L. Varone *, R. Catoni, A. Bonito, E. Gini, L. Gratani

Department of Environmental Biology, Sapienza University of Rome, P. le A. Moro, 5, 00185 Rome, Italy

**Abstract**

Substrate salinity is one of the main abiotic factors limiting plant establishment, growth and distribution in coastal habitats. Nevertheless, few studies have investigated the interaction between salt concentration and duration of exposure on the physiology and growth of **Carpobrotus edulis**, an important invasive plant species growing in coastal dune habitats. In this study, four salinity treatment cycles of different length (three, six, twelve and twenty-four days) at salinity of 0 M, 0.1 M, 0.2 M and 0.3 M were imposed. A significant response in plant growth was elicited after 24 days of treatment. The main shoot length (MSL) and stem biomass (SBMS) increased by 11% and 25%, respectively at 0.1 M and by 25% and 6% at 0.2 M compared with the control. At 0.3 M MSL did not significantly differ from the control while SBMS was 18% lower. Moreover, **C. edulis** showed a high photoprotection mechanism efficiency resulting in a high carotenoid to chlorophyll ratio increase which was two, three and four times higher than the control at 0.1 M, 0.2 M and 0.3 M, respectively. Photochemically, the quantum yield of photosynthesis (ΦPSII) was 17%, 50% and 52% lower than the control at 0.1 M, 0.2 M and 0.3 M. The ΦPSII decrease was associated with a low leaf nitrogen content (NL) decrease (16%, 21% lower than the control at 0.1 M and 0.2 M, respectively). By contrast, NL had the highest decrease (41% lower than the control) at 0.3 M, which constrains the growth capacity. Overall, **C. edulis** was able to modulate its response to salinity. The salt stimulated shoot elongation at low or moderate salt concentrations could confer a competitive advantage making **C. edulis** even more efficient in establishing within the areas which it colonizes. Since the expansion of **C. edulis** may be enhanced by the forecasted increase in soil salinity, it will be of paramount importance to apply effective management practices in areas invaded by **C. edulis** to limit its expansion and preserve the native plant biodiversity.

© 2017 SAAB. Published by Elsevier B.V. All rights reserved.

---

1. Introduction

Substrate salinity is one of the main abiotic factors limiting plant establishment, growth and distribution in coastal habitats where the salinity substrate level represents the most important threshold, which separates the unvegetated zone from the area colonized by plants (Fenu et al., 2012). Usually, substrate salinity ranges from 0.1% to 3% (Barbour et al., 1985) even though salt concentrations depend on time of year, distance from the sea and sea storm phenomena (Weber and D’Antonio, 1999). For example, substrate salinity varies from 0 after abundant rainfall to 0.5 M NaCl after a sea overwash in coastal sand dunes of dry areas (Sykes and Wilson, 1989). Moreover, exposure to high soil salinity levels induces detrimental effects on plant establishment and growth causing osmotic stress, which in turn reduces water uptake (Sucre and Suárez, 2011) leading through internal signals to decrease the cell expansion rate in growing tissues (Shabala et al., 2012). Ion toxicity due to Na⁺ and Cl⁻ accumulation in chloroplast and nutritional imbalance contribute to inhibiting plant growth (Greenway and Munns, 1980). At a metabolic level, salt stress restricts the activity of various enzymes (Morais et al., 2012), alters the nitrogen metabolism (Nazar et al., 2011) and reduces carbon assimilation because of stomatal and biochemical limitations (Chaves et al., 2009). Moreover, although the effect of salt stress on photochemistry is not fully understood, there is evidence that Na⁺ and Cl⁻ accumulation in chloroplasts affects electron transport and secondary processes that influence the photosynthetic machinery (Larcher, 2003).

