1 (B), and WD2 (C) treated plants. Ultrastructure of chloroplasts from mesophyll cells of *Rosmarinus officinalis*, (D) control plants, (E) WD1 plants, and (F) WD2 plants. (G) Detail of a plastid from a sclerenchyma cell of a WD2 plant. Capitulate glands of control (H) and WD2 (I) treated plants. Chl = chloroplast; DB = dense body; Dm = dense material; LB = lipid body; N = nucleus; Pt = plastoglobuli; V = vacuole. Bars: A, B, C, H, I = 5 µm, D, E, F, G = 1 µm.

Discussion

Plants submitted to drought stress had lower biomass at the end of the experiment than control plants, as found in many species submitted to drought stress (Chaves and Pereira, 1992; De Herralde et al., 1998; Sánchez-Blanco et al., 2004a, b). During the experimental period, there were important reductions in the leaf water potential of water stressed plants with respect to control plants. The leaf osmotic potential of WD1 and WD2 plants was more negative as a consequence of tissue dehydration leading to a reduction in turgor, which is often considered to be responsible for growth. Several authors have reported the capacity for osmotic adjustment of *Rosmarinus officinalis* plants under drought stress conditions (Sánchez-Blanco et al., 2004a). This capacity was enhanced when rosemary plants were inoculated with *Glomus deserticola* (Sánchez-Blanco et al., 2004b). However, in this experiment no leaf osmotic adjustment ability was observed, perhaps because the water stress intensity sustained by the plants was lower than in the previous experiments. A relationship between drought and oxidative stress has been shown in several Mediterranean plants: species that show resistance to drought,

such as *R. officinalis* or *Cistus clusii*, show lower oxidative stress than species sensitive to drought stress (Munné-Bosch et al., 2001). Such model systems have allowed the establishment of a positive relationship between antioxidant production and the degree of water stress tolerance and, as rosemary plants show a high production of antioxidant compounds (Munné-Bosch et al., 1999), it can be considered that this is part of a natural tolerance to water deficit. Nevertheless, tolerance to drought stress is linked not only to a higher antioxidant capacity but also to other mechanisms (i.e. structural changes, stomatal control, light absorption) that permit plants to tolerate drought (Munné-Bosch et al., 2001).

Anatomic studies of *Rosmarinus officinalis* demonstrated that long-term drought stress modified the structure of the leaves, and that the plants adapted to such conditions in different ways. (I) Leaves of stressed plants had an abaxial epidermis that was wrinkled compared with control plants. This strategy allows stressed plants to reduce transpiration and thus water loss. (II) Similarly, we observed that the cuticle was modified in size and structure, reducing epidermal conductance and, therefore, non-stomatal transpiration. (III) The re-

