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# Growth and Photosynthetic Responses to Salinity of the Salt-marsh Shrub *Atriplex portulacoides*

SUSANA REDONDO-GÓMEZ[1,*], ENRIQUE MATEOS-NARANJO[1], ANTHONY J. DAVY[2], FRANCISCO FERNÁNDEZ-MUÑOZ[1], ELOY M. CASTELLANOS[3], TERESA LUQUE[1] and M. ENRIQUE FIGUEROA[1]

[1]*Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Apartado 1095, 41080–Sevilla, Spain,* [2]*Centre for Ecology, Evolution and Conservation, School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK and* [3]*Departamento de Biología Ambiental y Salud Pública, Facultad de Ciencias Experimentales, Universidad de Huelva, 21071–Huelva, Spain*

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• *Background and Aims Atriplex* (*Halimione*) *portulacoides* is a halophytic, $C_3$ shrub. It is virtually confined to coastal salt marshes, where it often dominates the vegetation. The aim of this study was to investigate its growth responses to salinity and the extent to which these could be explained by photosynthetic physiology.
• *Methods* The responses of young plants to salinity in the range 0–700 mol $m^{-3}$ NaCl were investigated in a glasshouse experiment. The performance of plants was examined using classical growth analysis, measurements of gas exchange (infrared gas analysis), determination of chlorophyll fluorescence characteristics (modulated fluorimeter) and photosynthetic pigment concentrations; total ash, sodium, potassium and nitrogen concentrations, and relative water content were also determined.
• *Key Results* Plants accumulated $Na^+$ approximately in proportion to external salinity. Salt stimulated growth up to an external concentration of 200 mol $m^{-3}$ NaCl and some growth was maintained at higher salinities. The main determinant of growth response to salinity was unit leaf rate. This was itself reflected in rates of $CO_2$ assimilation, which were not affected by 200 mol $m^{-3}$ but were reduced at higher salinities. Reductions in net photosynthetic rate could be accounted for largely by lower stomatal conductance and intercellular $CO_2$ concentration. Apart from possible effects of osmotic shock at the beginning of the experiment, salinity did not have any adverse effect on photosystem II (PSII). Neither the quantum efficiency of PSII ($\Phi_{PSII}$) nor the chlorophyll fluorescence ratio ($F_v/F_m$) were reduced by salinity, and lower mid-day values recovered by dawn. Mid-day $F_v/F_m$ was in fact depressed more at low external sodium concentration, by the end of the experiment.
• *Conclusions* The growth responses of the hygro-halophyte *A. portulacoides* to salinity appear largely to depend on changes in its rate of photosynthetic gas exchange. Photosynthesis appears to be limited mainly through stomatal conductance and hence intercellular $CO_2$ concentration, rather than by effects on PSII; moderate salinity might stimulate carboxylation capacity. This is in contrast to more extreme halophytes, for which an ability to maintain leaf area can partially offset declining rates of carbon assimilation at high salinity.

**Key words:** *Atriplex portulacoides*, chlorophyll fluorescence, growth rate, halophyte, leaf area, photosynthesis, photosystem II, salt tolerance, salt marsh, stomatal conductance.

## INTRODUCTION

*Atriplex* (=*Halimione*) *portulacoides* (L.) Aellen is a perennial, shrubby $C_3$ halophyte that is widespread on salt marshes around the coasts of Europe, North Africa and South-West Asia. It is frequently the physiognomic dominant on well-drained and upper marshes, often fringing channels and pools that are flooded at full tide (Chapman, 1950). Consequently, *A. portulacoides* typically experiences salinities oscillating around that of seawater, although its habitats can become markedly hypo- or hypersaline (Carvalho *et al.*, 2001). *Atriplex portulacoides* has been shown experimentally to maintain growth over a range of salinities, particularly under high nitrate availability (Jensen, 1985). It is also well known that, like other halophytes, it accumulates high concentrations of salts in its leaves (Jensen, 1985; Freitas and Breckle, 1992), notwithstanding the ability to remove salt through epidermal bladders on both surfaces of its leaves (Baumeister and Kloos, 1974; Freitas and Breckle, 1992). Salt accumulation and removal, along with an ability to accumulate quaternary ammonium compounds as compatible osmotica (Stewart *et al.*, 1979), appear to be important components of its osmoregulation (Rozema *et al.*, 1985). Despite the evident salt tolerance of *A. portulacoides*, it is far from clear how photosynthesis and underlying growth processes respond to salinity. Light-induced fixation of $CO_2$ in cell cultures has been reported to increase with the addition of NaCl in the culture medium and, in salt-adapted cultures, $CO_2$-fixation was not impaired by even higher concentrations of NaCl in the assay medium (Plaut *et al.*, 1991). However, Lorenzen *et al.* (1990) found the fastest rates of net photosynthesis in plants grown in a nutrient solution that was hyposaline with respect to seawater.

Exposure of halophytes to increasing salinity may result in partial closure of the stomata, in order to limit both

* For correspondence. E-mail susana@us.es

transpiration and the transport of salts to the leaves (Véry *et al.*, 1998). The resulting lower $CO_2$ diffusion rates limit photosynthetic capacity. This, in turn, can lead to over-reduction of the reaction centres of photosystem II (PSII), if the plant is unable to dissipate excess energy otherwise, and hence cause damage to the photosynthetic apparatus (Demmig-Adams and Adams, 1992). However, some halophytes have shown no evidence of such photoinhibition in response to salinity stress (Qiu *et al.*, 2003; Redondo-Gómez *et al.*, 2006). Salinity may potentially affect many different aspects of growth, including those mediated through water stress and those more specific to NaCl (Munns, 2002).

The aim of the present study was to elucidate the growth and photosynthetic responses of *A. portulacoides* to salinity. The specific objectives were to: (1) analyse the growth of plants in experimental salinity treatments ranging from 0 to 700 mol $m^{-3}$ NaCl; (2) investigate the extent to which growth responses could be explained by changes in photosynthetic gas exchange and impairment of the integrity or function of PSII; and (3) examine possible role of concentrations of mineral matter (ash), sodium, potassium and nitrogen accumulated in response to increasing external NaCl in explaining effects on growth.

