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Photosynthetic Responses of the Tropical Spiny Shrub *Lycium nodosum* (Solanaceae) to Drought, Soil Salinity and Saline Spray

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Water relations and photosynthetic characteristics of plants of *Lycium nodosum* grown under increasing water deficit (WD), saline spray (SS) or saline irrigation (SI) were studied. Plants of this perennial, deciduous shrub growing in the coastal thorn scrubs of Venezuela show succulent leaves which persist for approx. 1 month after the beginning of the dry season; leaf succulence is higher in populations closer to the sea. These observations suggested that *L. nodosum* is tolerant both to WD and salinity. In the glasshouse, WD caused a marked decrease in the xylem water potential (ψ), leaf osmotic potential (ψ_s) and relative water content (RWC) after 21 d; additionally, photosynthetic rate (A), carboxylation efficiency (CE) and stomatal conductance (g_s) decreased by more than 90 %. In contrast, in plants treated for 21 d with a foliar spray with 35 ‰ NaCl or irrigation with a 10 % NaCl solution, ψ and RWC remained nearly constant, while ψ_s decreased by 30 %, and A, CE and g_s decreased by more than 80 %. An osmotic adjustment of 0·60 (SS) and 0·94 MPa (SI) was measured. Relative stomatal and mesophyll limitations to A increased with both WD and SS, but were not determined for SI-treated plants. No evidence of chronic photoinhibition due to any treatment was observed, since maximum quantum yield of PSII, F_v/F_m, did not change with either drought in the field or water or salinity stress in the glasshouse. Nevertheless, WD and SI treatments caused a decrease in the photochemical (q_P) and an increase in the non-photochemical (q_N) quenching coefficients relative to controls; q_N was unaffected by the SS treatment. The occurrence of co-limitation of A by stomatal and non-stomatal factors in plants of *L. nodosum* may be associated with the extended leaf duration under water or saline stress. Additionally, osmotic adjustment may partly explain the relative maintenance of A and g_s in the SS and SI treatments and the tolerance to salinity of plants of this species in coastal habitats.

Key words: Drought, *Lycium nodosum*, fluorescence, mesophyll limitation, saline stress, stomatal limitation, water deficit.

INTRODUCTION

Plant species growing in the semi-arid regions of the world need to be adapted to an environment in which drought strongly affects plant growth. Water availability is the main environmental factor limiting photosynthesis and growth even in plants well adapted to arid conditions. Another source of stress is soil salinity in non-tolerant species (Greenway and Munns, 1980).

Water deficit affects stomatal conductance, g_s, thus diminishing photosynthetic rate, A; stomatal closure is assumed to be the main cause of decreased A, because it decreases CO_2 availability in the mesophyll (Cornic, 2000). However, there is strong evidence that drought also affects mesophyll metabolism, reducing photosynthetic capacity (Tezara *et al.*, 1999; Lawlor, 2002). Stomatal closure was not the main factor for the reduction in A under drought in plants of *Amaranthus palmeri* (Ehleringer, 1983), *Encelia farinosa* (Ehleringer and Cook, 1984) or *Helianthus annuus* (Tezara *et al.*, 1999). An increase in the relative stomatal limitation (L_s) calculated from the response curve of A to the intercellular CO_2 concentration (C_i) allows it to be ascertained as to whether A is reduced solely because of decreased g_s, and not because of an increase in mesophyll limitation (Farquhar and Sharkey, 1982). Jacob and Lawlor (1991) defined the mesophyll limitation, L_m, as the proportional reduction in C_i-saturated A (A_{sat}) of plants subjected to stress. Processes in photosynthetic metabolism which may be impaired by water stress include Rubisco enzyme activity, RuBP regeneration, ATP supply, electron transport rate (J) and efficiency of light capture in the photosystems (Lawlor, 2002; Lawlor and Cornic, 2002). Therefore, changes in L_m may reflect changes in parameters such as chlorophyll *a* fluorescence and carboxylation efficiency (CE), among others.

During water stress, restricted CO_2 availability due to stomatal closure may lead to increased susceptibility to photodamage (Powles, 1984). However, such damage was not found to occur during the dry season in *Quercus petraea* (Epron *et al.*, 1992), suggesting that the mechanisms of protection against an excess of absorbed excitation energy are efficient. The major process involved in protection against photo-damage is probably the increase in non-

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photochemical quenching energy dissipation, which reduces the relative quantum yield of PSII (ϕ_{PSII}) in order to maintain an adequate balance between photosynthetic electron transport and carbon metabolism (Weis and Berry, 1987; Krause and Weis, 1991).

In a similar manner to water deficit, salinity stress may alter photosynthesis mainly through a reduction of g_s and/or of the mesophyll capacity to fix CO_2 (Seemann and Critchley, 1985; Bethke and Drew, 1992; Delfine *et al*., 1998). A 50 % reduction in A of plants of *Olea europaea* occurred when they were irrigated with 200 mM NaCl, due to a reduction in g_s and CE (Tattini *et al*., 1997). Salinity caused an 18 % reduction in Rubisco activity of leaves of *Cicer arietinum* (Soussi *et al*., 1998). In contrast, maize plants grown with low concentrations of NaCl (20–50 mM) showed slight increases in A and q_N, but a decrease in q_P (Shabala *et al*., 1998).

Changes in leaf anatomy, including an increase in thickness and succulence, are commonly observed in plants subjected to increased soil salinity, as in the case of many mangroves such as *Laguncularia racemosa* (Biebl and Kinzel, 1965) and *Avicennia germinans* (Suárez and Sobrado, 2000). Since salt-stressed leaves are generally thicker than in control plants and leaf thickening is likely to increase resistance to CO_2 diffusion in the mesophyll (Delfine *et al*., 1998), these changes may have an influence on gas exchange (Bongi and Loreto, 1989).

Lycium nodosum Miers. (Solanaceae) is a perennial, deciduous shrub growing in coastal scrubs of Venezuela. Plants occupy exposed as well as shaded sites, but tend to grow more vigorously under the partial shade of evergreen trees, such as *Prosopis juliflora*. Foliage persists for approx. 1 month into the dry season. Leaf succulence, as well as the occurrence of appreciable photosynthetic rates at the beginning and middle of the dry season in the field, suggested the occurrence of particular strategies of drought resistance in *L. nodosum*. Since adult (approx. 2 m tall) individuals have a main root not longer than 1 m and soil water content in the field at that depth is less than 4 % dry mass (DM) (Herrera *et al*., 1994), it seems unlikely that plants tap any significant soil water during the dry season.

