DOI: 10.1007/s11099-013-0005-6 PHOTOSYNTHETICA 51 (1): 109-114, 2013

Leaf morphological and ultrastructural performance of eggplant (*Solanum melongena* L.) in response to water stress

Q.S. FU[*,**], R.C. YANG[*], H.S. WANG[**], B. ZHAO[*], C.L. ZHOU[*], S.X. REN[***,+] and Y.-D. GUO[*,+]

Beijing Key Laboratory of Growth and Developmental Regulation for Protected Vegetable Crops,
College of Agriculture & Biotechnology, China Agricultural University, Beijing 100193, China[*]
Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China[**]
School of Agriculture, Virginia State University, PO Box 9061, Petersburg VA23806, USA[***]

Abstract

The effects of water stress on leaf surface morphology (stomatal density, size, and trichome density of both adaxial and abaxial surfaces) and leaf ultrastructure (chloroplasts, mitochondria, and cell nuclei) of eggplant (*Solanum melongena* L.) were investigated in this study. Higher stomata and trichome densities were observed on abaxial surface compared with the adaxial surface. Compared with well watered (WW) plants, the stomata and trichome density of the abaxial surface increased by 20.39% and 26.23% under water-stress condition, respectively. The number of chloroplasts per cell profile was lesser, the chloroplasts became round in a shape with more damaged structure of membranes, the number of osmiophilic granules increased, and the number of starch grains decreased. The cristae in mitochondria were disintegrated. The cell nuclei were smaller and the agglomerated nucleoli were bigger than those of WW plants. Our results indicated that the morphological and anatomical responses enhanced the capability of plants to survive and grow during stress periods.

Additional key words: eggplant; stomata; trichome; ultrastructure; water stress.

Introduction

Eggplants (*Solanum melongena* L.) are very sensitive to water stress during their entire growing period. They require considerable amount of water for their growth and development. Plants respond to water stress differently depending on the species, duration, and severity of soil water depletion (Dias and Brüggemann 2007, Fang *et al.* 2011, Song *et al.* 2011).

Stomata play an important role in determining the water and carbon cycle between the plants and the atmosphere (Weng *et al.* 2011). The features of stomata, such as stomatal size, stomata density, and position, occupy a central position in the gas-exchange process (Jones 1989, Fu *et al.* 2010). Stomata control the flux of CO_2 and water vapor by varying their aperture and they restrict the diffusion of gases. Stomatal resistance depends mainly upon stomata size and number (Meidner and Mansfeld 1968). Various environmental factors (*e.g.* water availability, light intensity, photoperiod) can affect stomata opening-closing behavior (Al-Ani and Bierhuizen 1971, Pallardy and Kozlowski 1979, Bondada *et al.* 1994). In addition to stomatal characteristics, other epidermal features, such as a shape and a number of the epidermal cells, trichomes, and epicuticular waxes, also play a crucial role in gas exchange processes (Poulson and Vogelmann 1990, Martin *et al.* 1991).

Fewer studies have been undertaken on drought-induced stomata behavior and ultrastructural changes in cells and organelles (Yordanov *et al.* 2000, Dias and Brüggemann 2007). The ability of plants to acclimate to water availability involves alterations of morphological and anatomical characteristics at the leaf level. These changes may enhance the capability of plants to survive and to grow during stress periods. The aim of this study was to delineate the differences in the morphological (on epidermal surface) and anatomical (chloroplasts, mitochondria, cell nuclei) changes involved in drought tolerance, which enabled plants to adapt to water stress and to maintain growth, development, and productivity.

Received 30 April 2012, *accepted* 14 November 2012.

[+] Corresponding authors; Y.-D. Guo, tel: 86-10-62734845, fax: 86-10-62734389, e-mail: yaguo@cau.edu.cn; S.X. Ren, tel: 804-524-3094, fax: 804-524-5186, e-mail: sren@vsu.edu.cn

Abbreviations: FC – field capacity; WS – water-stressed; WW – well watered.

Acknowledgements: This work was partly supported by the grants (2009CB119000) and Chinese Universities Scientific Fund 2009-2-06. The first two authors contributed equally to this work.