## MATERIAL AND METHODS

### *Plant material and stress treatments*

In mid-October 2004 ripe achenes were collected from *Atriplex portulacoides* growing in a well-drained and accreting lagoon (the 'Laguna de Don Claudio' of Castellanos *et al.*, 1994) at Odiel Marshes (37°15′N, 6°58′W; south-west Iberian Peninsula). Achenes were placed in a germinator (ASL Aparatos Científicos M-92004, Spain), and subjected to an alternating diurnal regime of 10 h of light (photon flux rate, 400–700 nm, 35 μmol $m^{-2}$ $s^{-1}$) at 20 °C and 14 h of darkness (5 °C), for 30 d. This temperature regime was chosen to mimic the autumn conditions in the Odiel Marshes when this species germinates. Seedlings were planted in individual plastic pots filled with pearlite and placed in a glasshouse (37 °23′N, 5 °59′W; south-west Iberian Peninsula) with controlled temperature of 21–25 °C, 40–60 % relative humidity and natural daylight (maximum light flux: 1000 μmol $m^{-2}$ $s^{-1}$). Pots were carefully irrigated with 20 % modified Hoagland's solution (Hoagland and Arnon, 1938) as necessary.

In spring 2005, when seedlings were between 10 and 15 cm in height (after 3 months), the pots were allocated to five NaCl treatments in shallow trays (15 pots per tray, with one tray per salinity treatment): 0, 20, 200, 400 and 700 mol $m^{-3}$, in the same glasshouse, and their growth examined for a period of 60 d. Salinity treatments were established by combining 20 % Hoagland's solution and NaCl of the appropriate concentration. The salt concentrations were increased in two stages.

At the beginning of the experiment, 3 L of the appropriate solution was placed in each of the trays to a depth of 1 cm. During the experiment, the levels in the trays were monitored and they were topped up to the marked level with 20 % Hoagland's solution (without NaCl) whenever necessary to maintain the salt concentration. In addition, the entire solution (including NaCl) was changed every 2 weeks.

### *Growth*

At the beginning and the end of the experiment, five plants from each treatment were dried at 80 °C for 48 h and weighed. Plants used for dry mass measurement had not been used for other measurements, in order to avoid damage that might have affected the final biomass. Dried, ground samples were ignited in lidded, ceramic crucibles and ash weights were recorded; the furnace temperature was raised slowly over 6 h to 550 °C and this temperature was maintained for a further 8 h. At the end of the experiment, leaves of five plants were selected at random from each treatment and their area measured by superimposition on millimetre-squared paper. The dry mass of these leaves was determined after drying at 80 °C for 48 h. In addition, circular leaf sections were taken from other plants in each treatment (see measurement of relative water content), dried at 80 °C for 48 h and weighed in order to determine the relationship between the area and the dry mass for *A. portulacoides* ($n = 50$ per treatment).

Classical growth analysis (Evans, 1972) was carried out with ash-free dry masses. The relative growth rate in whole plant dry mass (RGR; see Table 1 for abbreviations) was calculated and partitioned into its three components, unit leaf rate (ULR), specific leaf area (SLA) and leaf mass fraction (LMF, i.e. RGR = ULR × SLA × LMF), using the software tool of Hunt *et al.* (2002):

$$(1/W)/(\mathrm{d}W/\mathrm{d}t) = (1/L_A)/(\mathrm{d}W/\mathrm{d}t) \times L_A/L_W \times L_W/W \quad (1)$$

where $t$ is time, $W$ is total dry mass per plant, $L_A$ is total leaf area per plant and $L_W$ is total leaf dry mass per plant.

TABLE 1. *List of abbreviations used in the text*

| Abbreviation | Definition |
|---|---|
| $A$ | Net photosynthetic rate |
| Chl *a* | Chlorophyll *a* |
| Chl *b* | Chlorophyll *b* |
| C$x + c$ | Carotenoids |
| $F_0$ | Minimal fluorescence level in the dark-adapted state |
| $F_m$ | Maximal fluorescence level in the dark-adapted state |
| $F_s$ | Steady-state fluorescence yield |
| $F_v$ | Variable fluorescence level in the dark-adapted state |
| $F_v/F_m$ | Maximum quantum efficiency of PSII photochemistry |
| $\Phi_{PSII}$ | Quantum efficiency of PSII |
| $g_s$ | Stomatal conductance |
| LMF | Leaf mass fraction |
| NPQ | Non-photochemical quenching |
| RGR | Relative growth rate |
| SLA | Specific leaf area |
| ULR | Unit leaf rate |

*Determination of sodium, potassium and nitrogen*

At the end of the experiment, leaf, stem and root samples were dried at 80 °C for 48 h and ground. Then, 0·5-g samples were digested with 6 mL $HNO_3$, 0·5 mL HF and 1 mL $H_2O_2$. $Na^+$ and $K^+$ were measured by inductively coupled plasma (ICP) spectroscopy (ARL-Fison 3410, USA). Total N concentration was determined for undigested dry samples with an elemental analyser (Leco CHNS-932, Spain).

*Leaf relative water content*

Ten circular leaf sections of 7 mm diameter were collected for each pot ($n = 5$ per treatment), except for the 700 mol $m^{-3}$ treatment, where smaller leaves limited diameter to 5 mm. After 2 months of salinity treatment, relative water content (RWC) was calculated as

$$\mathrm{RWC} = (FW - DW)/(TW - DW) \times 100 \qquad (2)$$

where *FW* is the fresh mass of the leaf section, *TW* is the turgid mass after re-hydrating the leaf section in distilled water for 24 h, and *DW* is the dry mass after oven-drying at 80 °C for 48 h (Medrano and Flexas, 2004).