Gas exchange, water relations and parameters of chlorophyll *a* fluorescence of plants growing in the glasshouse, under frequent irrigation and water deficit (WD) and under two salinity treatments [foliar spray with 35 ‰ NaCl (SS) and irrigation with 10 ‰ NaCl (SI)] were measured to investigate the possible mechanisms of tolerance of *L. nodosum* plants to drought and salinity. To find out whether the reduction in A due to drought or salinity was attributable solely to stomatal closure or not was of particular interest. Plants were also measured in the field during the dry and the rainy season at locations either subject to or not subject to soil salinity and marine spray.

MATERIALS AND METHODS

Field sites and plant material

Measurements in the field were carried out in Venezuela in a thorn scrub near the city of Coro (11°25′N, 69°36′W) at approx. 20 m above sea level (a.s.l.) during the rainy season (February 2000), by the seashore in Adícora (11°57′N, 69°48′W) during the rainy season (February 2000) and Chichiriviche de la Costa (10°33′N, 67°12′W) during the dry season (August 1999). At Coro, plants of *L. nodosum* were growing under the canopy of the evergreen tree *Prosopis juliflora,* whereas in both Adícora and Chichiriviche de la Costa they were unshaded and exposed to full sunlight. *Lycium nodosum* is a spiny shrub with profuse decumbent branches less than 2 m long and small (approx. 1 cm^2) partly fleshy, sessile leaves. Leaves appear fully expanded a few centimetres from the branch tip, with lamina area and thickness showing a slight increase towards the main stem. For all measurements described below, only the youngest fully expanded leaves were sampled.

Glasshouse experiments

Whole plants collected in Chichiriviche de la Costa were grown in 15-L pots filled with commercial fertile garden soil in the glasshouse in Caracas (approx. 1000 m a.s.l.). Daily watering for 1 month ensured the production of abundant foliage; plants were fertilized weekly with a commercial fertilizer (N : P : K, 15 : 15 : 15). Three separate experiments were conducted: (1) WD, by suspension of watering; (2) SS, by spraying the leaves with a 35 ‰ NaCl solution three times a day for 21 d; and (3) SI, by irrigation every other day with 500 cm^3 of a 10 % NaCl solution, and with tap water on alternate days to avoid water deficit. Ten plants were used per treatment. Measurements of the variables indicated below were taken on leaves or leaf-bearing branches from three to ten different individuals.

Microclimatic parameters

Photosynthetic photon flux density (PPFD) was measured with a quantum sensor model 190-S connected to a meter model LI-185 (LI-COR Inc., Lincoln, NB, USA). Air temperature was measured with YSI 400 thermistors connected to a telethermometer (Yellow Springs Instruments, OH, USA), and relative humidity with a hair strand hygrometer (Abbeon model AB167B; Abbeon Cal., Santa Barbara, CA, USA). Soil salinity was measured with a refractometer model S/Mill-E (Atago Co., Ltd, Tokyo, Japan). Soil electrical conductivity was measured with an electrical conductivity meter, model 19101–00 (Cole-Parmer Instrument Co., Vernon Hills, IL, USA).

Water relations

Xylem water potential (ψ) was measured at 0600 h ($n = 6$) with a pressure bomb (PMS, Corvallis, OR, USA). Leaf osmotic potential (ψ_s) was measured in the sap expressed from frozen and defrosted leaves previously used for the determination of ψ, using a Wescor 5000 osmometer (Wescor, Inc., Logan, UT, USA). In order to eliminate the salt deposited on them, leaves sprayed with NaCl were rinsed with distilled water and blotted dry prior to freezing. Leaf water content was determined in leaves ($n = 10$) taken at 0800–0900 h as LWC = (FM – DM)/DM). Relative water

TABLE 1. *Values of xylem water potential, leaf water content, photosynthetic rate, leaf conductance and maximum quantum yield of PSII in plants of* L. nodosum *growing in the field at different locations and seasons*

	Location		
	Chichiriviche de la Costa	Coro	Adícora
Parameters	Dry season	Rainy season	Rainy season
Soil salinity (‰)	3·4	1·4	5·3
Soil salinity (mmohs)	6·6	3·3	9·5
ψ (MPa)	–4·16 ± 0·08	–1·82 ± 0·12	–1·57 ± 0·03
LWC (proportion of dry mass)	0·84 ± 0·04	0·78 ± 0·01	0·84 ± 0·01
A (μmol m^{-2} s^{-1})	0·2 ± 0·05	2·4 ± 0·2	4·6 ± 1·5
g_s (mmol m^{-2} s^{-1})	55 ± 5	133 ± 3	172 ± 18
F_v/F_m	0·82 ± 0·01	0·81 ± 0·01	0·84 ± 0·01

Values are means ± s.e. (n = 6).

content (RWC) was determined in leaves (n = 10) collected at 0700 h and floated on distilled water in the dark at 4 °C for 1 h. Osmotic potential at full turgor ($\psi_s{}^{100}$) was measured in the sap expressed from frozen and defrosted leaves (n = 6) of branches re-saturated in the dark for 1 h. Osmotic adjustment was calculated as the difference between $\psi_s{}^{100}$ of control plants and that of plants after 21 d of treatment.

Leaf gas exchange

Gas exchange in attached leaves was measured with a portable IRGA model CIRAS 1 used in conjunction with a PLC(B) assimilation chamber (PP Systems plc, Hitchin, Herts., UK). Measurements were made at an ambient CO_2 concentration (C_a) of 350 µmol mol^{-1}, unless otherwise stated. Instantaneous A was measured at 1000 h with a PPFD of 800 µmol m^{-2} s^{-1} and leaf temperature of 28 °C ± 0·5, the time at which daily maximum A was determined to occur in control plants prior to treatments. A/C_i curves (n = 3) were determined in WD and SS plants by decreasing C_i [calculated by the program in the IRGA (infra-red gas analyser), after Farquhar *et al.*, 1980] from approx. 220 µmol mol^{-1} (when A at C_a = 350 µmol mol^{-1} was initially measured) to zero, and then progressively increasing C_i to 1400 µmol mol^{-1}. CO_2 was provided by a cylinder filled with pure gas connected to the IRGA. A/C_i curves were fitted to the empirical equation $A = b + de^{kC_i}$, where b = CO_2-saturated photosynthetic capacity (A_{sat}) and $(b + d) = y$ – intercept (Tezara *et al.*, 1998). CE was calculated from the initial slope of the curve**.** The relative stomatal limitation of the photosynthetic rate was calculated as $L_s = 100 \times (A_o - A)/A_o$, where A_o is the photosynthetic rate at $C_i = C_a$ (Farquhar and Sharkey, 1982). The relative mesophyll limitation was calculated as $L_m = 100 \times (A_C - A_S)/A_C$, where A_C is A of control leaves at C_i = 800 µmol mol^{-1} and A_S the rate of stressed leaves at the same C_i (Jacob and Lawlor, 1991). Thus, L_m is a measure of the capacity of the mesophyll to fix CO_2 at saturating C_i and its value in control plants is zero. Conditions during measurements were: leaf temperature 28 ± 0·5 °C, and PPFD 700 ± 20 µmol m^{-2} s^{-1}. Previous determinations showed that increasing PPFD to 1200 µmol m^{-2} s^{-1} did not increase C_i-saturated A, but leaf temperature increased up to 34 °C. Response curves of A *vs.* PPFD (n = 3) were carried out by varying incident PPFD with neutral filters (Balzers, Handelsbank, Zürich); the light source was either the sun or a 50 W dichroic lamp (Phillips, Caracas, Venezuela).

