South African Journal of Botany 111 (2017) 258–266

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Photochemical performance of *Carpobrotus edulis* in response to various substrate salt concentrations

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ARTICLE INFO

Article history:
Received 20 October 2016
Received in revised form 8 February 2017
Accepted 9 March 2017

Edited by K Balkwill

Keywords:
Chlorophyll fluorescence
Invasive alien plant species
Leaf nitrogen content
Leaf photosynthetic pigment content
Salt stress
Shoot elongation

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 (MS_L) and stem biomass (SB_{MS}) increased by 11% and 4%, respectively at 0.1 M and by 25% and 6% at 0.2 M compared with the control. At 0.3 M MS_L did not significantly differ from the control while SB_{MS} 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 (Φ_{PSII}) was 17%, 50% and 52% lower than the control at 0.1 M, 0.2 M and 0.3 M. The Φ_{PSII} decrease was associated with a low leaf nitrogen content (N_L) decrease (16%, 21% lower than the control at 0.1 M and 0.2 M, respectively). By contrast, N_L 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.

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 over-wash 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 injure 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

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http://dx.doi.org/10.1016/j.sajb.2017.03.035
0254-6299/

(Calvo et al., 2011), which will further lead to intensifying the sea spray phenomena.

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 proprieties 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 (41°53′40.99″N; 12°09′45.24″E). This area is characterized by a Mediterranean type climate, with mean minimum air temperature (T_{min}) of 12.0 ± 5.6 °C and mean maximum air temperature (T_{max}) of 23.7 ± 8.5 °C. The total annual rainfall is 822 mm with the majority occurring in autumn and in winter and a dry period in summer from June to August with a total rainfall of 46.3 mm (data from Meteorological Station of Capocotta, SIARL, Arsial, for the period 2004–2013).

Each ramet was transplanted into a pot (68 cm diameter and 60 cm depth) filled with sand from the natural environment. Pots were placed in a glasshouse at the experimental garden of Sapienza University of Rome (41°54′N, 12°31′E; 41 m a.s.l.). During the experimental period (from July 1st to August 18th, 2013) T_{max} was 29.1 ± 1.7 °C and T_{min} was 23.1 ± 1.9 °C, relative air humidity was 40–60% and the photosynthetic photon flux density (PPFD, μmol photons m^{-2} s^{-1}) was 1500–1800 μmol photons m^{-2} s^{-1} in the glasshouse.

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).

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).

2.3. 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 (MS_L, cm) over the entire experiment. MS_L 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 was recorded.

Main Stems were harvested at the end of the last salinity treatment cycle (i.e. twenty-four days long) to determine stem biomass. Drying at 90 °C until constant mass was reached preceded stem (excluding leaves) biomass MS (SB_{MS}, g) determinations.

2.4. Chlorophyll fluorescence

Chlorophyll fluorescence measurements, including maximum PSII photochemical efficiency (F_V/F_M), actual quantum yield of photosynthesis of light-adapted leaves (Φ_{PSII}) and electron transportation rate (ETR), were carried out by a portable modulated fluorometer (OS5p, Opti-Sciences, USA) on fully expanded leaves on each MS.

For measurements of F_V/F_M, leaves were first dark-adapted for 30 min by leaf clips then a saturating pulse was applied to measure

Table 1
Effect of different NaCl concentrations on main shoot length and stem biomass of the main shoots of *C. edulis* through the applied treatment cycles differing in their duration (Cycle 1 = three days; Cycle 2 = six days; Cycle 3 = twelve days; Cycle 4 = twenty-four days).

Treatment cycle	1				2			
[NaCl]	0 M	0.1 M	0.2 M	0.3 M	0 M	0.1 M	0.2 M	0.3 M
MS_L [cm]	14.2 ± 0.3a	14.0 ± 0.1a	14.0 ± 0.2a	14.3 ± 0.2a	14.4 ± 0.2a	14.7 ± 0.4a	14.6 ± 0.4a	14.6 ± 0.3a
Treatment cycle	3				4			
[NaCl]	0 M	0.1 M	0.2 M	0.3 M	0 M	0.1 M	0.2 M	0.3 M
MS_L [cm]	15.1 ± 0.4a	15.0 ± 0.4a	15.1 ± 0.5a	15.3 ± 0.3a	15.5 ± 0.4a	17.2 ± 0.3b	19.4 ± 0.3c	15.9 ± 0.2a
SB_{MS} [g]*					2.750 ± 0.010a	2.834 ± 0.008b	2.908 ± 0.003b	2.246 ± 0.014c