Materials and methods

Plants and growth conditions: All experiments were conducted in the greenhouse at China Agricultural University, Beijing (Lat/Lon: 39.9°N 116.3°E). The study was carried out with eggplants (*S. melongena* L. cv. HQ-2) grown from seeds. Seeds were soaked overnight and allowed to germinate on wet tissue paper in darkness. They were then sown in plastic pots (20 cm diameter) with a mixture of field soil and vermiculite (2:1, v/v) in January 2008. The water-stress treatment was initiated, when the plants had 5–6 fully expanded leaves. The average temperature in the greenhouse was 25/18°C day/night, relative humidity was 65/85% day/night under a natural light (the maximal irradiance was around 1,000 μmol m^{-2} s^{-1}), light intensities were monitored using a *PAR Quantum Sensor SKP216 (Skye Instruments Ltd.*, UK). Forty plants were randomly assigned to two different watering regimes as follows: WW treatment [at field capacity (FC) of 100%] and water-stressed (WS) treatment (30% of FC, WS), following the methods of Desclaux and Roumet (1996). During the experiment, the pots were rewatered to different FC by replacing the amount of water transpired every day. The experiment lasted for 30 d. Each treatment was replicated 5 times (1 seedling per pot and 4 pots per replication).

Analysis of epidermal structure: Scanning electron microscope observation was done according to Chen *et al.* (2008). The leaf samples (2 mm × 2 mm) were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), then transferred to 1% OsO_4 fixative for 30‑40 min and washed several times with distilled water and then dehydrated firstly using 30% ethanol, followed by gradual series of increasing ethanol (30, 50, and 70%) and finally with 100% ethanol for 20‑30 min. Leaf samples were then transferred to propylene oxide for 20‑40 min and washed with isoamyl acetate for 40 min and then oven-dried at 40°C overnight and subsequently the samples were coated with a thin layer of gold for 5‑10 min prior to scanning electron microscope analysis (*S-3400N*, *Hitachi*, Japan).

Analysis of leaf ultrastructure: The mature leaf samples (2 mm × 2 mm) were preserved in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and then transferred to 1% OsO_4 to increase the fixation and washed several times with 0.1 M phosphate buffer and then dehydrated through series of acetone, and finally embedded in Spurr's epoxy resin (Spurr 1969). Thin sections (1–2 μm) were cut on an ultramicrotome (*EM UC6*, *Leica*, Germany) and then stained with 2.5% uranyl acetate, followed by lead citrate, and then examined by transmission electron microscopy (*JEOL-1230*, *Jeol*, Japan).

Statistical analysis: Data were subjected to analysis of variance (*ANOVA*) and mean values were compared by the *Duncan*'s multiple range test ($p<0.05$), when a significant difference was detected. The statistical analysis was performed using *SPSS 13.0 for Windows* (*SPSS*, Chicago, IL, USA).

Results

Stomatal characteristics: The higher stomata density was observed on the abaxial surface compared with the adaxial surface. The stomata on the abaxial surface were smaller (length × width) than those on the adaxial surface (Fig. 1, Table 1). The stomata density of the abaxial surface increased by 20.4% under the water stress compared with the well-water condition (Fig. 1, Table 1). For adaxial surface, there was no significant difference of stomatal density between the WW and WS plants (Table 1).

Trichome characteristics: The shape of trichomes was asteroidal. Trichome density of the abaxial surface was higher than that of adaxial surface (Fig. 2, Table 1). The trichome density of the abaxial surface increased by 26.2% under water-stress condition compared with the well-water condition (Table 1). For adaxial surface, no significant difference of trichome density between the WW and WS plants was observed (Fig. 2, Table 1).