Increase in soil salinity is becoming a serious concern in Mediterranean coastal habitats where climate change is exacerbating the stress conditions (e.g. low soil water-holding capacity). The forecasted decline in rainfall and the concomitant increase in evaporative demand due to warmer air temperature facilitate the substrate salt accumulation from aerosol spray (Greaver and Sternberg, 2007). The Mediterranean Basin could be hit by more extreme climatic events such as sea storm and intense wave episodes accompanied by wind intensification.
On the other hand, the new climatic conditions favor the colonization from non-native species (Davidson et al., 2011). Invasions by alien plant species is a worrisome phenomenon on a global scale because it can seriously compromise the survival of native flora (Chytrý et al., 2008). The threat to native plant species arises through the capacity of alien species to modify ecosystem functioning (Simberloff et al., 2013) through direct competition for abiotic resources, impact on pollination of native species, modification of the soil physical properties and by changes in the nutrient cycle (Brown et al., 2002; Ehrenfeld, 2003; Novoa et al., 2014). However, the extent of invasion differs among habitat types depending on different factors such as climate, historical and biogeographical characteristics (Chytrý et al., 2008). Coastal habitats are among those that are most vulnerable to invasion by alien species (García-de-Lomas et al., 2010). The cause of their vulnerability to biological invasions can be ascribed to the high level of disturbance that often characterizes these habitats (Affre et al., 2010). In fact, coastal habitats, especially in the Mediterranean Basin, have been severely degraded over time because of human activities (Fenu et al., 2012, 2013). In addition, the high tourist flux and extent of commercial exchange increase probability of alien plant introductions (García-de-Lomas et al., 2010). The invasiveness of alien plant species relies on specific physiological and morphological traits such as high maximum photosynthetic rate, low shoot/root ratio, high fecundity, high reproductive effort and high growth rate mainly due to clonal growth capacity (Roiloa et al., 2010). Moreover, in coastal habitats the capacity of alien plant species to tolerate the substrate salinity is an important factor affecting their spread (Weber and D’Antonio, 1999).

Few studies have aimed to analyze the ecophysiological response of invasive plant species to stress factors such as high salinity (Morais et al., 2012; Pintó-Marijuan and Munné-Bosch, 2013), although salinity tolerance may have an important role in determining the extent to which an invasive plant species can successfully colonize coastal habitats. Thus, understanding how invasive plant species respond to salinity stress could help to predict what salinity levels might influence future invasion rates.

Carpobrotus edulis (L.) N.E. Br. is a succulent perennial plant species from South Africa (Albert, 1995) introduced to Europe around 1680 (Fournier, 1952) and planted as an ornamental species or used to stabilize dunes and slopes (Traveset et al., 2008). Its growth has become rampant, causing a high impact on diversity, structure and dynamics of native plant communities, sometimes replacing them in many areas such as Southern Europe, California, and Australia, (Roiloa et al., 2010). Carpobrotus edulis is now considered to be one of the most harmful and aggressive invasive plant species of the Mediterranean coastal dunes (Sintes et al., 2007; Roiloa et al., 2010). Its clonal growth, which is achieved by the vegetative production of functional individuals (i.e. ramets) produced by the main shoots (i.e. stolons) by rooting at some shoot nodes, has been well studied (Traveset et al., 2008).

By contrast, research aimed at analyzing the physiological response to salinity stress in C. edulis is rare (Weber and D’Antonio, 1999; Madawala et al., 2014). Thus, the aim of this study was to analyze growth capacity, biomass production and photochemical functionality in response to a range of salinity levels. Moreover, since coastal habitats are characterized by temporal variations in substrate salinity (Sucre and Suárez, 2011), we hypothesized that the extent of the response of C. edulis depended not only on the salt concentration but also on the duration of the stress.

2. Materials and methods

2.1. Plant material

Individual ramets (n = 40) were collected at the end of June 2013 from an established sand dune along the Tyrrhenian coast near Rome (Calvo et al., 2011), which will further lead to intensifying the sea spray phenomena.

To evaluate the effect of the duration of exposure to salinity, plants were exposed to four consecutive cycles (Cycle 1, 2, 3 and 4) of three, six, twelve and twenty-four days, respectively. Salt concentrations in pots were achieved using a mixture of artificial seawater (Instant Ocean®, 3.5% salt content) with a Hogland’s nutrient solution (Weber and D’Antonio, 1999). Plants were irrigated with 1 l of salt solution containing 0, 17%, 34% and 50% artificial seawater to achieve the final NaCl concentrations. Control pots (i.e. 0 M NaCl) were irrigated with Hogland’s nutrient solution without salt. The reason for using twenty-four days as a maximum duration for the salinity treatment was to ensure substantial but not lethal salinity stress conditions (Zinnert et al., 2012). Ten pots for each salinity treatment were randomly arranged in four trays and measurements of plant growth, chlorophyll fluorescence, photosynthetic pigment content and leaf nitrogen content were carried out one day after each cycle end (i.e. on July 4th, 11th, 24th and on August 18th, 2013) on six individuals randomly chosen. After measurements had been taken and before the next cycle started, plants were irrigated with tap water to avoid salt increase in the substrate (Weber and D’Antonio, 1999).