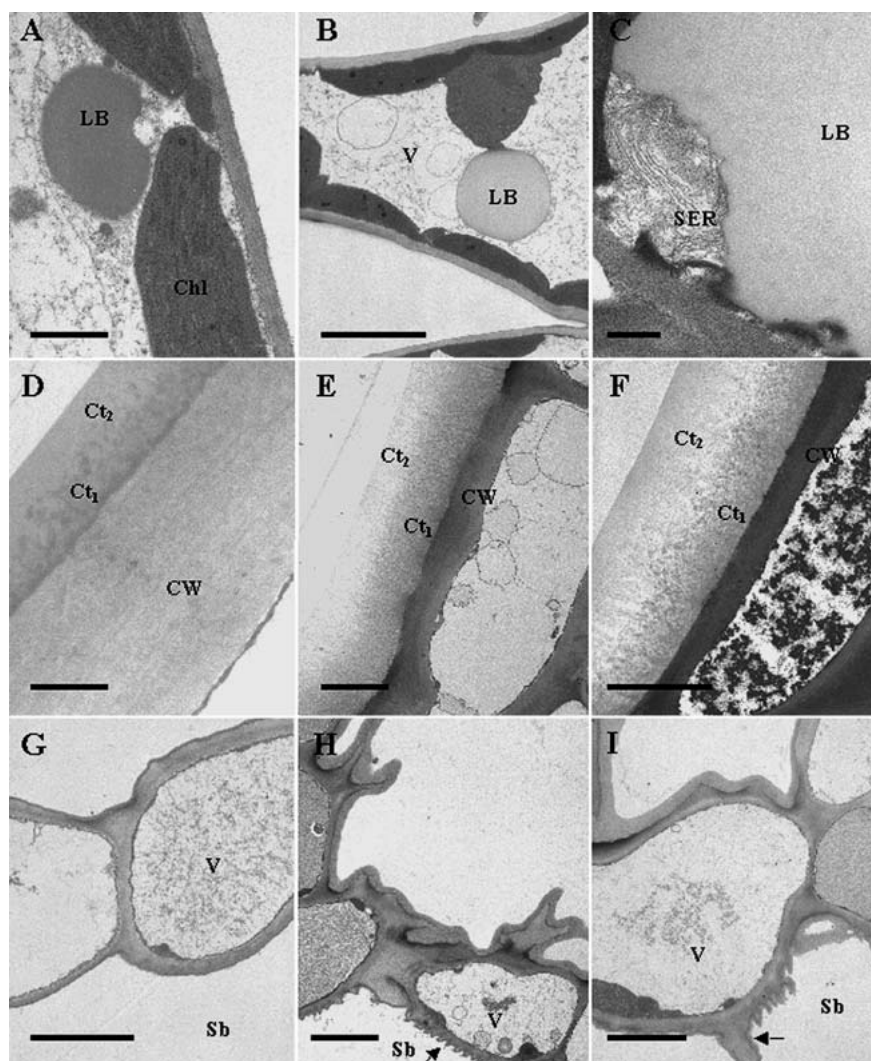


Fig. 4 Details of electron micrographs from mesophyll cells showing the presence of lipid bodies in control (A), WD1 (B), and WD2 (C) treated plants. Ultrastructure of cuticle from epidermis of control (D), WD1 (E), and WD2 (F) treated plants. Detail of cuticle of the internal cell wall of abaxial epidermal cells in control (G), WD1 (H, arrow), and WD2 (I, arrow) treated plants. Chl = chloroplast; C_{t1} = internal zone of cuticle; C_{t2} = external zone of cuticle; CW = cell wall; LB = lipid body; Sb = substomatal cavity; SER = smooth endoplasmic reticulum; V = vacuole. Bars: A, D = 1 μ m, C = 0.5 μ m, B, E, F, G, H, I = 5 μ m.

duction of cellular size of the adaxial epidermal cells and increased cell wall size and increased lignification of the collenchyma and sclerenchyma could help to reduce cellular collapse induced by long-term drought stress (Oertli et al., 1990). Xylem and phloem structures were unaffected but phloem fibres were highly lignified, increasing resistance of the vascular structure.

In drought-stressed plants we also observed a significant reduction in intercellular air spaces in the spongy mesophyll. This may affect CO_2 diffusion from the substomatal cavities to carboxylation sites. It is known that CO_2 assimilation is regulated by the photosynthetic capacity of the mesophyll and the conductance of CO_2 from the ambient air to the carboxylation sites in the chloroplasts (Syvertsen et al., 1995). The physiological data obtained by Munné-Bosch et al. (1999) demonstrate that, for *Rosmarinus officinalis* under conditions of severe drought (about -3 MPa), CO_2 assimilation was reduced by about 80%.

Munné-Bosch and Alegre (2000) observed that *Rosmarinus officinalis* avoids subcellular damage to the photosynthetic apparatus during long periods of drought stress. They proposed that this capacity was mediated by activation of the xanthophyll cycle and accumulation of tocopherol, avoiding damage induced by the production of free radicals in drought-stressed plants (Munné-Bosch and Alegre, 2000). However, chlorophyll content was highly reduced in rosemary plants (65–85%) under drought conditions (Sánchez-Blanco et al., 2004a). This decrease may be caused by a degradation of chlorophyll by photo-oxidation, and this may be a protective adaptive mechanism in stressed plants (Kyparissis et al., 1995). In our experiments, we observed that the ultrastructure of thylakoids and stroma was unaffected by either of the two stress conditions. However, in drought-stressed plants of *Rosmarinus officinalis*, a higher number of large plastoglobuli was observed in chloroplasts compared to well-watered plants. The number and size of plastoglobuli increase substantially in leaf chloroplasts following exposure to stresses such as high levels of ozone (Sakaki et al., 1990; Oksanen et al., 2001), fungal infection (Losel, 1978), viral infection (Hernández et al., 2004), chilling (Nordby and Yelenosky, 1984), freezing and thawing (Nordby and Ye-