Leaf ultrastructure: The chloroplasts of WW plants looked like shuttles in a shape, with tightly stacked grana thylakoids. Fewer number of osmiophilic granules was observed in the chloroplasts (Fig. 3*A*). Water stress significantly changed the internal structure of the chloroplast. The number of the chloroplasts per cell profile was smaller (Table 2). The length of the chloroplasts decreased and the width increased (Table 2), the chloroplasts became round in a shape with a bigger damage in the membrane structure, the number of osmiophilic granules increased, and the lamellae arranged disorderly (Fig. 3*A,B*). The number of starch grains (per chloroplast) decreased significantly (Fig. 3*A,B*, Table 2). The cristae in mitochondria were disintegrated (Fig. 3*C,D*). The cell nuclei were smaller and the agglomerate nucleoli were bigger than those of WW plants (Fig. 3*E,F*).

Discussion

The stomata play an important role in a regulation of a leaf water status (Baldini *et al.* 1997). Many xerophytes, in order to save internal water, develop their stomata in local leaf epidermal depressions or in crypts (Fahn 1986). We found the stomata were mostly located on the abaxial side below the trichome layer, the leaves of the WS plants had more and smaller stomata than those of WW plants (Table 1, Fig. 1). The increase in the number of stomata could be explained by the decrease in epidermal cell expansion and leaf area under water stress. Bosabalidis and Kofidis (2002) showed that the increase in stomata density contributed to better transpirational control. Under water stress, most stomata were closed (Fig. 1) which resulted in the reduction of the transpiration. This process entailed in parallel a reduction of the rate of photosynthesis. Wilkinson and Davies (2002) indicated that the major events of plant response to dehydration stresses were perception and transduction of the stress signals through signalling components. When water supply was limited, the abscisic acid (ABA) triggered release of anions and K^+ from guard cells. The decrease in guard cell osmotic pressure and volume resulted in stomata closure, reducing a loss of water during the leaf transpiration. The initial steps in ABA signal transduction have been shown to activate guard cell anion channels in a Ca-dependent as well as -independent manner (Keller *et al.* 1989, Lebaudy *et al.* 2007).

To avoid excessive water loss, higher trichome density was also observed on leaf surfaces of eggplants. Leaf pubescence is a common feature of xeric genotypes (Karabourniotis and Bornman 1999, Liakoura *et al.* 1999). This feature is thought to decrease transpirational water loss (Fahn 1986, Baldini *et al.* 1997) and to increase a water-use efficiency by increasing of leaf boundary layer resistance (Pallioti *et al.* 1994). Bongi and Palliotti (1994) observed that trichomes were a barrier to the diffusion of CO_2 and H_2O, and that they lowered the

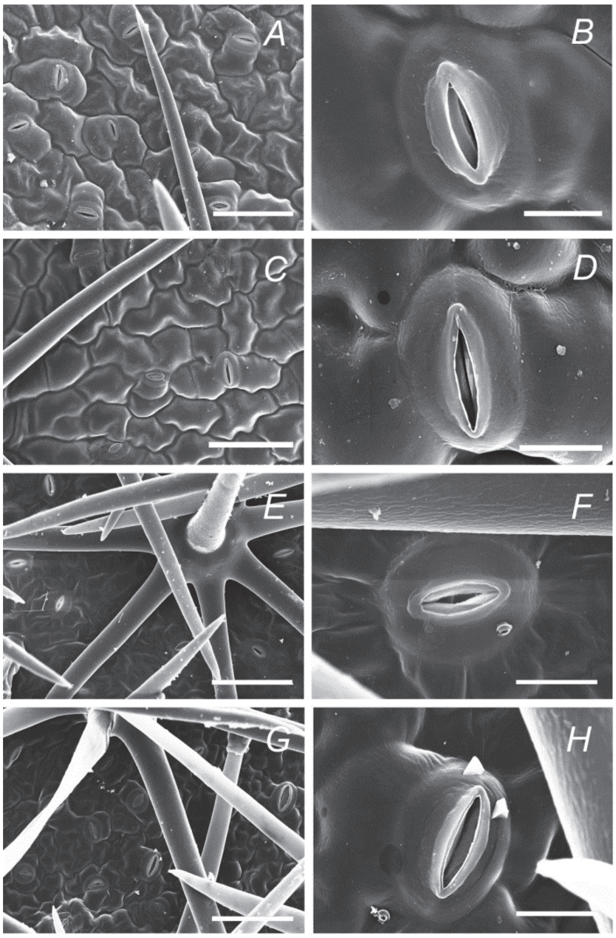

Fig. 1. A comparison of leaf epidermal structure of eggplant (cv. HQ-2) under well watered and water-stress conditions. Stomata and epidermal cells on the adaxial (*A–D*) and abaxial (*E–H*) leaf epidermis of eggplant grown under well water (*A,B,E,F*) and water stress (*C,D,G,H*) conditions. Bar – 50 µm (*A,C,E,G*), 10 µm (*B,D,F,H*).