2.2. Salinity treatment

Starting from July 1st 2013, plants of the same length (i.e. 14 cm long) were subjected to four salt concentrations (0 M, 0.1 M, 0.2 M and 0.3 M NaCl). To evaluate the effect of the duration of exposure to salinity, plants were exposed to four consecutive cycles (Cycle 1, 2, 3 and 4) of three, six, twelve and twenty-four days, respectively. Salt concentrations in pots were achieved using a mixture of artificial seawater (Instant Ocean®, 3.5% salt content) with a Hogland’s nutrient solution (Weber and D’Antonio, 1999). Plants were irrigated with 1 l of salt solution containing 0, 17%, 34% and 50% artificial seawater to achieve the final NaCl concentrations. Control pots (i.e. 0 M NaCl) were irrigated with Hogland’s nutrient solution without salt. The reason for using twenty-four days as a maximum duration for the salinity treatment was to ensure substantial but not lethal salinity stress conditions (Zinnert et al., 2012).

2.3. Main shoot elongation and stem biomass

At the beginning of the experiment, ten main shoots (MS) for each salinity treatment (i.e. one MS per pot) were labeled in order to monitor MS length (MSL, cm) over the entire experiment. MS, was calculated by summing the length of all internodes produced on a shoot, where internodes are the distance between two nodes, and a node is the point on the shoot at which leaves (one or more) are inserted (Sintes et al., 2007). In addition, at the end of each cycle the number of new shoots had been taken and before the next cycle started, plants were irrigated with tap water to avoid salt increase in the substrate (Weber and D’Antonio, 1999).

2.4. Chlorophyll fluorescence

Chlorophyll fluorescence measurements, including maximum PSII photochemical efficiency (Fv/Fm), actual quantum yield of photosynthesis of light-adapted leaves (ΦPSII) and electron transportation rate (ETR), were carried out by a portable modulated fluorometer (OFSp, Opti-Sciences, USA) on fully expanded leaves on each MS.

For measurements of Fv/Fm, leaves were first dark-adapted for 30 min by leaf clips then a saturating pulse was applied to measure
Initial (F₀) and maximum (Fₘ) fluorescence. Fₐ/Fₘ was estimated according to Maxwell and Johnson (2012) as:

\[ Fₐ = \frac{Fₘ - F₀}{Fₘ} \]

Φₜ was calculated on light-adapted leaves as:

\[ Φₜ = \frac{Fₘ'}{Fₘ} \]

where Fₘ' was the maximum fluorescence obtained with a light-saturating pulse (~8000 μmol m⁻² s⁻¹) and Fₘ was the steady-state fluorescence of illuminated leaves (1600 μmol m⁻² s⁻¹).

ETR (μmol e⁻ m⁻² s⁻¹) was calculated according to Krall and Edwards (1992) as:

\[ ETR = (Φₜ) × PPFD × 0.5 × 0.84 \]

2.5. Leaf photosynthetic pigments and nitrogen content

Chlorophyll (Chl, mg g⁻¹) and carotenoid (Car, mg g⁻¹) content were determined after grinding leaves of each MS in acetone (1.5 g of leaf fresh mass per replicate). The homogenates were centrifuged in a refrigerated centrifuge (4237R. A.L.C., I). Absorbance of the supernatants was measured by a Jasco model 7800LCD (Japan) spectrophotometer at the wavelengths of 645, 663, and 440 μm for Chl a, Chl b, and Car, respectively. Chl content was determined according to Maclachlan and Zalik (1963) and Car content according to Holm (1954). Total Chl content (Chlₐ + b), Chlₐ/Chlₜ ratio and Car/Chlₜ ratio were calculated.

Leaf nitrogen content (Nₑ, mg g⁻¹) was determined by the Kjeldahl method using 0.5 g of leaf dry mass per replicate.