*Gas exchange*

Gas exchange measurements were taken on random, fully expanded leaves by using an infrared gas analyser in an open system (LCi; Analytical Development Company Ltd, Hoddesdon, UK) after 6, 30 and 60 d of treatment. Net photosynthetic rate ($A$), intercellular $CO_2$ concentration ($C_i$) and stomatal conductance to $CO_2$ ($g_s$) were determined at an ambient $CO_2$ concentration of 360 μmol $mol^{-1}$, temperature of 25/28 °C, 50 ± 5 % relative humidity and a photon flux density of 1000 μmol $m^{-2}$ $s^{-1}$. $A$, $C_i$ and $g_s$ were calculated using the standard formulae of von Caemmerer and Farquhar (1981). Photosynthetic area was calculated by superimposing the surface of each leaf over a millimetre-square paper. The water-use efficiency (WUE) was calculated as the ratio between $A$ and transpiration rate [mmol ($CO_2$ assimilated) $mol^{-1}$ ($H_2O$ transpired)].

*Chlorophyll fluorescence*

Chlorophyll fluorescence was measured in random, fully expanded leaves using a portable modulated fluorimeter (FMS-2; Hansatech Instruments Ltd, Kings Lynn, UK) after 6, 30 and 60 d of treatment. Measurements were made on ten plants from each of the five salinity treatments. Light- and dark-adapted fluorescence parameters were measured at dawn (stable, 50 μmol $m^{-2}$ $s^{-1}$ ambient light) and at mid-day (1600 μmol $m^{-2}$ $s^{-1}$) to investigate whether salt concentration affected the sensitivity of plants to photoinhibition (Qiu *et al.*, 2003).

Plants were dark-adapted for 30 min by using leaf-clips designed for this purpose. The minimal fluorescence level in the dark-adapted state ($F_0$; see Table 1 for abbreviations) was measured by using a modulated pulse (<0·05 μmol $m^{-2}$ $s^{-1}$ for 1·8 μs) too small to induce significant physiological changes in the plant (Schreiber *et al.*, 1986). The data stored were averages taken over a 1·6 second period. Maximal fluorescence in this state ($F_m$) was measured after applying a saturating actinic light pulse of 15 000 μmol $m^{-2}$ $s^{-1}$ for 0·7 s (Bolhàr-Nordenkampf and Öquist, 1993). The value of $F_m$ was recorded as the highest average of two consecutive points. Values of the variable fluorescence ($F_v = F_m - F_0$) and maximum quantum efficiency of PSII photochemistry ($F_v/F_m$) were calculated from $F_0$ and $F_m$. This ratio of variable to maximal fluorescence correlates with the number of functional PSII reaction centres and dark-adapted values of $F_v/F_m$ can be used to quantify photoinhibition (Maxwell and Johnson, 2000).

The same leaf section of each plant was used to measure light-adapted parameters. Steady-state fluorescence yield ($F_s$) was recorded after adapting plants to ambient light conditions for 30 min. A saturating actinic light pulse of 15 000 μmol $m^{-2}$ $s^{-1}$ for 0·7 s was then used to produce the maximum fluorescence yield ($F_m'$) by temporarily inhibiting PSII photochemistry.

Using fluorescence parameters determined in both light- and dark-adapted states, the following were calculated: quantum efficiency of PSII [$\Phi_{PSII} = (F_m' - F_s)/F_m'$] (Genty *et al.*, 1989) and non-photochemical quenching [$NPQ = (F_m - F_m')/F_m'$; Schreiber *et al.*, 1986].

*Photosynthetic pigments*

At the end of the experiment period, photosynthetic pigments in fully expanded leaves (a randomly selected mixture of old and young leaves) from each treatment were extracted using 0·05 g of fresh material in 10 mL of 80 % aqueous acetone. After filtering, 1 mL of the suspension was diluted with a further 2 mL of 80 % aqueous acetone, and chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids ($Cx + c$) contents were determined with a Hitachi U-2001 spectrophotometer (Hitachi Ltd, Japan), using three wavelengths (663·2, 646·8 and 470·0 nm). Concentrations of pigments [μg g fresh weight (f. wt)$^{-1}$] were obtained by calculation, using the method of Lichtenthaler (1987).

*Statistical analysis*

Statistical analysis was carried out using Statistica v. 6·0 (Statsoft Inc.). Pearson coefficients were calculated to assess correlation between different variables. Data were analysed using one- and two-way analysis of variance ($F$-tests). Data were first tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variance with the Brown–Forsythe test. Significant test results were followed by Tukey tests for identification of important contrasts (Day and Quinn, 1989). Differences between measurements of fluorescence at dawn and mid-day were compared by Student's test ($t$-test).

## RESULTS

### *Growth analysis*

Mean relative growth rate was stimulated by moderate external salinity, reaching a peak at 200 mol $m^{-3}$ NaCl that was nearly double the value in the absence of NaCl (Fig. 1A). Further increase in salinity caused a reduction in RGR to a very low value at 700 mol $m^{-3}$ NaCl. The same pattern of response was followed very closely by unit leaf rate (Fig. 1B). The other components of RGR showed less clear trends: specific leaf area was somewhat higher at 20 and 200 mmol $m^{-3}$ NaCl than at higher and lower salinities (Fig. 1C), whereas leaf mass fraction was greater at the highest salinities (Fig. 1D).

Total leaf area after 60 d of salinity treatment was also greatest in the 200 mmol $m^{-3}$ NaCl treatment (Fig. 1E) and was highly correlated with mean RGR during the experiment ($r = 0{\cdot}94$, $P < 0{\cdot}01$). Individual leaf area did not differ significantly between salinity treatments, except for 700 mol $m^{-3}$ where it was smaller (ANOVA, Tukey test, $P < 0{\cdot}0001$; Fig. 1F); hence, the differences in total leaf area between salinity treatments were due mainly to differences in the numbers of leaves per plant.

### *Sodium, potassium and nitrogen concentrations*

There was a marked increase in the mineral (ash) fraction of both the leaves and the roots (but not the stems) with increasing external NaCl concentration (Fig. 2A).