Table 1. Effects of drought stress on leaf epidermal structure of eggplant. Data represent means ± SE of 5 replications (*n* = 5), and 20 visual fields of each replication. The *different letters* represent significant differences using *Duncan*'s multiple range test at $P<0.05$ after *ANOVA*.

Parameters	Well watered plants		Water-stressed plants	
	Adaxial	Abaxial	Adaxial	Abaxial
Stomatal density [numbers mm^{-2}]	206.3 ± 13.6^c	269.8 ± 16.2^b	203.1 ± 17.3^c	324.8 ± 22.3^a
Stomatal length [µm]	21.24 ± 1.74^a	18.78 ± 1.74^b	19.45 ± 2.06^b	18.17 ± 1.26^c
Stomatal width [µm]	15.63 ± 0.87^a	14.93 ± 1.92^a	14.06 ± 1.74^b	13.46 ± 1.03^c
Trichome density [numbers mm^{-2}]	5.76 ± 0.89^c	11.17 ± 1.73^b	4.64 ± 0.82^c	14.10 ± 1.21^a

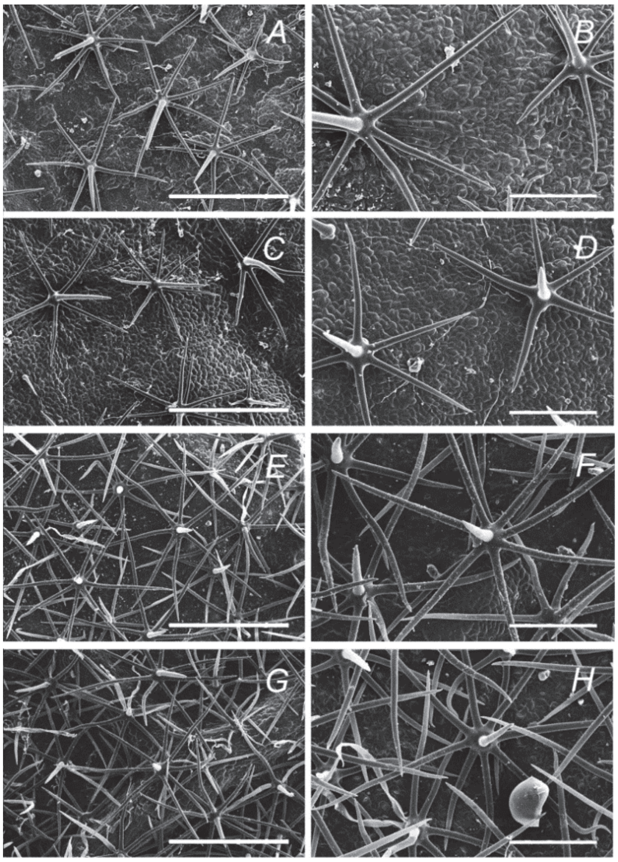

Fig. 2. The structure of eggplant (cv. HQ-2) trichome on adaxial (*A–D*) and abaxial (*E–H*) surface under well watered (*A,B,E,F*) and water-stressed (*C,D,G,H*) conditions. Bar – 500 µm (*A,C,E,G*), 200 µm (*B,D,F,H*).

boundary layer conductance in the air surrounding the stomata. After removing trichomes from leaves, they found that the total boundary layer resistance was reduced more than fivefold. Karabourniotis *et al*. (1992) also found that nonglandular hairs in a number of plant species contained flavonoids and other pigments. These compounds were located in the cell walls of the mature hairs (Karabourniotis and Bornman 1999). Besides, previous studies of *F. farinosa* showed that leaf pubescence reduced radiation absorption, and it was a cost-effective mechanism for maintaining favorable leaf temperatures in very hot, dry, and desert climates (Ehleringer 1983). Moreover, trichome layers that cover the surface of plant organs often act as an effective filter, protecting the underlying tissues against damage.