Chlorophyll a *fluorescence of PSII*

Chlorophyll fluorescence was measured on attached dark-adapted leaves (n = 6) with a Mini PAM fluorometer (Walz, Effeltrich, Germany) using the protocol described by Genty *et al.* (1989). The maximum quantum yield of PSII (F_v/F_m) was measured in attached dark-adapted leaves. The relative quantum yield of PSII at steady-state photosynthesis was calculated as $\phi_{PSII} = (F'_m - F_s)/F'_m$, where F_s and F'_m are fluorescence at steady-state photosynthesis and maximum fluorescence in the light, respectively. The coefficients q_P and q_N were calculated from measurements of fluorescence. Electron-transport rate of PSII was estimated by the method of Krall and Edwards (1992) as $J = \phi_{PSII} \times \text{PPFD} \times a \times 0{\cdot}5$, where a is the fraction of incident PPFD absorbed by the leaf, assumed to be 0·85 (leaves of *L. nodosum* are glabrous). Leaf chlorophyll content was determined after Bruinsma (1963) in acetone extracts of leaves (n = 4).

Statistical analysis

Results are presented as means ± s.e. Significance at $P < 0{\cdot}05$ was assessed by ANOVA using the statistical packages Statistica, Excel and Sigmaplot.

RESULTS

Field measurements

Values of variables in plants from the three field sites are presented in Table 1. Soil was more saline at Chichiriviche de la Costa and Adícora than at Coro. Plants had lower values of ψ in the dry season (Chichiriviche de la Costa) than in the rainy season (Coro and Adícora). Values of LWC were lower in Coro than at the other two, more saline, sites. Values of A and g_s were lower during the dry season, whereas the average F_v/F_m did not change with either site or season.

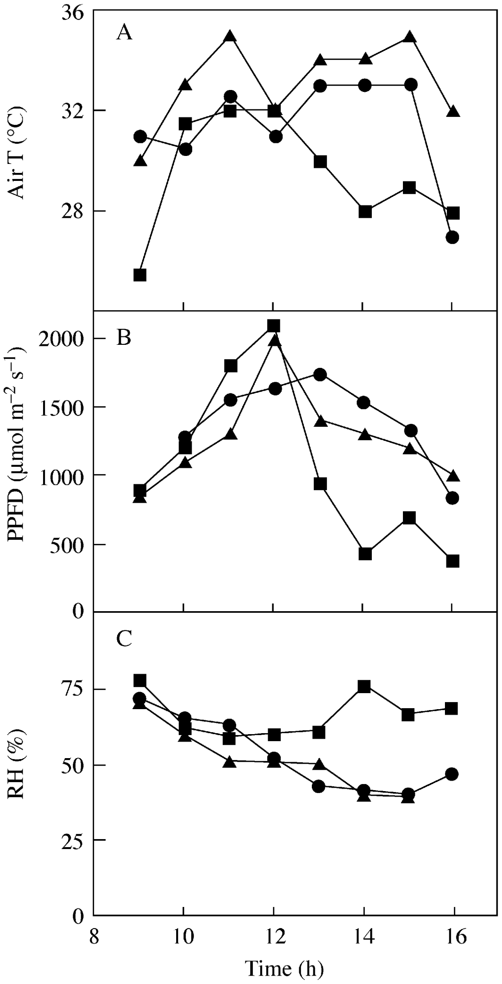

FIG. 1. Time course of changes on a typical clear day in (A) air temperature, (B) photosynthetic photon flux density and (C) relative humidity in the glasshouse (circles) and at the field sites of Coro during the rainy season (triangles, February 2000) and Chichiriviche de la Costa during the dry season (squares, August 1999).

Glasshouse experiments

Leaf water status. Microclimatic conditions in the glasshouse resembled values measured in Coro and Chichiriviche de la Costa (Fig. 1). Treatment effects in the parameters of water relations are shown in Fig. 2. After 21 d under the WD treatment, a decrease in ψ to –4·1 MPa was observed with a 10 % reduction in RWC. Treatments SS and SI had no effect on ψ, whereas RWC was significantly reduced by 21 d of treatment SI. An average 30 % reduction in ψ_s was observed after 21 d for all treatments. Leaf water content was significantly reduced by WD (13 %) and SI (4 %), but not by SS. Control values were similar to those measured in Chichiriviche de la Costa and Adícora, whereas values measured in Coro resembled those obtained in the glasshouse after 14 d of WD.

The apparent occurrence of negative turgor pressures due to higher values of ψ_s than expected may have been the consequence of dilution of cell sap by apoplastic water; nevertheless, an osmotic adjustment of 0·60 ± 0·13 (SS) and 0·94 ± 0·10 MPa (SI) was measured after 14 and 21 d of treatment, respectively. The ψ_s^{100} was not affected by treatment WD, averaging –1·44 MPa.

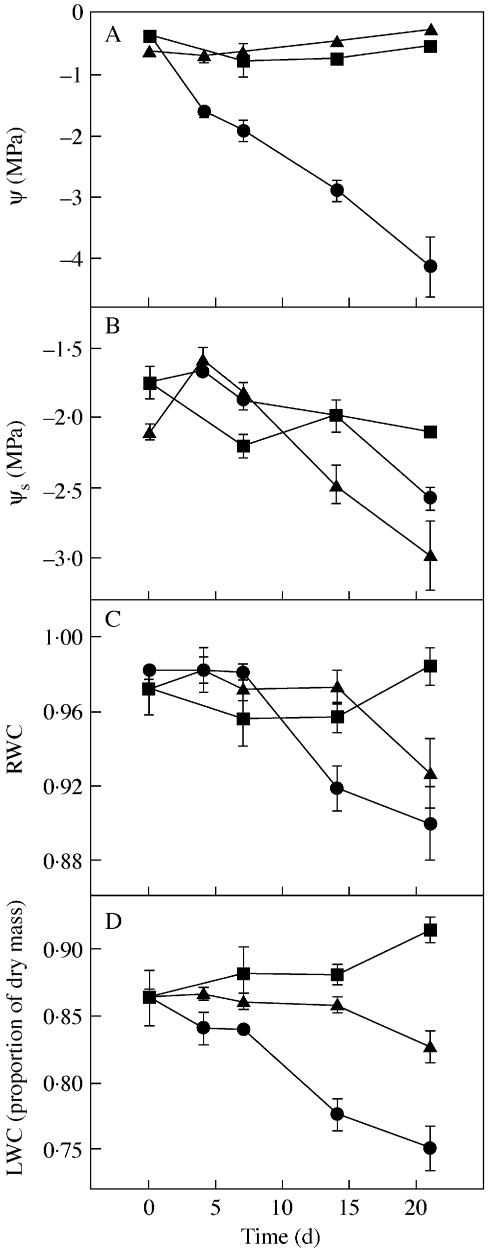

FIG. 2. Time-course of changes in (A) xylem water potential, (B) leaf osmotic potential, (C) relative water content and (D) leaf water content in plants of *Lycium nodosum* subjected to water deficit (circles), saline spray (squares) or saline irrigation (triangles). Values are means ± s.e. (n = 6).