MS_L = main shoots length; SB_{MS} = 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).
* SB_{MS} was calculated only during the last salinity treatment cycle (i.e. Cycle 4).

initial (F_0) and maximum (F_M) fluorescence. F_V/F_M was estimated according to Maxwell and Johnson (2012) as:

$$F_V/F_M = (F_M - F_0)/F_M.$$

Φ_{PSII} was calculated on light-adapted leaves as:

$$\Phi_{PSII} = (F_M' - F_S)/F_M'$$

where F_M' was the maximum fluorescence obtained with a light-saturating pulse (~8000 μmol m^{-2} s^{-1}) and F_S 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:

$$ETR = (\Phi_{PSII}) \times PPFD \times 0.5 \times 0.84.$$

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 (Chl_{a+b}), Chl_a/Chl_b ratio and Car/Chl_{a+b} ratio were calculated.

Leaf nitrogen content (N_L, 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 SB_{MS}. 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.

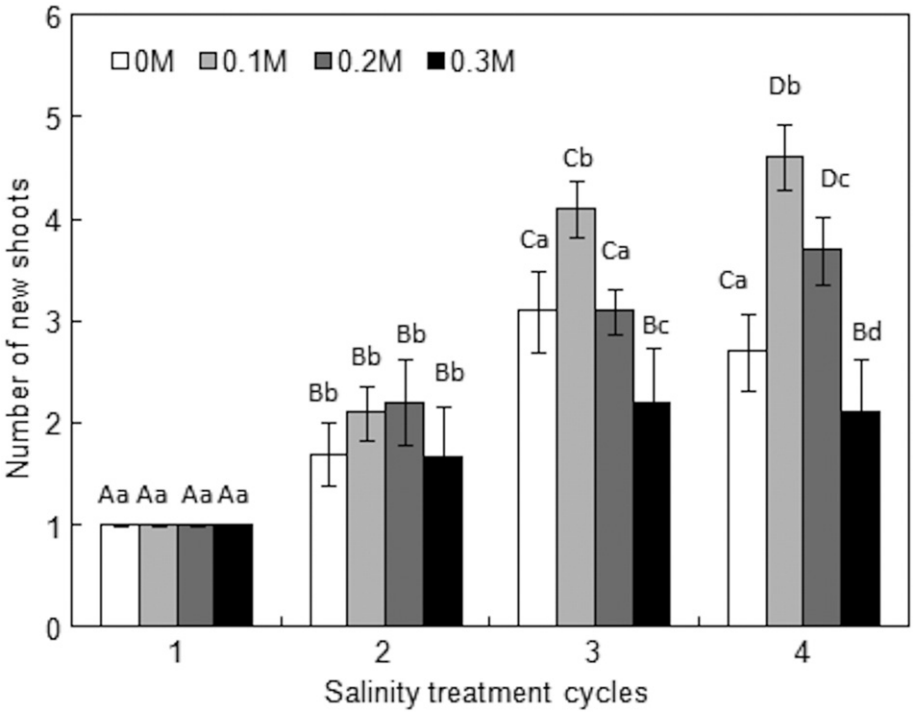

Fig. 1. Number of new shoots during the applied salinity treatment cycles differing in their duration (Cycle 1 = three days; Cycle 2 = six days; Cycle 3 = twelve days; Cycle 4 = twenty-four days). Mean values (± S.E.) are shown (n = 10). Mean values with different letters indicate significant differences (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.