The changes in chloroplasts were typical in plants stressed by extreme temperatures, low light, saline stress, and water stress (Munné-Bosch *et al*. 2001, Navarro *et al*. 2007, Fu *et al*. 2011). The increase in the number of osmiophilic granules (plastoglobuli) was observed in our study (Fig. 3*A,B*). It is assumed that plastoglobuli may play a role in the synthesis and recycling of lipophilic products and oxidative stress defense. Plastoglobuli have a function in the storage of thylakoid components, such as lipids, plastohydroquinone, and tocopherol (Munné-Bosch *et al*. 2001). The mechanisms that underlie cell shape determination in WS plants are still not fully understood. Potential interactions between sugar metabolism and actin organization dynamics were suggested by previous studies (Li *et al*. 2003, Chary *et al*. 2008). Eastmond *et al*. (2002) also reported that trehalose-6-P might be acting as a regulatory molecule involved in metabolism and embryo development. There could be two reasons: one possibility was that some sugar catabolic enzymes or related metabolites could serve as a signal in controlling cell shape determination; another possibility was that the related metabolites could be involved in the transcriptional regulation of genes encoding proteins involved in cytoskeletal organization (Chary *et al*. 2008).

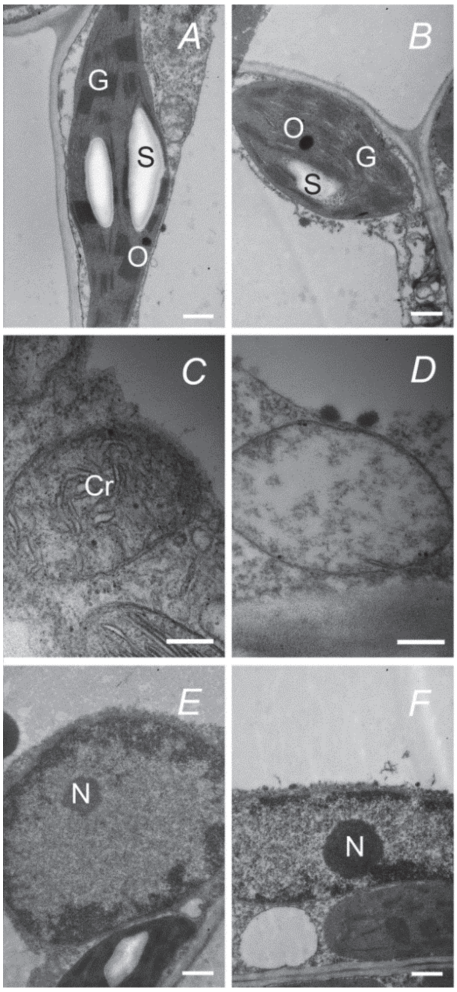

Fig. 3. A comparison of the ultrastructure of chloroplasts (*A,B*), mitochondria (*C,D*), and cell nuclei (*E,F*) under well watered (*A,C,E*) and water-stressed (*B,D,F*) conditions of eggplant (cv. HQ-2). G – grana lamellae; S – starch grain; O – osmiophilic granules; Cr – cristae; N – nucleoli. Bar – 500 nm (*A,B,E,F*), 200 nm (*C,D*).

Table 2. Effects of drought stress on the ultrastructure of chloroplast of eggplant. Data represent means ± SE of 5 replications ($n = 5$), and 20 visual field of each replication. The *different letters* represent significant differences using *Duncan*'s multiple range test at $P<0.05$ after *ANOVA*.