Leaf gas exchange. Control values of A and g_s were approx. five and two times higher, respectively, than in plants growing in the field during the rainy season, showing the effect of frequent watering on glasshouse-grown plants. After 21 d of treatment, A and g_s decreased by 92 and 94 % (WD), 75 and 82 % (SS) and 51 and 70 % (SI), respectively (Fig. 3). The relationship between A and g_s was a straight

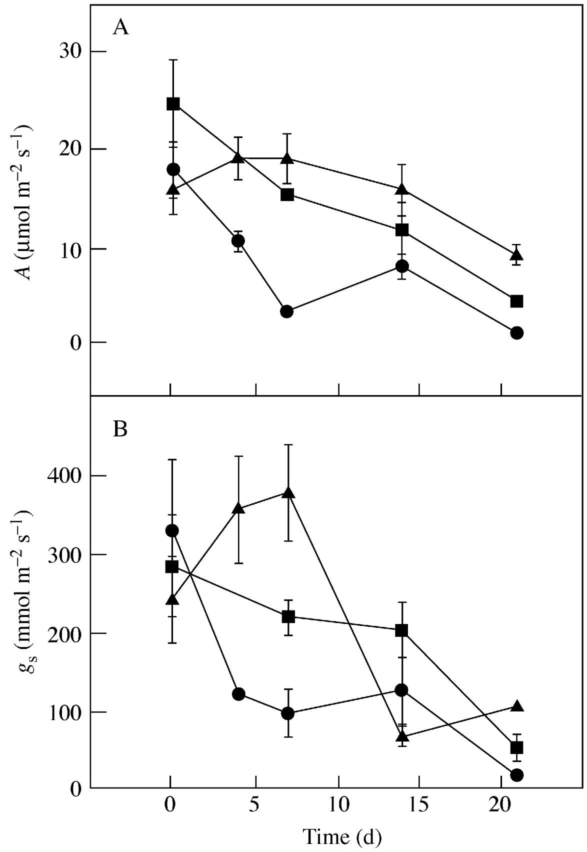

FIG. 3. Effects of time under treatment on (A) photosynthetic rate and (B) stomatal conductance in leaves of plants of *L. nodosum* subjected to water deficit (circles), saline spray (squares) or saline irrigation (triangles). Values are means ± s.e. (n = 4).

line of zero intercept, which explained 87 % of the variation in A (Fig. 4).

Both A_{sat} and CE declined with WD and SS treatments (Figs 5 and 6A and B). The CO_2 compensation point was 38 and 42 μmol mol^{-1} in control plants of the WD and SS treatments, respectively, and increased to 100 and 62 μmol mol^{-1} with length of time under treatment, remaining nearly constant in SS from day 4 to day 21. The L_s increased by 10 % a few days after the onset of the WD or SS treatments and remained constant thereafter, whereas L_m increased by 80 and 40 % with WD or SS, respectively (Fig. 6C and D).

Response of A *to PPFD.* In control plants, A was light-saturated at a PPFD of 800 μmol m^{-2} s^{-1}. As water deficit developed, saturating PPFD, saturated A and apparent quantum yield of CO_2 fixation progressively decreased (Fig. 7A). The same trend was observed under the SS treatment, although saturated A was approx. twice as large after 14 and 21 d as in WD plants (Fig. 7B).

Chlorophyll a *fluorescence.* No significant changes in chlorophyll content per unit area occurred under any treatment (Fig. 8A). The average F_v/F_m was 0·83 ± 0·01 and this was not affected by any treatment (Fig. 8B). However, a decrease in ϕ_{PSII} at PPFD = 1300 μmol m^{-2} s^{-1}

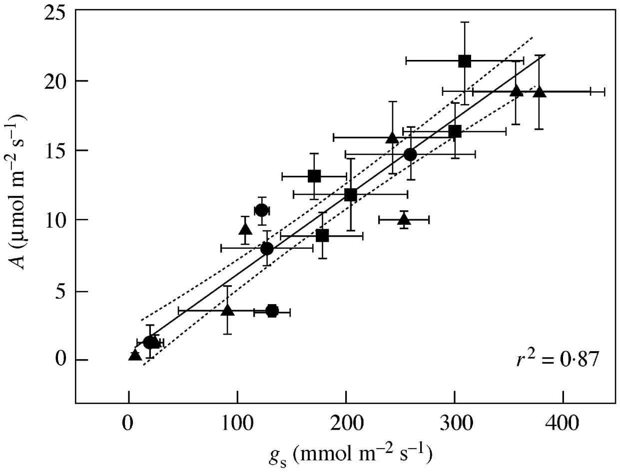

FIG. 4. Relationship between instantaneous photosynthetic rate (A) and stomatal conductance (g_s) in leaves of plants of *Lycium nodosum* subjected to water deficit (circles), saline spray (squares) or saline irrigation (triangles). Values are means ± s.e. (n = 4). The regression line (continuous) and the confidence intervals (dotted, $P < 0·05$) are shown. The value of the determination coefficient is included.