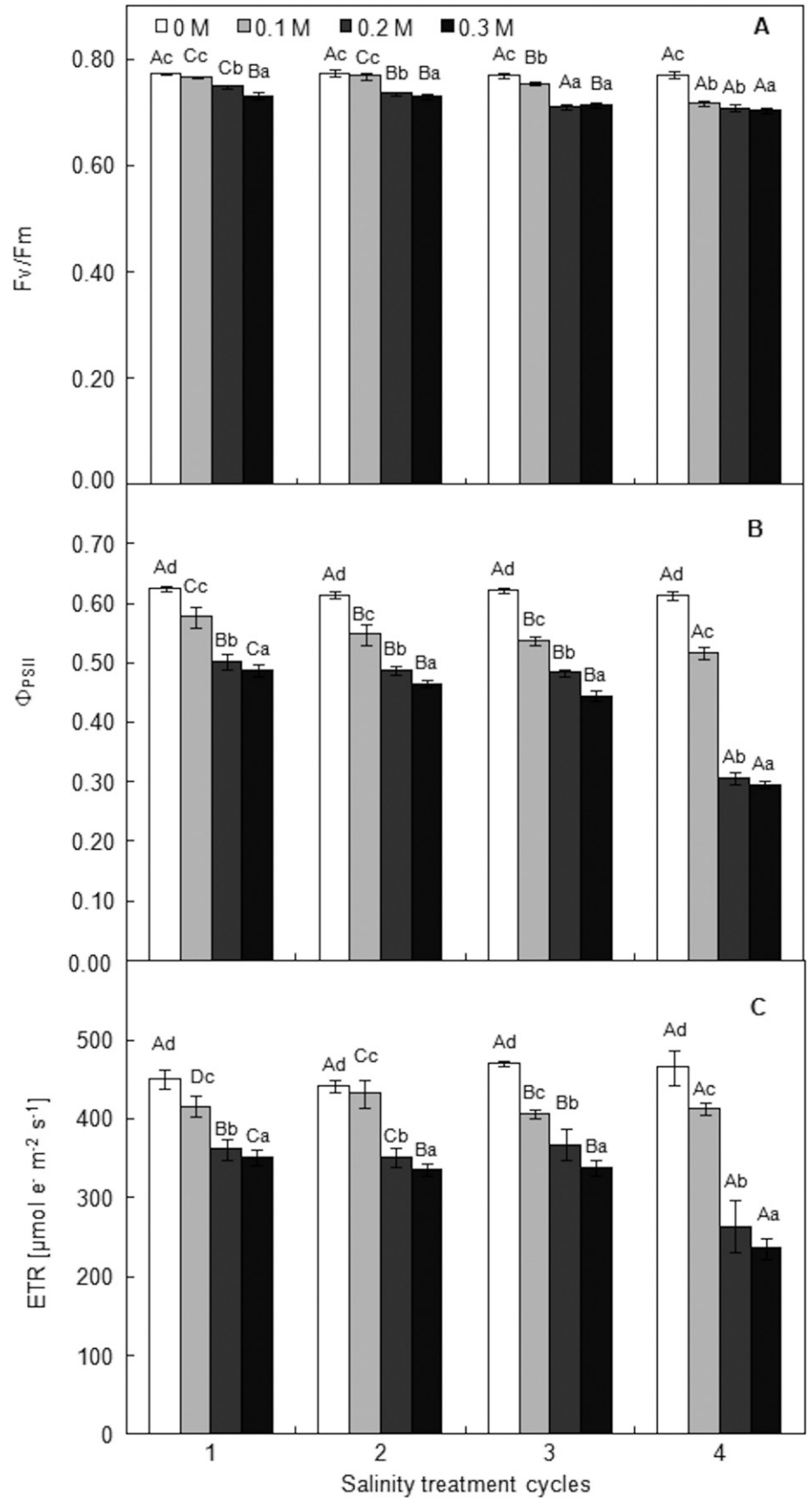

Fig. 2. Maximum PSII photochemical efficiency (F_V/F_M), actual quantum yield of photosynthesis (Φ_{PSII}) and electron transportation rate (ETR) at different salt concentrations during the applied salinity treatment cycles differing in their duration (Cycle 1 = three days; Cycle 2 = six days; Cycle 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, $P \leq 0.05$). 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 SB_{MS} 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_L as dependent variable and F_V/F_M, Φ_{PSII}, ETR, Chl_{a+b}, Chl_a/Chl_b, Car, Car/Chl_{a+b} and N_L 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_L compared with 0 M (Table 1). At the end of Cycle 4 differences were observed in MS_L depending on salt concentration. MS_L was 11% and 25% higher than the control at 0.1 M and 0.2 M, respectively. No significant differences in MS_L were found between the 0 M and 0.3 M treatments.

At the end of Cycle 4, SB_{MS} 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, SB_{MS} 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

F_V/F_M 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 F_V/F_M was 2% lower than 0 M. F_V/F_M 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 F_V/F_M 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).