Parameters	Well watered plants	Water-stressed plants
Chloroplast [cell numbers profile^{-1}]	13.71 ± 1.70^a	10.26 ± 1.26^b
Starch grain [numbers chloroplast^{-1}]	2.00 ± 0.35^a	0.50 ± 0.07^b
Chloroplast length [μm]	5.69 ± 0.70^a	4.18 ± 0.56^b
Chloroplast width [μm]	1.82 ± 0.21^b	2.21 ± 0.32^a
Starch grain length [μm]	1.43 ± 0.14^a	1.31 ± 0.17^a
Starch grain width [μm]	0.71 ± 0.06^a	0.54 ± 0.07^a

Conclusions: Our results indicated that the morphological, and anatomical characteristics collectively conferred eggplant a high degree of plasticity in a response to water stress. The leaves of the WS plants had more and smaller stomata than those of WW plants. Most of the stomata were closed and covered by trichomes to prevent water loss. The chloroplasts became round in shape with more damaged membrane structure, the number of osmiophilic granules increased, and the number of starch grain decreased. The cristae in mitochondria were disintegrated. The cell nuclei were smaller, the nucleoli were agglomerated and bigger than those of WW treatment.

References

Al-Ani, T.A., Bierhuizen J.F.: Stomatal resistance, transpiration, and relative water content as infuenced by soil moisture stress. – Acta Bot. Neerl. **20**: 318-326, 1971.

Baldini, E., Facini, O., Nerozzi, F., *et al.*: Leaf characteristics and optical properties of different woody species. – Trees **12**: 73-81, 1997.

Bondada, B.R., Oosterhuis, D.M., Wullschleger, S.D. *et al.*: Anatomical considerations related to photosynthesis in cotton (*Gossypium hirsutum* L.) leaves, bracts, and the capsule wall. – J. Exp. Bot. **45**: 111-118, 1994.

Bongi, G., Paliotti, A.: Olive. – In: Schaffer, B., Andersen, P.C. (ed.): Handbook of Environmental Physiology of Fruit Crops, Vol. I, Temperate Crops. Pp. 165-187. CRC Press, Boca Raton 1994.

Bosabalidis, A.M., Kofidis, G.: Comparative effects of drought stress on leaf anatomy of two olive cultivars. – Plant Sci. **163**: 375-379, 2002.

Chary, S.N., Hicks, G.R., Choi, Y.G. *et al.*: Trehalose-6-phosphate synthase/phosphatase regulates cell shape and plant architecture in *Arabidopsis*. – Plant Physiol. **146**: 97-107, 2008.

Chen, Q., Wang, L.F., Su, N. *et al.*: Photosystem 2 photochemistry and pigment composition of a yellow mutant of rice (*Oryza sativa* L.) under different irradiances. – Photosynthetica **46**: 35-39, 2008.

Desclaux, D., Roumet P.: Impact of drought stress on the phenology of two soybean (*Glycine max* L. Merr) cultivars. – Field Crop. Res. **46**: 61-70, 1996.

Dias, M.C., Brüggemann, W.: Differential inhibition of photosynthesis under drought stress in *Flaveria* species with different degrees of development of the C_4 syndrome. – Photosynthetica **45**: 75-84, 2007.

Eastmond, P.J., van Dijken, A.J., Spielman, *M. et al.*: Trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose synthesis, is essential for *Arabidopsis* embryo maturation. – Plant J. **29**: 225-235, 2002.

Ehleringer, J.: Characterization of a glabrate *Encelia farinosa* mutant: morphology, ecophysiology, and field observations. – Oecologia **57**: 303-310, 1983.

Fang, X.W., Turner, N.C., Li, F.M. *et al.*: *Caragana korshinskii* seedlings maintain positive photosynthesis during short-term, severe drought stress. – Photosynthetica **49**: 603-609, 2011.

Fahn, A.: Structural and functional properties of trichomes of xeromorphic leaves. – Ann. Bot. **57**: 631-637, 1986.

Fu, Q.S., Zhao, B., Wang, X.W. *et al.*: The responses of morphological trait, leaf ultrastructure, photosynthetic and biochemical performance of tomato to differential light availabilities. – J. Integr. Agr. **10**: 1887-1897, 2011.

Fu, Q.S., Zhao, B., Wang, Y.J. *et al.*: Stomatal development and associated photosynthetic performance of capsicum in response to differential light availabilities. – Photosynthetica **48**: 189-198, 2010.