2.6. Statistical analysis

To test salt concentration effect in relation to salinity treatment duration, a Two-Way ANOVA was carried out using all data except SBMS. Two-Way ANOVA was based on a completely randomized 4 × 4 factorial design with salt concentrations (four levels: 0 M, 0.1 M, 0.2 M and 0.3 M NaCl) and salinity treatment duration (four levels: Cycles 1, 2, 3 and 4) as factors. In the Two-Way ANOVA design, there were 10 pots for each treatment. Of these, six pots were randomly selected for measurements for each treatment for each sampling period.

Table 1

| Treatment cycle | 1 | 2 | 3 | 4 |
|-----------------|---|---|---|---|
| [NaCl]          | 0 M | 0.1 M | 0.2 M | 0.3 M |
| MSL [cm]        | 14.2 ± 0.3a | 14.0 ± 0.1a | 14.0 ± 0.2a | 14.3 ± 0.2a |
| MSL [cm]        | 15.1 ± 0.4a | 15.0 ± 0.4a | 15.1 ± 0.5a | 15.3 ± 0.3a |
| SBMS [g]        | 2.750 ± 0.010a | 2.834 ± 0.008b | 2.908 ± 0.003b | 2.246 ± 0.014c |

MSL = main shoots length; SBMS = stem biomass of the main shoots. Within each treatment cycle mean values followed by the same letters are not significantly different (Tukey’s test, P ≥ 0.05).

* SBMS was calculated only during the last salinity treatment cycle (i.e. Cycle 4).

Fig. 1. Number of new shoots during the applied salinity treatment cycles differing in their duration (Cycle 1 = three days; Cycle 2 = six days; Cycle 3 = twelve days; Cycle 4 = twenty-four days). Mean values (± S.E.) are shown (n = 10). Mean values with different letters indicate significant differences (Tukey’s test, P ≤ 0.05). Capital letters refer to differences for the same salt concentrations among the four cycles. Lowercase letters refer to differences in the same cycle among the different salt concentrations.
Fig. 2. Maximum PSII photochemical efficiency (Fv/Fm), actual quantum yield of photosynthesis (ΦPSII) and electron transportation rate (ETR) at different salt concentrations during the applied salinity treatment cycles differing in their duration (Cycle 1 = three days; Cycle 2 = six days; Cycle 3 = twelve days; Cycle 4 = twenty-four days). Mean values (± S.E.) are shown (n = 6). Mean values with different letters indicate significant differences (Tukey's test, P ≤ 0.05). Capital letters refer to differences for the same salt concentrations among the four cycles. Lowercase letters refer to differences in the same cycle among the different salt concentrations.
Since SBMS was determined only at the end of Cycle 4, it was analyzed by One-Way ANOVA to test the differences among the different salt concentrations. Moreover, a multiple regression analysis was carried out using MS as dependent variable and Fv/Fm, ΦPSII, ETR, Chl_a+b, Chl_a/Chl_b, Car, Car/Chl_a+b and Nl as independent variables.

All data are shown as mean ± standard error. All statistical tests were performed using Statistica 8.0 (Stasoft, USA).

3. Results

3.1. Shoot elongation and stem biomass

The application of 0.1 M, 0.2 M and 0.3 M NaCl over Cycles 1, 2 and 3 did not produce differences in MS compared with 0 M (Table 1). At the end of Cycle 4 differences were observed in MS depending on salt concentration. MS was 11% and 25% higher than the control at 0.1 M and 0.2 M, respectively. No significant differences in MS were found between the 0 M and 0.3 M treatments.

At the end of Cycle 4, SBMS increased on average by 3% and 6% at 0.1 M and 0.2 M, respectively, compared with 0 M (Table 1) whereas at salinity of 0.3 M, SBMS was 18% lower than 0 M.

New shoots began to develop during Cycle 2. At the end of Cycle 4, plants subjected to 0.1 M and 0.2 M had the highest number of new shoots (Fig. 1). By contrast, at 0.3 M the production of new shoots was lower than the control.

3.2. Chlorophyll fluorescence

Fv/Fm was slightly impaired in response to increase in salt concentration (Fig. 2a). Significant differences between 0.1 M and 0 M were found from Cycle 3 when Fv/Fm was 2% lower than 0 M. Fv/Fm significantly differed from the control at salinity of 0.2 M and 0.3 M from Cycle 1, resulting in on average 5% and 6% lower than 0 M at 0.2 M and 0.3 M, respectively (mean value of the Cycles 1, 2 and 3). The lowest Fv/Fm was measured at the end of Cycle 4 (0.716 ± 0.005, 0.709 ± 0.004 and 0.704 ± 0.004 at 0.1 M, 0.2 M and 0.3 M, respectively).