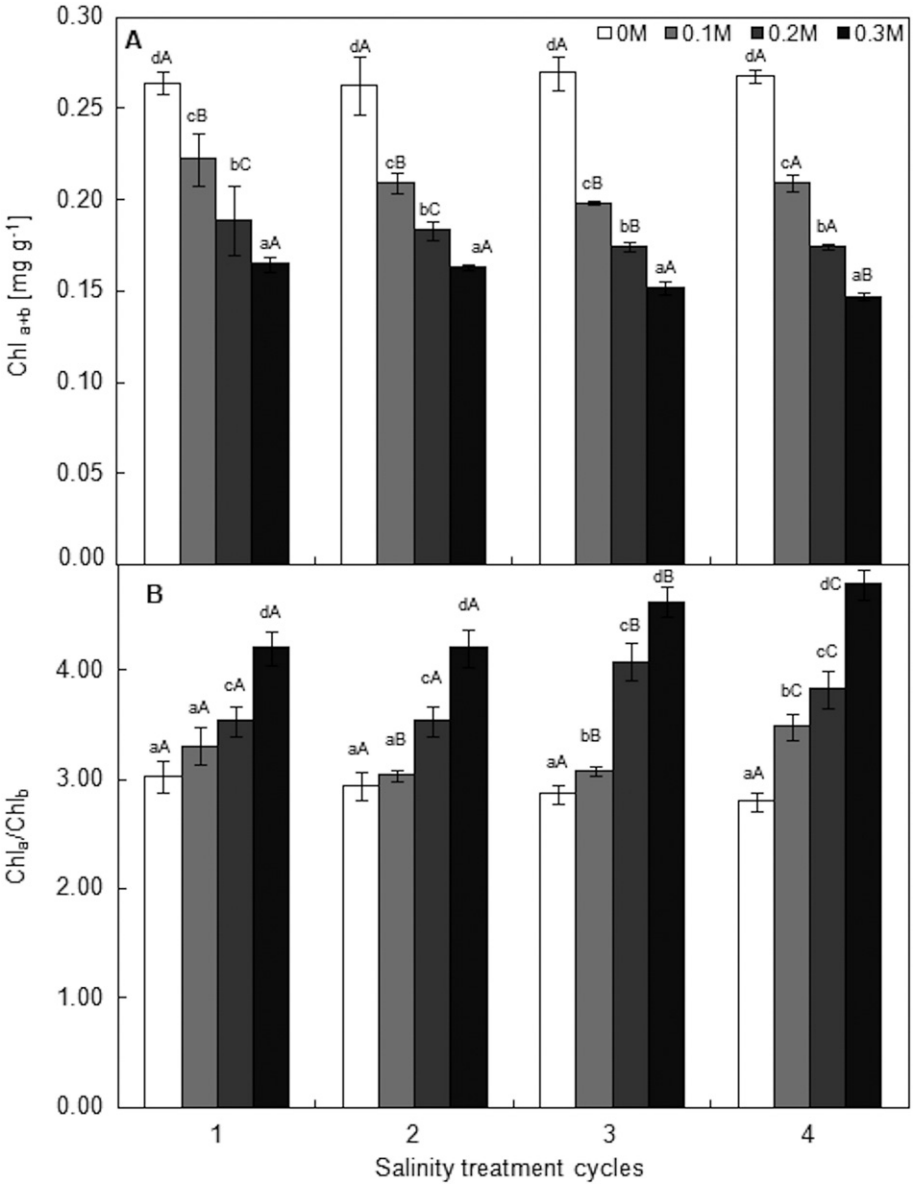

Fig. 3. Total chlorophyll content (Chl_{a+b}) and carotenoid content (Car) at different salt concentrations during the applied salinity treatment cycles differing in their duration (Cycle 1 = three days; Cycle 2 = six days; Cycle 3 = twelve days; Cycle 4 = twenty-four days). Mean values (± S.E.) are shown ($n = 6$). Mean values with different letters indicate significant differences (Tukey's test, $P \leq 0.05$). 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.

Φ_{PSII} was more impaired than F_V/F_M 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 Φ_{PSII} by 8%, 20% and 22% at 0.1 M, 0.2 M and 0.3 M compared with 0 M. Φ_{PSII} 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, Φ_{PSII} 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 Φ_{PSII} (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, Chl_{a+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, Chl_{a+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). Chl_b contributed more to the reduction of Chl_{a+b} as highlighted by the increase in Chl_a/Chl_b ratio at all salinity treatments (Fig. 3b). In control plants, Chl_a/Chl_b 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 03 M, respectively at the end of Cycle 4.