Jones, R.W., Pike, L.M., Yourman, L.F.: Salinity influences cucumber growth and yield. – J. Amer. Soc. Hort. Sci. **114**: 547-551, 1989.

Karabourniotis, G., Bornman, J.: Penetration of UV-A, UV-B and blue light through the leaf trichome layers of two xeromorphic plants, olive and oak, measured by optical fibre microprobes. – Physiol. Plant. **105**: 655-661, 1999.

Karabourniotis, G., Papadopoulos, K., Papamarkou, M., Manetas, Y.: Ultaviolet-B radiation absorbing capacity of leaf hairs. – Physiol. Plant. **86**: 414-418, 1992.

Keller, B.U., Hedrich, R., Raschke, K.: Voltage-dependent anion channels in the plasma membrane of guard cells. – Nature **341**: 450-453, 1989.

Lebaudy, A., Very, A.A., Sentenac, H.: K^+ channel activity in plants: Genes, regulations and functions. – FEBS Lett. **581**: 2357-2366, 2007.

Liakoura, V., Stavrianakou, S., Liakopoulos, G., *et al.*: Effects of UV-B radiation on *Olea europaea*: comparisons between a greenhouse and a field experiment. – Tree Physiol. **19**: 905-908, 1999.

Li, S., Blanchoin, L., Yang, Z., Lord, E.M.: The putative *Arabidopsis* Arp2/3 complex controls leaf cell morphogenesis. – Plant Physiol. **132**: 2034-2044, 2003.

Martin, G.D., Myers, A., Vogelmann, T.C.: Characterization of plant epidermal lens effects by a surface replica technique. – J. Exp. Bot. **42**: 581-587, 1991.

Meidner, H., Mansfield, T.A.: The role of rhythms in stomatal behaviour. – In: Meidner, H., Mansfield, T.A. (ed.): Physiology of Stomata. Pp. 102-169. McGraw-Hill, New York 1968.

Munné-Bosch, S., Jubany-Marí, T., Alegre, L.: Drought-induced senescence is characterized by a loss of antioxidant defences in chloroplasts. – Plant Cell Environ. **24**: 1319-1327, 2001.

Navarro, A., Bañon, S., Olmos, E., Sánchez-Blanco, M.J.: Effects of sodium chloride on water potential components, hydraulic conductivity, gas exchange and leaf ultrastructure of *Arbutus unedo* plants. – Plant Sci. **172**: 473-480, 2007.

Pallardy, S.G., Kozlowski, T.T.: Frequency and length of 21 Populus clones. – Can. J. Bot. **57**: 2519-2523, 1979.

Pallioti, A., Bongi, G., Rocchi, P.: Peltate trichomes effects on photosynthetic gas exchange of *Olea europaea* L. leaves. – Plant Physiol. **13**: 35-44, 1994.

Poulson, M.E.T., Vogelmann, C.: Epidermal focusing and effects on photosynthetic light-harvesting in leaves of *Oxalis*. – Plant Cell Environ. **13**: 803-811, 1990.

Song, X.S., Shang, Z.W., Yin, Z.P. *et al.*: Mechanism of xanthophyll-cycle-mediated photoprotection in *Cerasus humilis* seedlings under water stress and subsequent recovery. – Photosynthetica **49**: 523-530, 2011.

Spurr, A.R.: A low viscosity epoxy resin embedding medium for electron microscopy. – J. Ultrastruct. Res. **26**: 31-43, 1969.

Weng, J.H., Chien, C.T., Chen, C.W., Lai, X.M.: Effects of osmotic- and high-light stresses on PSII efficiency of attached and detached leaves of three tree species adapted to different water regimes. – Photosynthetica **49**: 555-563, 2011.

Wilkinson, S., Davies, W.J.: ABA-based chemical signalling: the co-ordination of responses to stress in plants. – Plant Cell Environ. **25**: 195-210, 2002.

Yordanov, I., Velikova, V., Tsonev, T.: Plant response to drought, acclimation, and stress tolerance. – Photosynthetica **38**: 171-186, 2000.