By the end of the experiment, tissue sodium concentrations were greater in the leaves than in stems or roots (ANOVA, Tukey test, $P < 0{\cdot}0001$), and increased markedly with external NaCl concentration (Fig. 2B). By contrast, leaf tissue potassium concentrations were highest in the non-saline control and dropped sharply when exposed to 20 mol $m^{-3}$ NaCl; leaf $K^+$ concentration did not respond to further increases in external NaCl concentrations, however (Fig. 2C). Stem and root $K^+$ concentrations were little affected by external NaCl concentration.

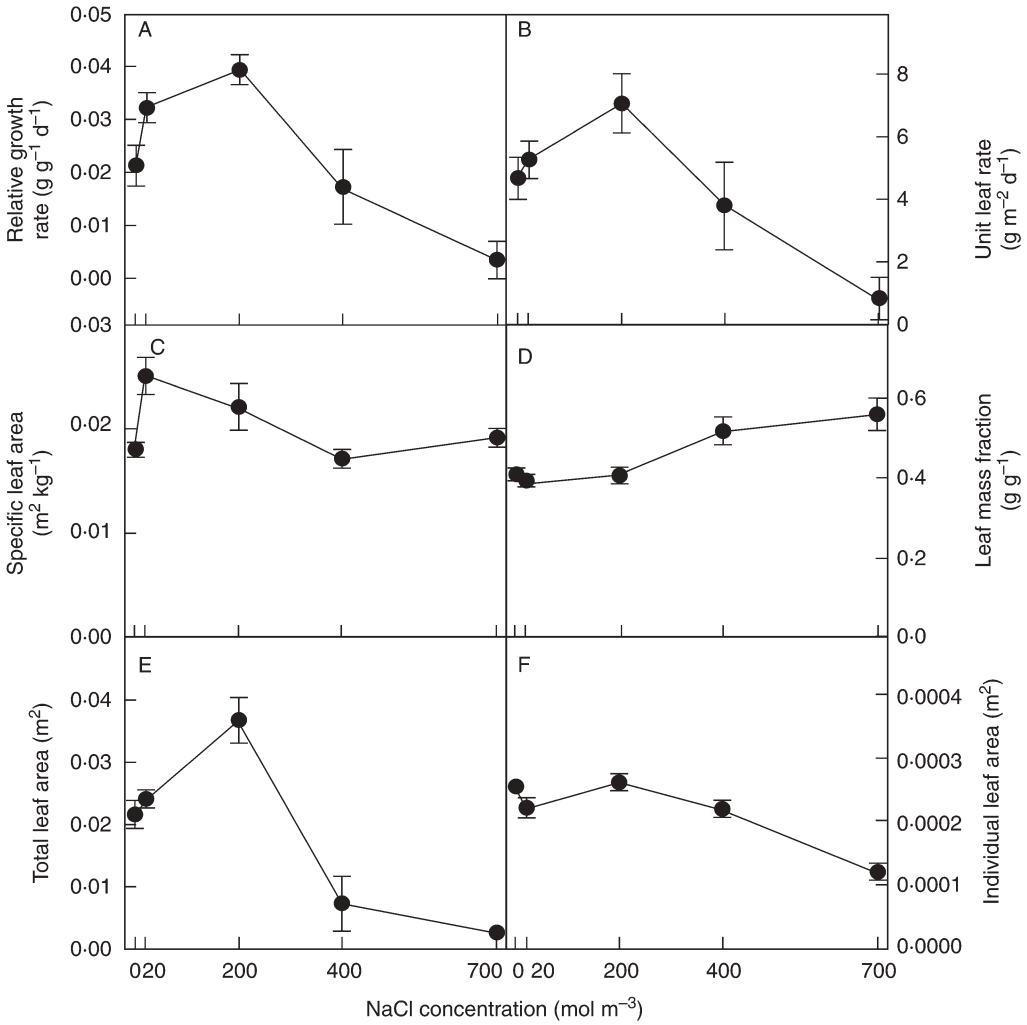


FIG. 1. Growth analysis of *Atriplex portulacoides* in response to treatment with a range of NaCl concentrations over 60 d. Relative growth rate (A), unit leaf rate (B), specific leaf area (C), leaf mass fraction (D), total leaf area (E) and individual leaf area (F). Values represent mean ± s.e., $n = 5$ ($n = 10$ for individual leaf area). The analysis was carried out on an ash-free dry mass basis.