Car (Fig. 4a) had an opposite trend compared to Chl_{a+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/ Chl_{a+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 N_L (Fig. 5) was measured in control plants (15.02 ± 0.05 mg g^{-1}, mean value of all cycles). The N_L trend was similar at 0.1 M (12.82 ± 0.22 mg g^{-1}, mean value of all cycles) and at 0.2 M

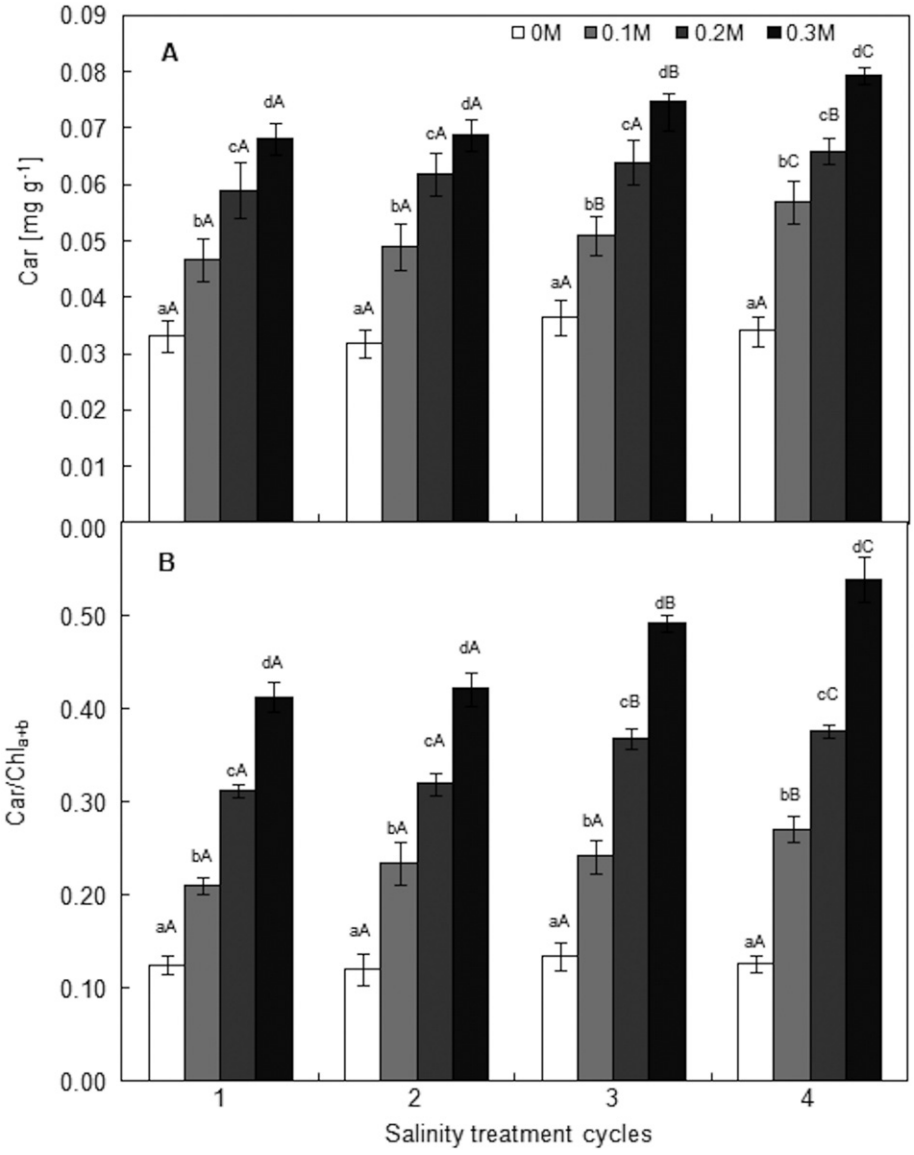

Fig. 4. Carotenoid to total chlorophyll content ratio (Car/Chl_{a+b}) at different salt concentrations during the applied salinity treatment cycles differing in their duration (Cycle 1 = three days; Cycle 2 = six days; Cycle 3 = twelve days; Cycle 4 = twenty-four days). Mean values (± S.E.) are shown ($n = 6$). Mean values with different letters indicate significant differences (Tukey's test, $P \leq 0.05$). 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.