lenosky, 1985), salinity (Hernández et al., 1995; Morales et al., 2001), and drought (Pääkkönen et al., 1998; Eymery and Rey, 1999; Mäkelä et al., 2000; Munné-Bosch et al., 2001). It is assumed that plastoglobuli function in the storage of thylakoid components such as lipids, plastohydroquinone, and tocopherol (Steinmüller and Tevini, 1985). It is possible that, under drought stress, plastoglobuli can be used as a storage site for thylakoid compounds degraded during stress (Eymery and Rey, 1999). There is also evidence for accumulation of triacylglycerols in the leaves of some species following ozone or drought stress that is coincident with increases in plastoglobuli size and abundance (Sakaki et al., 1990; Pääkkönen et al., 1998). However, they may also function in the synthesis and recycling of lipophilic products arising from oxidative metabolism during stress. Recent proteomic analyses have revealed that *Arabidopsis* leaf plastoglobuli contain more than 30 proteins that are probably involved in the metabolism of molecules derived from the isoprenoid and lipid pathways, as well as in carotenoid cleavage (Ytterberg et al., 2006).

In the cytoplasm of stressed plants we observed a lipid body that increased in size with the duration of stress. Lipid body enlargement occurred at a single cytoplasmic site within mesophyll cells. At the base of this lipid body a well-developed tubular membrane system is frequently observed that could be smooth endoplasmic reticulum. Accumulation of lipids is a common sign of stress (Holopainen et al., 1992). These lipid bodies also accumulate in *Picea abies* under drought and ozone stress (Kivimäenpää et al., 2001). The biochemical composition of this structure is unknown. However, analysis of similar structures in the seeds and mesocarp of fruits indicates that such lipid bodies are rich in triacylglycerols (Rangel et al., 1997). Therefore, it can be speculated that lipid bodies can be used as a reservoir for energy under severe drought stress, and can be metabolised by β -oxidation in the peroxisomes.

Peltate glandular hairs were highly affected by both stress treatments. These glands accumulate mixed secretions, with a major hydrophilic component and a minor lipophilic component containing essential oils (Bottega and Corsi, 2000). Peltate glandular hairs are probably involved in mechanical and chemical defence against herbivores and pathogens. However, the structure of capitate hairs (Types I and II) was slightly affected by drought stress. It is difficult to classify the capitate hairs into distinct types, since some differences are more quantitative than qualitative (Werker et al., 1985). Type I capitate hairs are similar to peltate hairs in relation to mode and type of secretion and probably supplement peltate hairs in mechanical defence. Type II capitate hairs are typical lipophilic glandular hairs. The fact that they contain large quantities of flavonoids combined with alkaloids suggests that they function in chemical defence (Bottega and Corsi, 2000).

In conclusion, the results presented here stress the importance of anatomic and ultrastructural studies in elucidating possible major mechanisms of resistance to drought in many wild species adapted to Mediterranean drought conditions. In this article, *Rosemary* has shown important anatomic and ultrastructural adaptations for resistance to drought. Such adaptations make this plant a useful subject to cultivate for drought amelioration in the Mediterranean area.