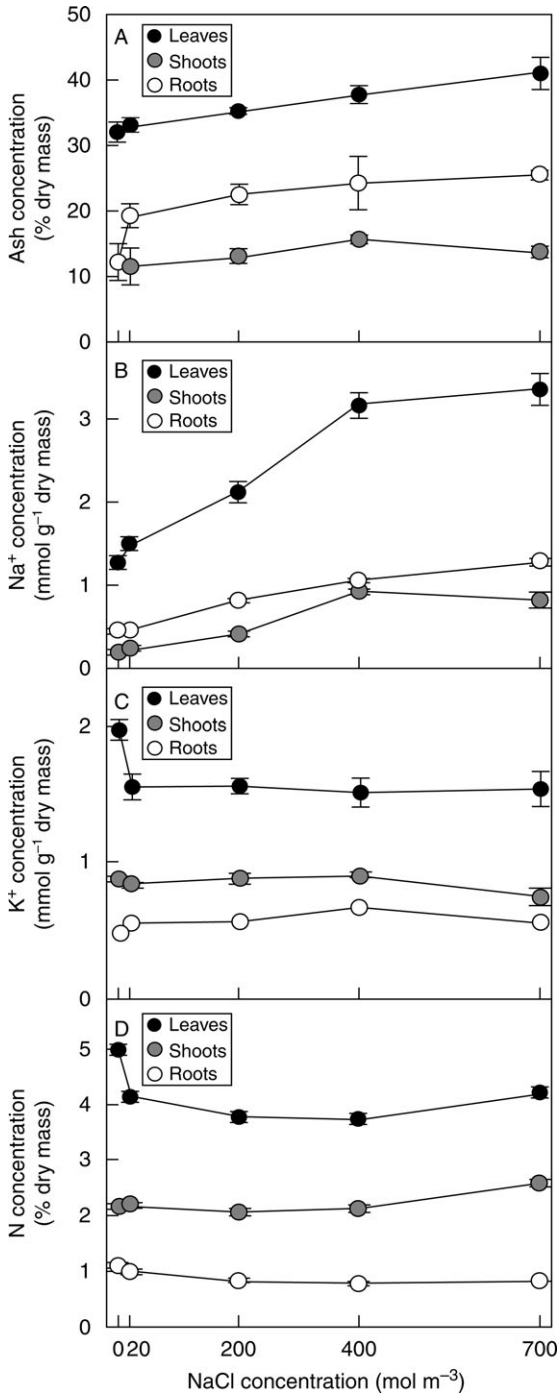


FIG. 2. Ash (A), total sodium (B), total potassium (C) and total nitrogen (D) concentrations for leaves, stems and roots of *Atriplex portulacoides* in response to treatment with a range of NaCl concentrations after 60 d. Values represent mean ± s.e., $n = 6$.

Total nitrogen concentrations were considerably higher in the stems than in the roots, and those in the leaves were much higher again than in the stems (Fig. 2D). Leaf N concentration was by far highest in the non-saline control; stem concentration tended to increase with external salinity, and root N concentration tended to decline with increasing salinity.

*Gas exchange*

Net photosynthetic rate ($A$) declined significantly with increasing external salinity after 6 d of treatment (Fig. 3A). By 30 and 60 d, there was a clear peak in $A$ at 200 mol $m^{-3}$ NaCl, before it declined with further increases in salinity (Fig. 3B, C). The values recorded at 200 mol $m^{-3}$ NaCl after 30 and 60 d were significantly higher than at other salinities (ANOVA, Tukey test, $P < 0{\cdot}001$). There was a strong linear relationship between $A$ and URL after 60 d ($r = 0{\cdot}97$, $P < 0{\cdot}01$).

At each of the three measurement times, stomatal conductance ($g_s$) declined significantly with increasing external salinity (Fig. 3D–F). However, $g_s$ tended to increase during the course of the experiment, across the whole range of external salinity. Intercellular $CO_2$ concentration ($C_i$) responded differently to salinity at the early stage of the experiment than at later stages: it increased significantly with salinity after 6 d of treatment but declined significantly after 30 and 60 d (Fig. 3G–I). Water use efficiency (WUE) after 60 d of treatment ranged from $1{\cdot}47 \pm 0{\cdot}08$ (s.e.) to $2{\cdot}38 \pm 0{\cdot}18$ mmol $mol^{-1}$ and increased significantly with salinity ($r = 0{\cdot}93$, $P < 0{\cdot}05$).

*Leaf relative water content*

Although relative leaf water content after 2 months was somewhat lower in the non-saline control, it was consistent across the salinity treatments at approx. 60 % and showed no significant overall response to salinity.

*Chlorophyll fluorescence*

Values of $F_v/F_m$ at dawn were uniformly high (Fig. 4), with values varying around 0·85. $F_v/F_m$ was always lower at mid-day and the reductions resulted mainly from lower values of $F_m$ (data not presented) at mid-day than at dawn ($t$-test, $P < 0{\cdot}05$). Furthermore, the mid-day $F_v/F_m$ values were significantly higher after 60 d of treatment than after 6 or 30 d (ANOVA, Tukey test, $P < 0{\cdot}0001$), again because of different values of $F_m$. No differences were recorded at dawn.

There were no significant relationships between $F_v/F_m$ and external NaCl concentration, at 6 or 30 d of treatment. By 60 d, however, $F_v/F_m$ increased with salinity up to 200 mol $m^{-3}$ NaCl, at mid-day and at dawn, although the changes at dawn were very small (Fig. 4). The increase at mid-day resulted mainly from lower values of $F_0$ (ANOVA, Tukey test, $P < 0{\cdot}0001$; data not presented). There was a positive linear relationship between $F_v/F_m$ at mid-day and total sodium concentration ($r = 0{\cdot}88$, $P < 0{\cdot}05$) after 60 d.

The quantum efficiency of PSII ($\Phi_{PSII}$) at dawn did not show a significant relationship with salinity at any time, although dawn values were significantly higher than mid-day values ($t$-test, $P < 0{\cdot}05$; Fig. 5A). Only in the early stages of the experiment (6 d), mid-day $\Phi_{PSII}$ declined significantly with increasing external salinity ($r = -0{\cdot}45$, $P < 0{\cdot}01$; Fig. 5A); this was in fact due to a substantial reduction at 700 mol $m^{-3}$ NaCl that was accompanied