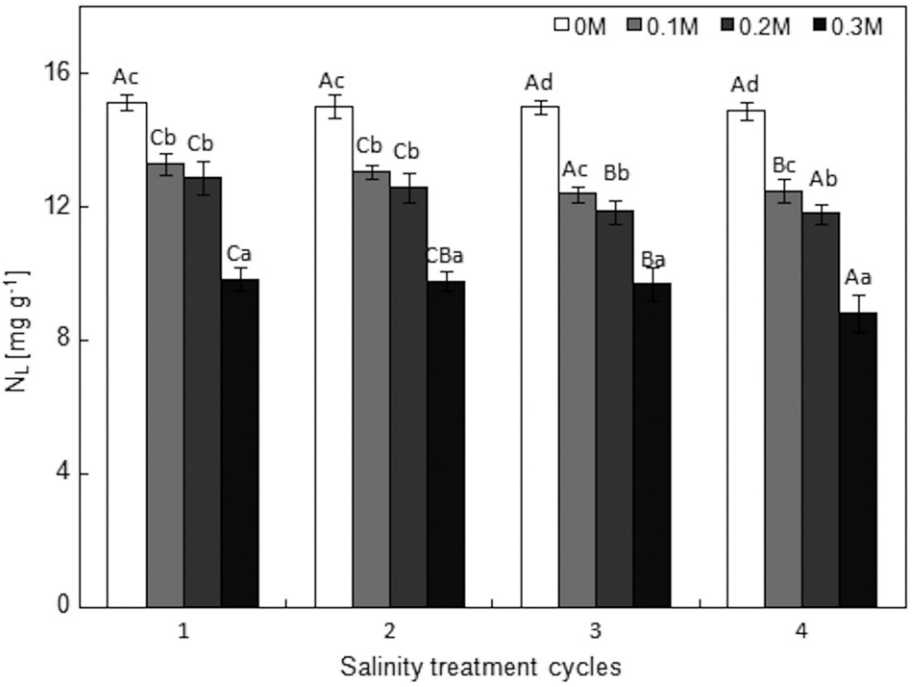

Fig. 5. Leaf nitrogen (N_L) content at different salt concentrations during the applied salinity treatment cycles differing in their duration (Cycle 1 = three days; Cycle 2 = six days; Cycle 3 = twelve days; Cycle 4 = twenty-four days). Mean values (± S.E.) are shown ($n = 6$). Mean values with different letters indicate significant differences (Tukey's test, $P \leq 0.05$). 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.

(12.30 ± 0.27 mg g^{-1}, mean value of all cycles) while plants subject to salinity of 0.3 M showed the lowest N_L 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 MS_L significantly depended on a linear combination of the considered physiological and photochemical variables. Nevertheless, Φ_{PSII}, Car, Car/Chl_{a+b} and N_L were the significant ($p \leq 0.05$) variables accounting for the ability to predict MS_L (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 MS_L, SB_{MS} 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 MS_L did not increase and SB_{MS} 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

Table 2
F value from two-way ANOVA carried out to test the effect of salt concentration (C), salinity treatment duration (T) and their interaction (C × T) on the considered variables.

Effect	MS_L	F_V/F_M	Φ_{PSII}	ETR	Chl_{a+b}	Chl_a/Chl_b	Car	Car/Chl_{a+b}	N_L
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***	2322.5***	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*

MS_L = main shoot length; F_V/F_M = maximum PSII photochemical efficiency; Φ_{PSII} = actual quantum yield of photosynthesis; ETR = electron transportation rate; Chl_{a+b} = total chlorophyll content; carotenoid content; Chl_a/Chl_b = chlorophyll a to chlorophyll b ratio; Car = carotenoid content; Car/Chl_{a+b} = Car to Chl_{a+b} ratio; N_L = Leaf nitrogen content.
*** $P \leq 0.001$.
* $P \leq 0.05$.

Table 3
Results of Multiple Regression Analysis. The multiple determination coefficient (R^2), adjusted R^2 ($AdjR^2$), residual standard error (RSE), regression degrees of freedom (DF_{Reg}), residual degrees of freedom (DF_{Res}), F-statistic (F) and the t-statistic (t) are shown.