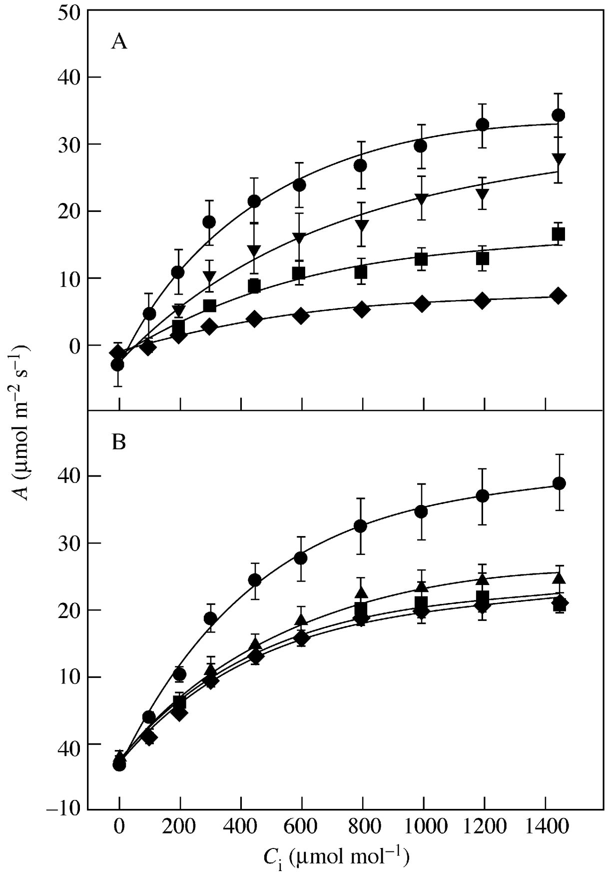

FIG. 5. Responses of photosynthetic rate to intercellular CO_2 concentration in leaves of plants of *Lycium nodosum* after 0 d (circles), 7 d (triangles), 14 d (squares) and 21 d (diamonds) of (A) water deficit and (B) saline spray. Values are means ± s.e. (n = 3); standard errors are shown when greater than the symbol.

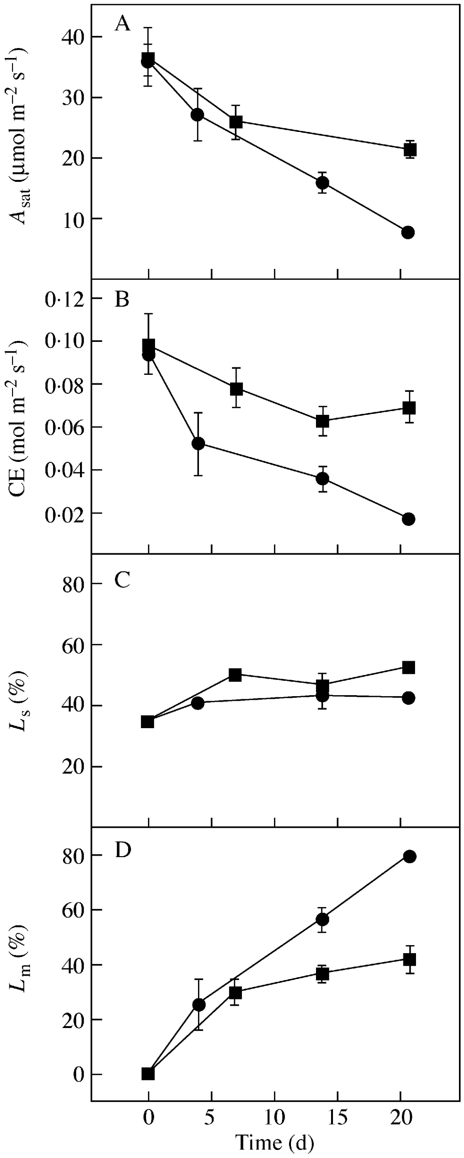

FIG. 6. Effect of time under treatment on (A) C_i-saturated photosynthetic rate, (B) carboxylation efficiency, (C) relative stomatal limitation and (D) relative mesophyll limitation. Circles, water deficit treatment; squares, saline spray treatment. Values are means ± s.e. (n = 3); standard errors are shown when greater than the size of the symbol.

was observed in all three treatments (Fig. 8C); consequently, J was reduced by 70, 52 and 45 % after 21 d of WD, SS and SI treatments, respectively (Fig. 8D). The q_P followed the same trend as $\phi_{PSII,}$ decreasing with time under each treatment (Fig. 8E), while q_N increased in WD and remained constant in SS and SI (Fig. 8F).

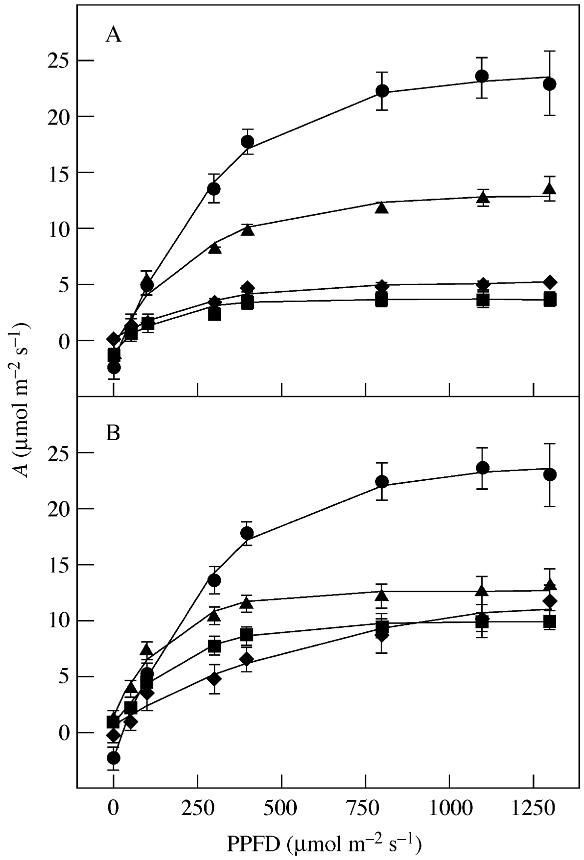

FIG. 7. Responses of photosynthetic rate to irradiance in leaves of plants of *Lycium nodosum* subjected for 0 d (circles), 7 d (triangles), 14 d (squares) and 21 d (diamonds) to (A) water deficit and (B) saline spray. Values are means ± s.e. (n = 3); standard errors are shown when greater than the size of the symbol.

DISCUSSION

Limiting supplies of water lead to dehydration of plants, shown by decreased RWC and ψ and loss of turgor of leaves, which can result in stomatal closure, increased concentration of solutes and decreased ψ_s (Lawlor, 1995). In the present study water deficit resulted in a reduction in RWC and ψ, while saline irrigation (but not spray) caused a reduction in ψ_s, RWC and LWC.

Leaves of *L. nodosum* showed variable succulence; in populations near the sea leaves were more succulent than in the inland, less saline, population, indicating that these plants possess mechanisms of salt tolerance. Similarly, plants subjected to SS or SI showed maintenance of, or even an increase in, succulence. Plant exposure to high salinity at the root caused increases in leaf succulence in seedlings of the mangroves *Aegiceras corniculatum* (Ball and Farquhar, 1984) and *Avicennia germinans* (Suárez and Sobrado, 2000). In contrast, no changes or even decreases in LWC were found in the halophyte *Halosarcia pergranulata* (Short and Colmer, 1999) and the mangrove *Avicennia marina* (Ball and Farquhar, 1984), and a decrease in mesophyll and total leaf thickness was found in spinach (Delfine *et al.*, 1998), suggesting that increased succulence is not a universal response to salinity.