References

- Bottega, S. and Corsi, G. (2000) Structure, secretion and possible functions of calyx glandular hairs of *Rosmarinus officinalis* L. (Labiatae). Botanical Journal of the Linnean Society 132, 325–335.
- Chaves, M. M. and Pereira, J. S. (1992) Water stress, CO₂ and climate change. Journal of Experimental Botany 43, 1131–1139.
- De Herralde, F., Biel, C., Savé, R., Morales, M. A., Torrecillas, A., Alarcón, J. J., and Sánchez-Blanco, M. J. (1998) Effect of water and salt stresses on the growth, gas exchange and water relations in *Argyranthemum coronopifolium* plants. Plant Science 139, 9–17.
- Eymery, F. and Rey, P. (1999) Immunocytolocalization of CDSP 32 and CDSP 34, two chloroplastic drought-induced stress proteins in *Solanum tuberosum* plants. Plant Physiology and Biochemistry 37, 305–312.
- Foyer, C. H., Lelandais, M., and Kunert, K. J. (1994) Photooxidative stress in plants. Physiologia Plantarum 92, 696–717.
- Franco, J. A., Bañon, S., Fernandez, J. A., and Leskovar, D. I. (2000) Effect of nursery regimes and establishment on root development of *Lotus creticus* seedlings following transplanting. Journal of Horticultural Science and Biotechnology 76, 174–179.
- Gucci, R., Xiloyannis, C., and Flore, J. A. (1991) Gas exchange parameters, water relations and carbohydrate partitioning in leaves of field-grown *Prunus domestica* following fruit removal. Physiologia Plantarum 83, 497–505.
- Hernández, J. A., Olmos, E., Corpas, F. J., Sevilla, F., and del Rio, L. A. (1995) Salt-induced oxidative stress in chloroplasts of pea plants. Plant Science 105, 151–167.
- Hernández, J. A., Rubio, M., Olmos, E., Ros-Barceló, A., and Martínez-Gómez, P. (2004) Oxidative stress induced by long-term plum pox virus infection in peach (*Prunus persica*). Physiologia Plantarum 122, 486–495.
- Holopainen, T., Anttonen, S., Wulff, A., Palomäki, V., and Kärenlampi, L. (1992) Comparative evaluation of the effects of gaseous pollutants, acidic deposition, and mineral deficiencies: structural changes in the cells of forest plants. Agriculture, Ecosystems and Environment 42, 365–398.
- Inada, N., Sakai, A., Kuroiwa, H., and Kuroiwa, T. (1998) Three-dimensional analysis of the senescence program in rice (*Oryza sativa* L.) coleoptiles – investigations by fluorescence microscopy and electron microscopy. Planta 206, 585–597.
- Kivimäenpää, M., Sutinen, S., Medin, E. L., Karlsson, P. E., and Sellén, G. (2001) Diurnal changes in microscopic structures of mesophyll cells of Norway spruce, *Picea abies* (L.) Karst., and the effects of ozone and drought. Annals of Botany 88, 119–130.
- Kyparissis, A., Petropoulou, Y., and Manetas, Y. (1995) Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., Labiatae) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. Journal of Experimental Botany 46, 1825–1831.
- Losel, D. M. (1978) Lipid-metabolism of leaves of *Poa pratensis* during infection by *Puccinia poarum*. New Phytologist 80, 167–174.
- Mäkelä, P., Kärkkäinen, J., and Somersalo, S. (2000) Effects of glycinebetaine on chloroplast ultrastructure, chlorophyll and protein content, RuBPCO activities in tomato grown under drought or salinity. Biologia Plantarum 43, 471–475.
- Morales, M. A., Olmos, E., Torrecillas, A., Sánchez-Blanco, M. J., and Alarcón, J. J. (2001) Differences in water relations, leaf ion accumulation and excretion rates between cultivated and wild species of *Limonium* sp. grown in conditions of saline stress. Flora 196, 345–352.
- Munné-Bosch, S., and Alegre, L. (2000) Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. Planta 210, 925–931.