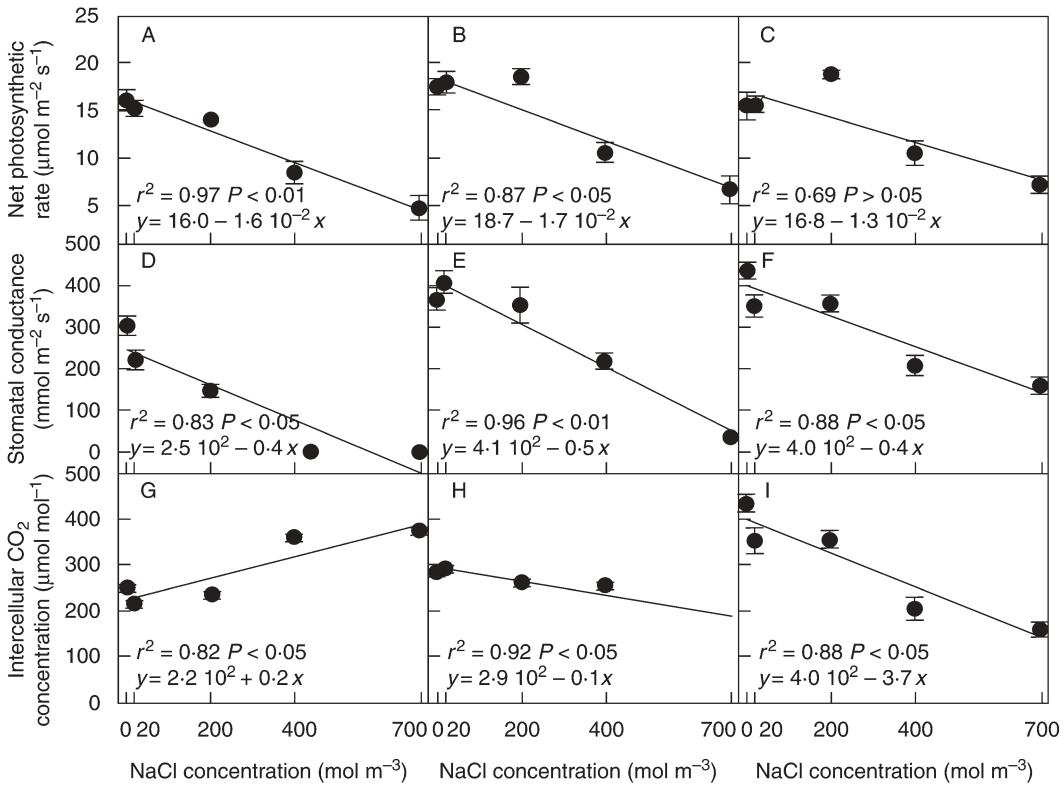


FIG. 3. Net photosynthetic rate, $A$ (A–C), stomatal conductance, $G_s$ (D–F) and intercellular $CO_2$ concentration, $C_i$ (G–I) in randomly selected, fully expanded leaves of *Atriplex portulacoides* in response to treatment with a range of NaCl concentrations after: 6 d (A, D, G); 30 d (B, E, H); and 60 d (C, F, I). Values represent mean ± s.e., $n = 10$.

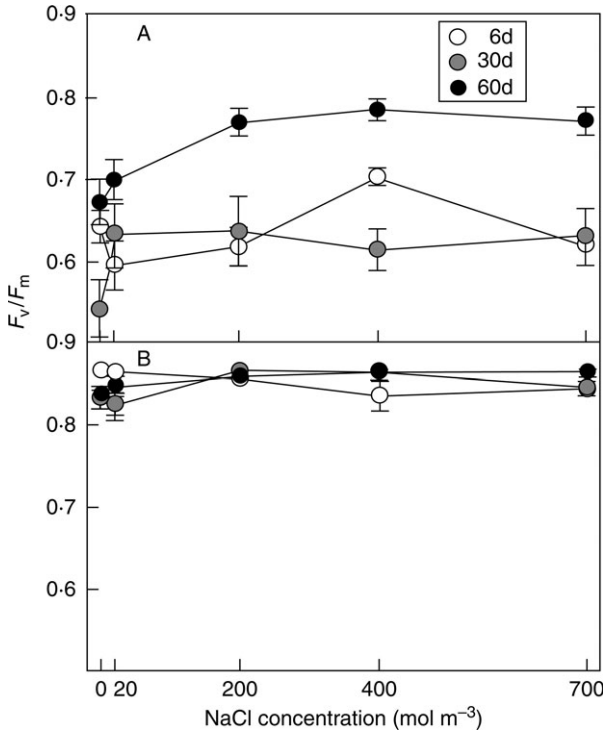


FIG. 4. Maximum quantum efficiency of PSII photochemistry ($F_v/F_m$) at mid-day (A) and at dawn (B) in randomly selected, fully expanded leaves of *Atriplex portulacoides* in response to treatment with a range of NaCl concentrations for 6, 30 and 60 d. Values represent mean ± s.e., $n = 10$.

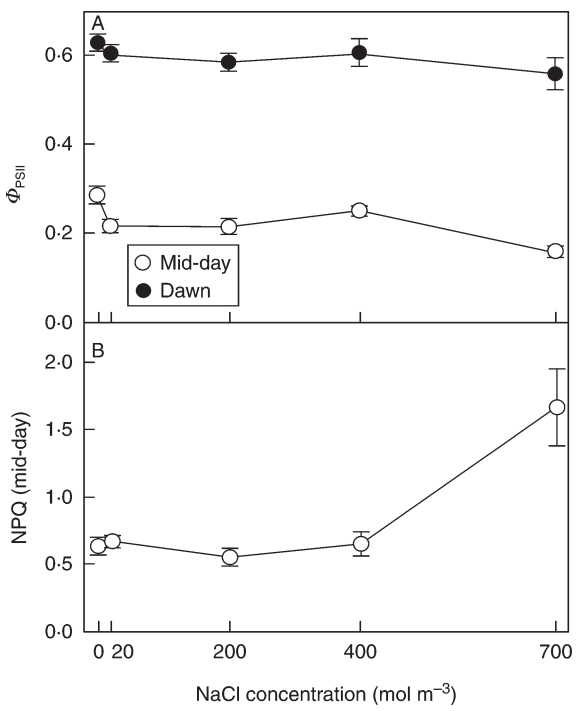


FIG. 5. Quantum efficiency of PSII (A) at mid-day and at dawn, and non-photochemical quenching (NPQ) at mid-day (B) in randomly selected, fully expanded leaves of *Atriplex portulacoides* in response to treatment with a range of NaCl concentrations for 6 d. Values represent mean ± s.e., $n = 10$.

by markedly increased non-photochemical quenching (Fig. 5B). There were no significant responses of $\Phi_{PSII}$ or NPQ at mid-day to external salinity after 30 or 60 d.