Considerable osmotic adjustment occurred in leaves of *L. nodosum* subjected to saline spray or irrigated with saline solution. Osmotic adjustment, the lowering of ψ_s by active accumulation of osmotically active solutes, is generally thought to be the main mechanism for turgor maintenance under stress, enabling plants to maintain metabolic activity during drought (Turner and Jones, 1980), and is a mech-

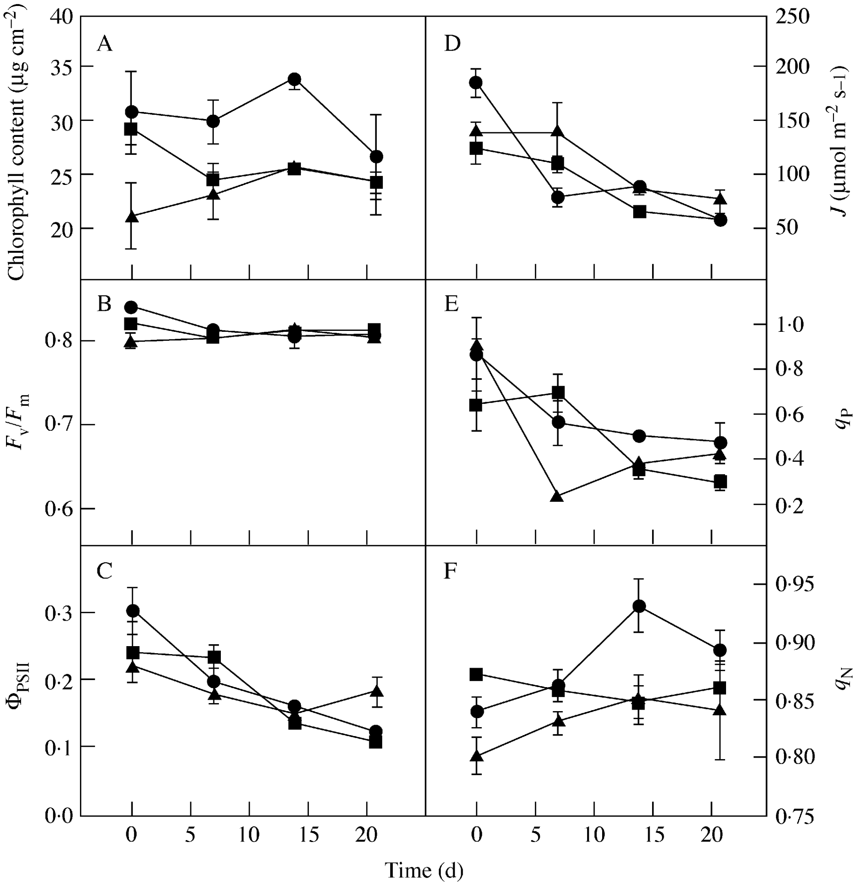

FIG. 8. Time-course of changes in plants of *Lycium nodosum* subjected to water deficit (circles), saline spray (squares) and saline irrigation (triangles) in (A) total chlorophyll content, (B) maximum quantum yield of PSII, (C) relative quantum yield of PSII; (D) total electron transport rate, (E) coefficient of photochemical quenching and (F) coefficient of non-photochemical quenching of fluorescence. The parameters ϕ_{PSII}, J, q_P and q_N were measured at a PPFD = 1300 µmol m^{-2} s^{-1}. Values are means ± s.e. (n = 4).

anism reported to operate in plants in response to salt (Morgan, 1984). The ψ_s of *H. pergranulata* decreased 2·5 times with an increase in NaCl concentration in the soil from 10 to 800 mM (Short and Colmer, 1999). Leaf osmotic adjustment in salt-stressed plants of *L. nodosum* may result in the maintenance of a favourable water status, contributing substantially to the salt tolerance of this species.

Both water and salinity stress markedly inhibited A, CE and g_s, although the effect of salinity on these variables was milder than water deficit. Similar results with water stress have been reported in *Ipomoea carnea* and *Jatropha gossypifolia*, species sympatric to *L. nodosum* (Herrera *et al.*, 1994; Tezara *et al.*, 1998). In flag leaves of barley (Belkhodja *et al.*, 1999) and in salt-sensitive rice cultivars, salinity caused a substantial reduction in A and g_s (Dionisio-Sese and Tobita, 2000), whereas leaf gas exchange was unaffected by high salinity (100–400 mM NaCl) in the halophyte, *Suaeda salsa* (Lu *et al.*, 2003).

The decrease in both A and C_i with declining ψ and the consequent decrease in g_s may indicate that stomata were imposing a larger limitation on A under water and saline stress conditions. Many studies have reported that both stomatal and non-stomatal components are responsible for a decrease in A (Tezara *et al.*, 1999; Lawlor, 2002). In the present study, in treatments WD and SI, L_s remained nearly constant after an initial increase a few days from the onset of treatments, whereas L_m increased to 80 % (WD) and 40 % (SS), suggesting that, as stress increased, metabolic regulation of photosynthesis became more important than stomatal closure.

The decreased CE and A_{sat} suggest loss of Rubisco activity with decreasing RWC and ψ. The amount and specific activity of Rubisco and the availability of RuBP affect CE and thus A. Conclusions relating to stomatal and non-stomatal limitations of A based on A/C_i curves may, in some cases, be misleading due to the erroneous calculation of C_i because of stomatal patchiness (Downton *et al.*, 1988; Terashima *et al.*, 1988) or to an increase in cuticular conductance to water vapour but not to CO_2 (Boyer *et al.*, 1997). Lawlor and Cornic (2002) attribute less importance to stomatal patchiness and more to the decrease in metabolism caused by a lowering of RWC. Little or no effect of patchy stomatal conductance on A measured at a given C_i has been reported (Cheeseman, 1991), and patchy stomatal distributions did not produce large differences in A compared with conditions of homogenous g_s (Buckley *et al.*, 1997). Patchiness has been shown to occur in heterobaric leaves, i.e. leaves in which the mesophyll continuity is interrupted by vascular bundles spanning the entire cross-section, but not in homobaric leaves (Terashima *et al.*, 1988), the latter being the case in *L. nodosum.*

As for the influence of cuticular transpiration on C_i, in the present study it may be negligible since g_s was lower than 30 mmol m^{-2} s^{-1} only in severely water-stressed leaves; this value is the lower limit, shown by Flexas *et al.* (2002) in water-stressed grapevine, of equality between C_i normally calculated (Farquhar *et al.*, 1980) and that corrected for cuticular transpiration (Boyer *et al.*, 1997). Also, Buckley *et al.* (1997) found that values of C_i became less reliable only when g_s was lower than 30 mmol m^{-2} s^{-1}.