Fig. 3. Total chlorophyll content (Chl_a+b) and carotenoid content (Car) at different salt concentrations during the applied salinity treatment cycles differing in their duration (Cycle 1 = three days; Cycle 2 = six days; Cycle 3 = twelve days; Cycle 4 = twenty-four days). Mean values (± S.E.) are shown (n = 6). Mean values with different letters indicate significant differences (Tukey’s test, P ≤ 0.05). Capital letters refer to differences for the same salt concentrations among the four cycles. Lowercase letters refer to differences in the same cycle among the different salt concentrations.
ΦPSII was more impaired than Fv/Fm in all Cycles showing significant differences at all salt concentrations compared with 0 M (Fig. 2b). In particular, the imposition of Cycle 1 led to a decrease of ΦPSII by 8%, 20% and 22% at 0.1 M, 0.2 M and 0.3 M compared with 0 M. ΦPSII further decreased over Cycles 2 and 3 being 0.543 ± 0.006, 0.487 ± 0.003 and 0.455 ± 0.010 at 0.1 M, 0.2 M and 0.3 M, respectively. At the end of Cycle 4, ΦPSII was 17%, 50% and 52% lower than 0 M at 0.1 M, 0.2 M and 0.3 M, respectively.

ETR trend was similar to ΦPSII (Fig. 2c). During Cycle 1, ETR ranged from 416 ± 12 μmol e− m−2 s−1 at 0.1 M to 351 ± 10 μmol e− m−2 s−1 at 0.3 M. The lowest ETR was measured at the end of Cycle 4 reaching values 12%, 43% and 49% lower than 0 M at 0.1 M, 0.2 M and 0.3 M, respectively.

3.3. Leaf photosynthetic pigment content and nitrogen content

Leaf photosynthetic pigments (Fig. 3a) showed a greater responsiveness to increases in salt concentration compared to chlorophyll fluorescence. After Cycle 1, Chla+b decreased by 16%, 28% and 38% at 0.1 M, 0.2 M and 0.3 M compared with 0 M (0.264 ± 0.006 mg g−1). In all successive treatments, Chla+b showed a consistent trend. The lowest values were measured at the end of Cycle 4 (0.209 ± 0.005, 0.174 ± 0.002 and 0.147 ± 0.002 mg g−1 at 0.1 M, 0.2 M and 0.3 M, respectively). Chlb contributed more to the reduction of Chla+b as highlighted by the increase in Chlb/Chla ratio at all salinity treatments (Fig. 3b). In control plants, Chlb/Chla ratio was 2.90 ± 0.05 (mean value of all cycles) progressively increasing up to 3.48 ± 0.11, 3.83 ± 0.16 and 4.78 ± 0.14 at 0.1 M, 0.2 M and 0.3 M, respectively at the end of Cycle 4.

Car (Fig. 4a) had an opposite trend compared to Chla+b increasing in response to salinity treatments up to more than 100% of the control at 0.3 M after Cycle 4. In addition, Car/Chla+b ratio increased strongly in all salinity treatments reaching values 2, 3 and 4 times higher than 0 M at 0.1 M, 0.2 M and 0.3 M, respectively by the end of Cycle 4 (Fig. 4b).

The highest Nt (Fig. 5) was measured in control plants (15.02 ± 0.05 mg g−1, mean value of all cycles). The Nt trend was similar at 0.1 M (12.82 ± 0.22 mg g−1, mean value of all cycles) and at 0.2 M

![Fig. 4. Carotenoid to total chlorophyll content ratio (Car/Chla+b) at different salt concentrations during the applied salinity treatment cycles differing in their duration (Cycle 1 = three days; Cycle 2 = six days; Cycle 3 = twelve days; Cycle 4 = twenty-four days). Mean values (± S.E.) are shown (n = 6). Mean values with different letters indicate significant differences (Tukey's test, P ≤ 0.05). Capital letters refer to differences for the same salt concentrations among the four cycles. Lowercase letters refer to differences in the same cycle among the different salt concentrations.](image-url)
(12.30 ± 0.27 mg g⁻¹, mean value of all cycles) while plants subject to salinity of 0.3 M showed the lowest Nₖ particularly after Cycle 4 (8.84 ± 0.05 mg g⁻¹).