Dependent variable	R^2	$AdjR^2$	RSE	DF_{Reg}	DF_{Res}	F
MS_L	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
Φ_{PSII}	−15.103			−2.761		0.015*
F_V/F_M	0.00573			0.862		0.402
ETR	−22.082			−1.554		0.141
Chl_{a+b}	70.762			1.430		0.173
Car	−47.243			−2.529		0.023*
Car/ Chl_{a+b}	−21.146			−3.289		0.004**
Chl_a/Chl_b	0.675			−1.055		0.308
N_L	0.547			2.390		0.030*

MS_L = main shoot length; Φ_{PSII} = actual quantum yield of photosynthesis; F_V/F_M = maximum PSII photochemical efficiency ETR = electron transportation rate; Chl_{a+b} = Total chlorophyll content; Car = carotenoid content; Car/ Chl_{a+b} = carotenid to total chlorophyll ratio; Chl_a/Chl_b = Chlorophyll a to chlorophyll b ratio; N_L = leaf nitrogen content.
* $P \le 0.05$.
** $P \le 0.01$.
*** $P \le 0.001$.

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 N_L 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 N_L is used for photosynthesis (Evans, 1989), the observed greater N_L 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 CO_2 fixation leading to a reduction in photosynthates available for growth.

Salinity stress constrains CO_2 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 photoinhibition. 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 photoinhibition by reducing the amount of light intercepted (Choinski 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/ Chl_{a+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/ Chl_{a+b} increase reflects a regulatory process allowing a reduction of over-excitation risk. At the level of chlorophyll fluorescence, the results showed a lower Φ_{PSII} than the control at any salt concentration. According to the results of Batista-Santos et al. (2015) for *Casuarina glauca*, a reduced Φ_{PSII} seems due to energy dissipation mechanisms that compete for light energy rather than to photochemical damage. The absence of damage to PSII is supported also by F_V/F_M values that were slightly decreased compared to the control. Thus, the absence of a strong photochemical impairment suggests that the effect of salt stress on CO_2 assimilation was not severe contributing to explain the observed MS_L increase at 0.1 M and 0.2 M. However, the relations between growth and photosynthetic performance should be further investigated by direct measurements of leaf photosynthetic activity. In particular, the role of stomatal conductance should be clarified as it is a target of salinity stress. As far as we know, studies on gas exchange measurements in *C. edulis* are scarce possibly because of the difficulty in enclosing its succulent leaf in a readily available leaf chambers. Overall, the results showed that photochemical parameters as well as photosynthetic pigment and nitrogen content are important parameters to analyze the growth capacity of *C. edulis* under salt stress. Indeed, *C. edulis* has an intermediate behavior between typical halophytes and salt-sensitive plants depending on NaCl concentration and duration of application. Accordingly, *C. edulis* may be defined as a facultative halophyte. Facultative halophytes live in less saline habitats and are able to cope with saline and non-saline conditions (Parida and Das, 2005). The capacity of *C. edulis* to modulate its response to salinity could enhance its colonization success. In particular, the salt stimulated shoot elongation at low or moderate salt concentrations makes *C. edulis* even more efficient in establishing within the areas which it colonizes since the MS functions to extend the plant into the environment increase the ability of a species to explore surrounding areas (Traveset et al., 2008).

Considering that *C. edulis* represents a dangerous threat to the Mediterranean flora and that few physiological data are available for adult plants, the obtained results may be used to advance hypotheses on the competitive capacity of *C. edulis* under saline conditions. However, comparative studies of Mediterranean native plant species from sand dune habitats will clarify whether *C. edulis* has a competitive advantage in these habitats. Debez et al. (2008) for example, reported that *Cakile maritima*, a psammophilous species growing in Mediterranean sand dune habitats, showed a salt stimulated growth at salinity ranging between 0.1 M and 0.2 M (up to 15% more than the control), but its growth capacity was lower compared with our results for *C. edulis* in the same salinity range.

Since, it is predicted that climate change will bring an increase in soil salinity, which might stimulate the expansion of *C. edulis*, it is thus important that effective management is implemented in areas that are prone to invasion by *C. edulis* in order to limit its expansion and to protect native biodiversity.

Acknowledgements

This paper was supported by the grant "Ricerche Universitarie 2013" (code: C26A13JZ89) from Sapienza University of Rome for the year 2013.

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