Salinity reduced A of spinach leaves through a combined reduction in stomatal and mesophyll conductance. The reduction in the latter was attributed to a decrease in mesophyll intercellular spaces in thinner leaves, highlighting that a decrease in mesophyll conductance may be physical, rather than biochemical (Delfine *et al.*, 1998). However, it has recently been demonstrated in salt-stressed olive that changes observed in the parameters of A/C_i curves between control and stressed plants were due to a decrease in mesophyll conductance to CO_2 (Centritto *et al.*, 2003).

The relationship between A and g_s for all treatments was a straight line of zero intercept, and the C_i under any stress treatment was nearly constant with an average $C_i/C_a = 0{\cdot}73$, which indicates that photosynthetic rate was co-limited by stomatal and non-stomatal factors (Schulze and Hall, 1982; Lawlor, 2002) under drought or salinity.

In *L. nodosum*, both apparent quantum yield and light-saturated A decreased with increasing water stress, similar to observations in the desert shrub *Encelia farinosa* (Ehleringer and Cook, 1984), and also with salinity. This suggests that either light harvesting or electron transport were affected by these stresses, or that enzymatic processes were responsible for decreasing CO_2 fixation, or all of these factors together. F_v/F_m was not affected by water deficit, in agreement with other studies (Tezara *et al.*, 1999; Lawlor and Cornic, 2002), or by salt stress, as previously reported by Dionisio-Sese and Tobita (2000) and Lu *et al.* (2003), suggesting that PSII activity is very resistant to these stresses. Also, q_P at steady-state photosynthesis was hardly affected by water deficit in sunflower (Scheuermann *et al.*, 1991) and was insensitive to water deficit in wheat (Biehler and Fock, 1993, 1996). However, the lower ϕ_{PSII} of stressed plants was associated with lower q_P, i.e. a higher reduction state of primary acceptors (the QA pool), than in control plants. In barley, salinity induced only small decreases in ϕ_{PSII} at midday steady-state photosynthesis, indicating that the photosynthetic electron transport was little affected by salinity (Belkhodja *et al.*, 1999). In *S. salsa*, F_v/F_m as well as ϕ_{PSII} were insensitive to salinity (Lu *et al.*, 2003). The higher light-saturated values of A in SS and SI plants than in WD plants after 21 d of treatment may have been the product of a slower reduction in J of saline-stressed plants. In contrast, J was reduced by approx. 40 % in maize leaves subjected to water deficit (Scheuermann *et al.*, 1991).

The reduction in ϕ_{PSII} and J due to water deficit, as well as salt stress, was lower than the decrease in A, possibly due to higher photorespiration at low ψ, as suggested by Lawlor and Cornic (2002). This is supported by the observation that the CO_2 compensation point in WD and SS plants was twice and half as high, respectively, than in their controls.

Water and saline stress increased q_N in plants of *L. nodosum*, indicating that a greater proportion of the energy was thermally dissipated, thus accounting for the apparent down-regulation of PSII and supporting the protective role of the non-photochemical quenching against photoinhibition. In contrast, q_N was unaffected by water deficit in wheat (Biehler and Fock, 1993), and salinity in salt-tolerant rice cultivars (Dionisio-Sese and Tobita, 2000) and in *S. salsa* (Lu *et al.*, 2003), but increased with salinity in salt-sensitive rice cultivars.

The observed reductions in ϕ_{PSII}, J, q_P and CE may partly explain the increase in L_m and the occurrence of co-limitation of photosynthesis in plants of *L. nodosum* under drought or salinity. The decrease in J of *L. nodosum* may have contributed to the increase in L_m through a diminution in ATP and/or RuBP contents (Tezara *et al.*, 1999; Lawlor and Cornic, 2002) since in A/C_i curves, A_{sat} equates to the maximum rate of RuBP regeneration and maximum J (Farquhar *et al.*, 1980). Increased L_m under stress may also be caused by decreased activity of some Calvin cycle enzymes (e.g. reduction in Rubisco activity and/or amount, which would be seen as a decrease in CE) and/or decreased mesophyll conductance to CO_2 (Flexas *et al.*, 2002; Centritto *et al.*, 2003).

It is concluded that, in plants of *L. nodosum* subjected to water and saline stress, photosynthesis is co-limited by stomatal and non-stomatal factors and that these stresses do not cause chronic photoinhibition. Co-limitation would operate as a mechanism that optimizes water use and resource allocation when carbon acquisition through almost closed stomata is impaired. Plants of *L. nodosum* are capable of osmotic adjustment when subjected to salinity, and this apparently bears a relationship to the observed lower reduction of A in the SI treatment. In coastal habitats characterized by large temporal and spatial variations in rain, salinity and salt spray, osmotic adjustment, co-limitation of photosynthesis by stomatal and mesophyll factors and tolerance to high irradiance allow the plants to maintain a positive carbon balance and growth in a stressful environment.

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LITERATURE CITED

Ball MC, Farquhar GD. 1984. Photosynthetic and stomatal responses of two mangrove species, *Aegiceras corniculatum* and *Avicennia marina*, to long term salinity and humidity conditions. *Plant Physiology* **74**: 1–6.

Belkhodja R, Morales F, Abadía A, Medrano H, Abadía J. 1999. Effects of salinity on chlorophyll fluorescence and photosynthesis of barley (*Hordeum vulgare* L.) grown under a triple-line-source sprinkler system in the field. *Photosynthetica* **36**: 375–387.

Bethke P, Drew MC. 1992. Stomatal and non-stomatal components to inhibition of photosynthesis in leaves of *Capsicum annuum* during progressive exposure to NaCl salinity. *Plant Physiology* **99**: 219–226.

Biebl R, Kinzel H. 1965. Blattbau und Salzhaushalt von *Laguncularia racemosa* (L) Gaertn. f. und anderer Mangrovebäume auf Puerto Rico. *Österreichischen Botanischen Zeitschrift* **112**: 56–93.

Biehler K, Fock H. 1993. P700 contributes to the dissipation of excessive light energy in water stressed wheat. *Photosynthetica* **4**: 55–558.

Biehler K, Fock H. 1996. Evidence for the contribution of the Mehler-peroxidase reaction in dissipating excess electrons in drought-stressed wheat. *Plant Physiology* **112**: 265–272.

Bongi G, Loreto F. 1989. Gas exchange properties of salt-stressed olive (*Olea europea* L.) leaves. *Plant Physiology* **90**: 1408–1416.

Boyer JS, Wong SC, Farquhar GD. 1997. CO_2 and water vapour exchange across leaf cuticle (epidermis) at various water potentials. *Plant Physiology* **114**: 185–191.