### *Photosynthetic pigment concentration*

Concentrations of Chl *a*, Chl *b* and C$x + c$ in leaf tissues were not affected by salinity treatment. Chl *a* ranged between $1{\cdot}27 \pm 0{\cdot}10$ and $1{\cdot}93 \pm 0{\cdot}07$, Chl *b* between $0{\cdot}50 \pm 0{\cdot}18$ and $0{\cdot}67 \pm 0{\cdot}03$, and C$x + c$ between $0{\cdot}42 \pm 0{\cdot}01$ and $0{\cdot}50 \pm 0{\cdot}02$ μg g f. wt$^{-1}$. Chl *a* values were positively correlated with $F_v/F_m$ after 60 d of treatment, both at mid-day and at dawn ($r = 0{\cdot}91$, $P < 0{\cdot}05$ and $r = 0{\cdot}88$, $P < 0{\cdot}05$, respectively).

## DISCUSSION

*Atriplex portulacoides* is highly tolerant of salinity. Growth was stimulated by an external salinity up to 200 mol m$^{-3}$ of NaCl and some growth was maintained even at 700 mol m$^{-3}$. This response was apparent as the RGR of ash-free dry mass, total leaf area and, by inference, the number of leaves produced. Enhanced growth at moderate salinities in this southern European population of *A. portulacoides* is consistent with results for Danish material, in which the fastest growth rate was in the range 85–170 mol m$^{-3}$ NaCl (Jensen, 1985). The progressive accumulation of $Na^+$ seen with increasing salinity treatment, particularly in the leaves, indicates the effective compartmentation of salt in the vacuoles that is a hallmark of halophytes (Munns, 2002). Jensen (1985) documented the accumulation of chloride ions under similar salinity treatments. Salt accumulation in our experiment was also manifested as the overall accumulation of mineral matter (ash); ash contents were consistent with those presented for *A. portulacoides* by Jensen (1985) and similar to those recorded for other *Atriplex* species (Ungar, 1996; Khan *et al.*, 2000*a*). The salt bladders of *A. portulacoides* (Osmond *et al.*, 1980; Freitas and Breckle, 1992) are functional for salt removal only in young leaves; most bladders from mature leaves are collapsed and ineffective (Baumeister and Kloos, 1974). The reduction in total potassium concentration when *A. portulacoides* was presented with an external supply of sodium is also characteristic of dicotyledonous halophytes and has been attributed to displacement of $K^+$ by $Na^+$. $K^+$ leakage from the root plasmalemma can occur as a result of $Ca^{2+}$ displacement by $Na^+$ (Cramer *et al.*, 1986). Furthermore, sodium uptake causes plasma membrane depolarization, leading to activation of outward-rectifying $K^+$ channels and a consequent $K^+$ loss (S. Shabala *et al.*, 2003; L. Shabala *et al.*, 2005). The unchanged concentration of $K^+$, in all organs, across the entire range of salinity treatments suggests remarkably efficient $K^+$ homeostasis in *A. portulacoides*. Interestingly, a degree of salt tolerance in the non-halophyte barley has recently been associated with the ability to retain $K^+$ at elevated salinity (Chen *et al.*, 2007). The reduction in total N concentration in our salinity treatments may reflect dilution in the tissues by increasing growth, although it is likely that at higher salinities an increasing fraction of this N would have been diverted to the production of glycine betaine as an osmolyte (Storey *et al.*, 1977; Stewart *et al.*, 1979).

Halophytic *Atriplex* species show stimulation of growth at NaCl concentrations that are inhibitory to non-halophytes (Osmond *et al.*, 1980). Ashby and Beadle (1957) reported that the growth of both *A. inflata* and *A. nummularia* was greater at 600 mol m$^{-3}$ NaCl than in nutrient-only controls. *A. griffithii* var. *stocksii* grew faster at 180 mol m$^{-3}$ NaCl than at higher and lower external salinities (Khan *et al.*, 2000*a*), whereas *A. centralasiatica* is able to perform well at 400 mol m$^{-3}$ NaCl (Qiu *et al.*, 2003). Other chenopod halophytes, such as *Halosarcia pergranulata* (Short and Colmer, 1999), *Suaeda fruticosa* (Khan *et al.*, 2000*b*) and *Sarcocornia fruticosa* (Redondo-Gómez *et al.*, 2006), have growth optima at moderate to high salinities.

The growth analysis of *A. portulacoides* in this experiment provides insight into the mechanisms underlying such salinity tolerance. The component of RGR that was most sensitive to salinity was clearly ULR, underlining the primary importance of the rate of assimilation per unit leaf area. There was also a tendency to allocate more biomass to the leaf fraction with increasing salinity; leaf mass fraction increased from approx. 0·4 to 0·56 over the salinity range. Although it was not associated with any consistent changes in specific leaf area, increased LMF would have contributed to the maintenance of leaf area at higher salinity. Except at the highest salinity, variation in leaf area could be attributed mainly to variation in the numbers of leaves rather than their mean area.

The pattern of ULR response to salinity was strongly supported by the direct, short-term measurements of photosynthetic carbon assimilation (*A*). After both 30 and 60 d of treatment, the highest rates of *A* were recorded consistently at 200 mol m$^{-3}$ NaCl, even though the overall trend was a reduction in *A* with salinity. Lorenzen *et al.* (1990) similarly reported higher net photosynthetic rates in plants grown in 50 % seawater than in either 0 or 100 % seawater, although the rates they measured in northern European material were substantially lower than those in our experiment. Rates recorded at 200 and 400 mol m$^{-3}$ NaCl, after at least 1 month of salinity treatment, in the present experiment are similar to those of Qiu *et al.* (2003) for *A. centralasiatica*. Responses of photosynthetic rate to salinity might be mediated by individual effects on the assimilatory enzyme systems, photochemical processes or resistances to gas exchange. Measurements of rates of light-driven incorporation of $^{14}CO_2$ into cell suspension cultures derived from leaves of *A. portulacoides* (Plaut *et al.*, 1991) indicated that at least 500 mol m$^{-3}$ NaCl in the assay medium was not inhibitory to salt-adapted cultures; furthermore, increasing NaCl up to at least 200 mol m$^{-3}$ in the culture medium stimulated subsequent incorporation rates. Hence, enzymic limitation under salt stress is unlikely, presumably because of compartmentation of NaCl in the cell vacuoles and the synthesis of compatible osmolytes, such as glycine betaine, in the cytoplasm. However, stimulation of carboxylation by salt might have contributed to the increased photosynthesis observed here at 200 mol m$^{-3}$ NaCl.