- Munné-Bosch, S. and Alegre, L. (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. *Functional Plant Biology* 31, 203–216.
- Munné-Bosch, S., Jubany-Mari, T., and Alegre, L. (2001) Drought-induced senescence is characterized by a loss of antioxidant defences in chloroplasts. *Plant, Cell and Environment* 24, 1319–1327.
- Munné-Bosch, S., Schwarz, K., and Alegre, L. (1999) Enhanced formation of α -tocopherol and highly oxidized abietane diterpenes in water-stressed rosemary plants. *Plant Physiology* 121: 1047–1052.
- Naveh, Z. (1987) Landscape ecology, management and conservation of European and Levant Mediterranean uplands. In *Plant Response to Stress. Functional Analysis in Mediterranean Ecosystems*, Vol. 15 (Tenhunen, J. D., ed.), New York, Berlin, Heidelberg: NATO ASI Series Ecological Sciences, Springer Verlag, pp. 641–647.
- Nordby, H. E. and Yelenosky, G. (1984) Effects of cold hardening on acyl lipids of citrus tissue. *Phytochemistry* 23, 41–45.
- Nordby, H. E. and Yelenosky, G. (1985) Change in citrus leaf lipids during freeze-thaw stress. *Phytochemistry* 24, 1675–1679.
- Oertli, J. J., Lips, S. H., and Agami, M. (1990) The strength of sclerophyllous cells to resist collapse due to negative turgor pressure. *Acta Oecologica* 11, 281–289.
- Oksanen, E., Sober, J., and Karnosky, D. F. (2001) Impacts of elevated CO₂ and/or O₃ on leaf ultrastructure of aspen (*Populus tremuloides*) and birch (*Betula papyrifera*) in the Aspen FACE experiment. *Environmental Pollution* 115, 437–446.
- Olmos, E. and Hellin E. (1998) Ultrastructural differences of hyperhydric and normal leaves from regenerated carnation plants. *Scientia Horticulturae* 75, 91–101.
- Pääkkönen, E., Vahala, J., Pohjola, M., Holopainen, T., and Kärenlampi, L. (1998) Physiological, stomatal and ultrastructural ozone responses in birch (*Betula pendula* Roth.) are modified by water stress. *Plant, Cell and Environment* 21, 671–684.
- Rangel, B., Platt, K. A., and Thomson, W. W. (1997) Ultrastructural aspects of the cytoplasmic origin and accumulation of oil in olive fruit (*Olea europaea*). *Physiologia Plantarum* 101: 109–114.
- Reynolds, E. S. (1963) The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology* 17, 208–212.
- Ristic, Z. and Cass, D. D. (1991) Chloroplast structure after water shortage and high temperature in two lines of *Zea mays* L. that differ in drought resistance. *Botanical Gazette* 152, 186–194.
- Sakaki, T., Saito, K., Kawaguchi, A., Kondo, N., and Yamada, M. (1990) Conversion of monogalactosyldiacylglycerols to triacylglycerols in ozone-fumigated spinach leaves. *Plant Physiology* 94, 766–772.
- Sánchez-Blanco, M. J., Ferrández, T., Navarro, A., Bañón, S., and Alarcón, J. J. (2004a) Effects of irrigation on water relations, growth and survival of *Rosmarinus officinalis* plants during and after transplanting. *Journal of Plant Physiology* 161, 1133–1142.
- Sánchez-Blanco, M. J., Ferrández, F., Morales, M. A., Morte, A., and Alarcón J. J. (2004b). Variations in water status, gas exchange, and growth in *Rosmarinus officinalis* plants infected with *Glomus deserticola* under drought conditions. *Journal of Plant Physiology* 161, 675–682.
- Sánchez-Blanco, M. J., Morales, M. A., Torrecillas, A., and Alarcón, J. J. (1998) Diurnal and seasonal osmotic potential changes in *Lotus creticus creticus* plants grown under saline stress. *Plant Science* 136, 1–10.
- Savé, R., Alegre, L., Pery, M., and Terradas, J. (1993) Ecophysiology after fire resprouts of *Arbutus unedo* L. *Orsis* 8, 107–119.
- Scholander, P. F., Hammel, H. T., Bradstreet, E. D., and Hemingsen, E. A. (1965) Sap pressure in vascular plants. *Science* 148, 339–346.
- Spurr, A. R. (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* 26, 31–43.
- Steinmüller, D. and Tevini, N. (1985) Composition and function of plastoglobuli. 1. Isolation and purification from chloroplasts and chromoplasts. *Planta* 163, 201–207.
- Syvertsen, J. P., Lloyd, J., McConchie, C., Kriedemann, P. E., and Farquhar, G. D. (1995) On the relationship between leaf anatomy and CO₂ diffusion through the mesophyll of hypostomatous leaves. *Plant, Cell and Environment* 18, 149–157.
- Turner, N. C. (1988) Measurement of plant water status by the pressure chamber technique. *Irrigation Science* 9, 289–308.
- Werker, E., Ravid, U., and Putievsky, E. (1985) Structure of glandular hairs and identification of the main components of their secreted material in some species of the Labiatae. *Israel Journal of Botany* 34, 31–45.
- Ytterberg, A. J., Peltier, J. B., and van Wijk, K. J. (2006). Protein profiling of plastoglobules in chloroplasts and chromoplasts; a surprising site for differential accumulation of metabolic enzymes. *Plant Physiology* 140, 998–1008.

E. Olmos

Departamento de Nutrición

CEBAS-CSIC

P.O. Box 164

30100 Espinardo-Murcia

Spain

E-mail: eolmos@cebas.csic.es

Editor: M. Riederer