Bruinsma J. 1963. The quantitative analysis of chlorophylls a and b in plant extracts. *Photochemistry and Photobiology* **2**: 241–249.

Buckley TN, Farquhar GD, Mott KA. 1997. Qualitative effects of patchy stomatal conductance distribution features on gas-exchange calculations. *Plant, Cell and Environment* **20**: 867–880.

Centritto M, Loreto F, Chartzoulakis K. 2003. The use of low [CO_2] to estimate diffusional and non-diffusional limitations of photosynthetic capacity of salt-stressed olive saplings. *Plant, Cell and Environment* **26**: 585–594.

Cheeseman JM. 1991. PATCHY: simulation and visualizing the effects of stomatal patchiness on photosynthetic CO_2 exchange studies. *Plant, Cell and Environment* **14**: 593–599.

Cornic G. 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture – not by affecting ATP synthesis. *Trends in Plant Sciences* **5**: 187–188.

Delfine S, Alvino A, Zacchini M, Loreto F. 1998. Consequences of salt stress on conductance to CO_2 diffusion, Rubisco characteristics and anatomy of spinach leaves. *Australian Journal of Plant Physiology* **25**: 395–402.

Dionisio-Sese ML, Tobita S. 2000. Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. *Journal of Plant Physiology* **157**: 54–58.

Downton WJ, Loveys BR, Grant WJ. 1988. Non-uniform stomatal closure induced by water stress putative non-stomatal inhibition of photosynthesis. *New Phytologist* **110**: 503–509.

Ehleringer J. 1983. Ecophysiology of *Amaranthus palmeri*, a sonoran desert summer annual. *Oecologia* **57**: 107–112.

Ehleringer J, Cook C. 1984. Photosynthesis in *Encelia farinosa* Gray in response to decreasing leaf water potential. *Plant Physiology* **75**: 688–693.

Epron D, Dreyer E, Breda N. 1992. Photosynthesis of oak trees (*Quercus petraea* (Matt.) Liebl.) during drought under field conditions: diurnal course of net CO_2 assimilation and photochemical efficiency of photosystem II. *Plant, Cell and Environment* **15**: 809–820.

Farquhar GD, Sharkey TD. 1982. Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology* **33**: 317–345.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. *Planta* **149**:78–90.

Flexas J, Bota J, Escalona JM, Sampol B, Medrano H. 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Functional Plant Biology* **29**: 461–471.

Genty B, Briantais JM, Baker NR. 1989. The relationships between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**: 87–92.

Greenway H, Munns R. 1980. Mechanisms of salt tolerance in non-halophytes. *Annual Review of Plant Physiology* **22**: 131–160.

Herrera A, Tezara W, Urich R, Montes G, Cuberos M. 1994. Mechanism of drought tolerance in the C3 deciduous shrub, *Ipomoea carnea*. *Ecotrópicos* **7**: 35–47.

Jacob J, Lawlor DW. 1991. Stomatal and mesophyll limitations of photosynthesis in phosphate deficient sunflower, maize and wheat plants. *Journal of Experimental Botany* **42**: 1003–1011.

Krall JP, Edwards GE. 1992. Relationship between photosystem II activity and CO_2 fixation in leaves. *Physiologia Plantarum* **86**: 180–187.

Krause GH, Weis E. 1991. Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**: 313–349.

Lawlor DW. 1995. The effects of water deficit on photosynthesis. In: Smirnoff N, ed. *Environment and plant metabolism.* Bios Scientific Publishers, Oxford: 129–161.

Lawlor DW. 2002. Limitation to photosynthesis in water stressed leaves: stomata versus metabolism and the role of ATP. *Annals of Botany* **89**: 871–885.

Lawlor DW, Cornic G. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell and Environment* **25**: 275–294.

Lu C, Qiu N, Wang B, Zhang J. 2003. Salinity treatment shows no effects on photosystem II photochemistry, but increases the resistance of photosystem II to heat stress in halophyte *Suaeda salsa. Journal of Experimental Botany* **54**: 851–860.

Morgan JM. 1984. Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology* **35**: 299–319.

Powles SB. 1984. Photoinhibition of photosynthesis induced by visible light. *Annual Review of Plant Physiology* **35**: 15–44.

Scheuermann R, Biehler K, Stuhlfauth T, Fock HP. 1991. Simultaneous gas exchange and fluorescence measurements indicate differences in the response of sunflower, bean and maize to water stress. *Photosynthesis Research* **27**: 189–197.

Schulze E-D, Hall AE. 1982. Stomatal responses, water loss and CO_2 assimilation rates of plants in contrasting environments. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, eds. *Physiological Plant Ecology. II. Water Relations and Carbon Assimilation.* Berlin: Springer-Verlag, 181–230.

Seemann JR, Critchley C. 1985. Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. *Planta* **164**: 151–162.

Shabala SN, Shabala, SI, Martynenko AI, Babourina O, Newman IA. 1998. Salinity effect on bioelectric activity, growth, Na^+ accumulation and chlorophyll fluorescence of maize leaves: a comparative survey and prospects for screening. *Australian Journal of Plant Physiology* **25**: 609–616.

Short DC, Colmer TD. 1999. Salt tolerance in the halophyte *Halosarcia pergranulata* subs. *pergranulata*. *Annals of Botany* **83**: 207–213.

Soussi M, Ocaña A, Lluch C. 1998. Effects of salt stress on growth, photosynthesis and nitrogen fixation in chick-pea (*Cicer arietinum* L.). *Journal of Experimental Botany* **49**: 1329–1337.

Suárez N, Sobrado MA. 2000. Adjustments in leaf water relations of mangrove (*Avicennia germinans*) seedlings grown in a salinity gradient. *Tree Physiology* **20**: 277–282.

Tattini M, Lombardini L, Gucci R. 1997. The effect of NaCl stress and relief on gas exchange properties of two olive cultivars differing in tolerance to salinity. *Plant and Soil* **197**: 87–93.

Terashima I, Wong S-C, Osmond CB, Farquhar GD. 1988. Characterisation of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. *Plant and Cell Physiology* **29**: 385–394.

Tezara W, Fernández MD, Donoso C, Herrera A. 1998. Seasonal changes in photosynthesis and stomatal conductance of five species from a semiarid ecosystem. *Photosynthetica* **35**: 399–410.

Tezara W, Mitchell VJ, Driscoll SD, Lawlor DW. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* **401**: 914–917.

Turner NC, Jones MM. 1980. Turgor maintenance by osmotic adjustment: a review and evaluation. In Turner N, Kramer P, eds. *Adaptation of plants to water and high temperature stress*. New York: John Wiley & Sons, 87–103.

Weis E., Berry J.A. 1987. Quantum efficiency of photosystem II in relation to energy-dependent quenching of chlorophyll fluorescence. *Biochemica et Biophysica Acta* **894**: 198–208.