3.4. Two-way ANOVA and multiple regression analysis

Two-Way ANOVA revealed that intensity and duration of the salinity treatments had a significant effect on plant growth and photosynthetic apparatus functionality of C. edulis with a combined effect of the two factors (Table 2). Multiple Regression analysis showed that MSL significantly depended on a linear combination of the considered physiological and photochemical variables. Nevertheless, ΦPSII, Car, Car/Chla+b and Nₖ were the significant (p ≤ 0.05) variables accounting for the ability to predict MSL (Table 3).

4. Discussion

Salt tolerance is defined as a plant’s capacity to grow in saline vs control conditions over a prolonged period of time (Munns, 2002). Overall, the extent of response by C. edulis to salinity depended on the intensity and duration of the salt stress. The most significant effects were observed after twenty-four days of salt treatment (i.e. at the end of Cycle 4). In this case, C. edulis showed two contrasting responses in terms of plant growth depending on salt concentration. First, at 0.1 M and 0.2 M there was a salt stimulated growth as highlighted by an increase in MSL, SBMS and in the production of new shoots compared with the control. This response reveals a halophytic-like behavior in C. edulis, since halophytes show optimal growth at NaCl concentrations ranging from 0.10 M to 0.25 M (Shabala and Mackay, 2011) even if some extreme halophytes tolerate a wider range of salinity from 0.17 M to 0.60 M (Redondo-Gómez et al., 2010). The capacity of C. edulis to take advantage of increases in salinity may be limited, however, considering that in natural environments it tends to colonize areas further from the shoreline (Carranza et al., 2010; Madawala et al., 2014) that are characterized by low or moderate substrate salinity as they are not exposed to sea inundation and to the effect of sea spray. In view of this, the imposed experimental concentrations of 0.1 M and 0.2 M reflect the natural growth conditions of C. edulis compared with the control. Thus, the improved plant growth performance may be the result of an acclimation response that in turn could be due to the achievement of a full osmotic adjustment in plant tissues (Shabala et al., 2012). Osmotic adjustments by synthesis of solutes such as proline, polyols, amino acids and proteins are involved in acclimation responses to salinity conditions (Chaves et al., 2009; Bazrafshan and Ehsanzadeh, 2014; Batista-Santos et al., 2015) by counteracting the osmotic stress, which is one of the main causes of lack of growth (Sucre and Suárez, 2011).

Second, at 0.3 M an inhibitory effect on plant growth began, since MSL did not increase and SBMS fewer new shoots were produced than in the control. This result is in accordance with Munns et al. (2006) who reported that limitations to plant growth occurred at salinity levels

| Effect | MSL | Fv/Fm | ΦPSII | ETR | Chla+a+b | Chla+b/Chlb | Car | Car/Chla+b | Nₖ |
|--------|-----|-------|-------|-----|---------|-------------|-----|------------|----|
| C      | 42.6*** | 521.4*** | 606.6*** | 17,262.1*** | 6947.4*** | 201.2*** | 4837.0*** | 14,990.6*** | 959.4*** |
| T      | 481.9*** | 169.4*** | 191.2*** | 2322.9*** | 82.3*** | 9.6*** | 177.8*** | 585.8*** | 23.9*** |
| C × T  | 50.5*** | 27.9*** | 38.5*** | 982.2*** | 33.9*** | 5.5*** | 26.1*** | 132.9*** | 3.216*** |

MSL = main shoot length; Fv/Fm = maximum PSII photochemical efficiency; ΦPSII = actual quantum yield of photosynthesis; ETR = electron transportation rate; Chla+a+b = total chlorophyll content; Car = carotenoid content; Car/Chla+b = Car to Chla+b ratio; Nₖ = Leaf nitrogen content.