There were very clear effects of NaCl on stomatal conductance in the present experiment, across the whole range of salinity. At all stages of the experiment $g_s$ showed an overall strong negative association with external salinity, although there was little evidence of a reduction at salinities up to 200 mol $m^{-3}$ in the later stages of the experiment. Consequently, changes in $g_s$ appear to provide an explanation for the concomitantly declining photosynthetic assimilation rates. After 30 and 60 d of treatment, the lower $g_s$ led to a reduction in intercellular $CO_2$ concentration, which in turn would have limited carboxylation. In the early stages of the experiment (6 d), during which plants were probably experiencing a degree of osmotic shock, there was almost complete stomatal closure at the highest two salinities; the opposite relationship pertained and intercellular $CO_2$ concentration increased with external salinity. Centritto *et al.* (2003) reported that salt stress affects both stomatal and mesophyll conductances, so the accumulation of $CO_2$ in the intercellular spaces could indicate that the photosynthetic decline at this time was nevertheless caused by diffusional limitation of photosynthesis, rather than any effect on carboxylation capacity. The decline in stomatal conductance was not accompanied by a loss of leaf relative water content in *A. portulacoides* and therefore is likely to be the result of a signalling process rather than a general loss of turgor. Essentially the same finding applies to *Sarcocornia fruticosa* (Redondo-Gómez *et al.*, 2006). At higher salinities, *A. portulacoides* was able to increase water use efficiency substantially, although values were not as high as in the extreme halophyte *Sarcocornia fruticosa*.

There was very little evidence that elevated salinity affected the integrity or function of the photochemical apparatus, and there was no effect on chlorophyll concentrations in the leaves. After 6 d of treatment, when plants are presumed to have been susceptible to osmotic shock, there was decreased quantum efficiency of PSII ($\Phi_{PSII}$) at mid-day at the highest salinity and the concomitant increase in non-photochemical quenching indicated that the plants were dissipating excess light energy as heat. Also at this time the general decline in $A$ was similar to that of $\Phi_{PSII}$. Otherwise, the response of $A$ to salinity did not track that of $\Phi_{PSII}$. This disparity could have been caused by changes in the relative rates of $CO_2$ fixation, photorespiration, nitrogen metabolism and electron donation to oxygen (the Mehler reaction; Fryer *et al.*, 1998). It has been suggested that salinity can increase photorespiration (Parida and Das, 2005) and cyclical electron transport (Bukhov and Carpentier, 2004). Redondo-Gómez *et al.* (2006) showed these two physiological processes could be mechanisms to protect *Sarcocornia fruticosa* against excess of radiation under high salinities, as the relatively stable NPQ and chlorophyll content across the salinity range suggested that salt did not produce an increase in thermal dissipation in the PSII antennae.

The maximum quantum efficiency of PSII photochemistry ($F_v/F_m$) did show a significant reduction at mid-day compared with dawn values, which is indicative of photoinhibition associated with an over-reduction of PSII. This photoinhibition would have been caused by a lower proportion of open reaction centres (lower values of $F_m$) resulting from a saturation of photosynthesis by light. This decrease seems to be dynamic photoinhibition as the low mid-day values recovered completely by dawn to optimal values for unstressed plants (Björkman and Demmig, 1987). The mid-day depression of $F_v/F_m$ was greater in the earlier stages of the experiment than in the older plants at the end. It was also largely independent of salinity treatment. However, the mid-day reduction in $F_v/F_m$ was much more marked at low salinity (0–20 mol $m^{-3}$ NaCl), particularly after 30 and 60 d, indicating that low salinity represents an environmental stress (Maxwell and Johnson 2000) for *A. portulacoides*. The increased $F_v/F_m$ values at mid-day with both the duration of treatment and the NaCl concentration could have been caused by a higher proportion of open reaction centres (higher values of $F_m$), which could be attributed to an increase in Chl *a* content. By contrast, studies on the non-halophyte barley have demonstrated that increased salinities cause highly detrimental effects on $F_v/F_m$ and leaf pigment composition, although these adverse effects on the leaf photochemistry of barley could be alleviated by increasing external $Ca^{2+}$ supply (S. Shabala *et al.*, 2005). The fact that photoinhibition was not more severe in salt-adapted plants, even when exposed to high light, suggests that they have mechanisms by which excess energy is dissipated safely (Qiu *et al.*, 2003). Kocheva *et al.* (2004) proposed that the changes in fluorescence intensity resulted from long-term structural/conformational changes, presumably in the PSII antennae, which led to increased energy dissipation. In addition, the decreases in mid-day $F_0$ after 60 d indicated lower photoinhibitory damage at higher salinity (Maxwell and Johnson, 2000).

The comparison of growth and photosynthetic responses of *A. portulacoides* has provided new insight into salinity tolerance in a widespread, competitive coastal hygrohalophyte, which experiences salinities oscillating around those of seawater. Differences in growth rate over this range of salinity can be accounted for largely by effects on net photosynthesis. Allowing for the effects of some osmotic shock in the early stages of the experiment, it is clear that salinity has little overall effect on the photochemical (PSII) apparatus. Similarly, the carboxylation capacity does not seem to be adversely affected by salinity, although its stimulation by moderate salt concentrations may contribute to increased growth rates. The greatest impact of salinity on photosynthesis appears to be via the regulation of stomatal conductance and its consequences for intercellular leaf $CO_2$ concentration. This finding is in contrast with results for a xero-halophyte (*Sarcocornia fruticosa*) in which lower rates of $CO_2$ assimilation could be more than compensated for by the development of greater photosynthetic area, allowing its fastest growth to occur at even higher salinities than for *A. portulacoides*, at least 500 mol $m^{-3}$ in this case (Redondo-Gómez *et al.*, 2006).

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