*** P ≤ 0.001.
* P ≤ 0.05.
Table 3

Results of Multiple Regression Analysis. The multiple determination coefficient (R²), adjusted R² (AdjR²), residual standard error (RSE), regression degrees of freedom (DFreg), residual degrees of freedom (DFres), F-statistic (F) and the t-statistic (t) are shown.

| Dependent variable | R² | AdjR² | RSE | DFreg | DFres | F | t | P |
|--------------------|----|-------|-----|-------|-------|---|---|---|
| MSₙ     | 0.948 | 0.920 | 0.455 | 8     | 15 | 34,090∗∗** |

Coefficient t P

| Intercept | 45.706 | 3.864 | 0.002** |

Independent variables Coefficient t P

| Φ₁₉ₛ₁ | -15.103 | -2.761 | 0.015* |
| Φ₁₉ₛ₆ | 0.00573 | 0.862 | 0.402 |
| ETR | -22.082 | -1.544 | 0.141 |
| Chlₐ + b | 70.762 | 1.430 | 0.173 |
| Car | -47.243 | -2.529 | 0.023* |
| Car/ Chlₐ + b | -21.146 | -3.289 | 0.004* |
| Chₖₜₐₜ + b | 0.675 | -1.055 | 0.308 |
| Nₑ | 0.547 | 2.390 | 0.030 |

MSₙ = main shoot length; Φ₁₉ₛ₁ = actual quantum yield of photosynthesis; Φ₁₉ₛ₆ = maximum PSHI photochemical efficiency ETR = electron transportation rate; Chlₐ + b = Total chlorophyll content; Car = carotenoid content; Car/ Chlₐ + b = carotenoid to total chlorophyll ratio; Chₖₜₐₜ + b = Chlorophyll a to chlorophyll b ratio; Nₑ = leaf nitrogen content.

* P ≤ 0.05.
** P ≤ 0.01.
*** P ≤ 0.001.

above 0.25 M for moderately salt-tolerant plants. The lack of salt-stimulated growth at 0.3 M may be explained by a lower nitrogen allocation to leaves. In fact, while Nₑ was slightly reduced at lower salt concentrations (on an average 16% lower than control), a strong reduction (41% lower than control) occurred at 0.3 M. In saline substrates nitrogen is an important factor for plant growth (Weber and D’Antonio, 1999). Nitrogen affects the structure and composition of the photosynthetic apparatus (Nazar et al., 2011) as well as the Rubisco content (Kumar et al., 2002). As the majority of Nₑ is used for photosynthesis (Evans, 1989), the observed greater Nₑ reduction at 0.3 M could impair Rubisco activity leading to reduced photosynthetic performance with negative effects on plant growth. If less nitrogen is allocated to Rubisco, its activity is reduced and thus the rate of CO₂ fixation leading to a reduction in photosynthates available for growth.

Salinity stress constrains CO₂ fixation through stomatal and non-stomatal limitations (Sucre and Suárez, 2011). As a consequence, the reducing power production rate is greater than its utilization rate by the Calvin cycle (Chaves et al., 2009) putting the plants at risk of photo-inhibition. These effects are magnified in coastal dune habitats which repeatedly experience stressful conditions such as high temperature, excessive radiation and low water availability during summer (Werner et al., 2002; Varone et al., 2012). Thus, plants from these environments evolved efficient photoprotective mechanisms (Debez et al., 2008), which are essential to limit the production of reactive oxygen species that are responsible for oxidative cellular damage. This is achieved via thermal dissipation in light-harvesting complexes by carotenoids including xanthophylls (Debez et al., 2008). Chlorophyll loss typically occurs in salt treated plants (Munns, 2002; Bazrafshan and Ehsanzadeh, 2014; Tabatabaei and Ehsanzadeh, 2016) and may be part of the strategy to prevent photo-inhibition by reducing the amount of light intercepted (Choiniski et al., 2003) or alternatively it may be a consequence of photosynthetic pigment degradation due to an overproduction of reactive oxygen species by chloroplasts (Tabatabaei and Ehsanzadeh, 2016). In our case, chlorophyll loss seems to act as a supplementary defense against photo-inhibition being associated with a strong Car increase especially at the end of Cycle 4 when the Car/ Chlₐ + b ratio had the highest values (two, three and four times higher than control at 0.1 M, 0.2 M and 0.3 M, respectively). Thus, the Car/ Chlₐ + b increase reflects a regulatory process allowing a reduction of over-excitation risk. At the level of chlorophyll fluorescence, the results showed a lower Φᵥₚₛᵢᵢ than the control at any salt concentration.
