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Effect of the foliar application of cyanobacterial hydrolysate (*Arthrospira platensis*) on the growth of *Petunia x hybrida* under salinity conditions

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Abstract
Salinity is one of the environmental factors inhibiting productivity in crop plants. Available strategies to mitigate the abiotic stresses are limited. Microalgae and cyanobacteria can mitigate the adverse effects of abiotic stress due to their biostimulant properties. The aim of this work was to study the effect of hydrolysate of the ciyanobacterium *Arthrospira platensis* on the growth of *Petunia* plants under salt stress conditions over two growing seasons (winter and spring). Plants were exposed to 2.0, 2.5, and 3.0 dS m^{-1} EC, with and without application of *A. platensis* hydrolysate. At the end of the trial, biometric parameters and plant analysis were determined. Petunias had a negative response when the salinity level rose from 2 to 3 dS m^{-1} and exhibited moderate tolerance to high internal Na and Cl concentrations. Results show that a high salinity reduces the Ca, Mg, K, and S contents in the leaves and provokes a sharp reduction in the K^+/Na^+ relationship. Applying *A. platensis* hydrolysate alleviates the effects of NaCl stress and stimulates shoot and bud formation in the petunia mother plant while inducing flowering in commercial *Petunia* plants. The *A. platensis* hydrolysate application increased the K^+/Na^+ relationship in treatments with an EC of 3.0 dS m^{-1}.

Keywords NaCl · Biostimulants · Abiotic stress · Ornamental plant

Introduction

Salinity is one of the most decisive environmental factors limiting the productivity of crop plants, especially in arid and semiarid regions (Zörb et al. 2019). More than 900 million ha of land worldwide are affected by salinity, which represents 20% of total agricultural land (Arroussi et al. 2017). There has been an increase in the incidence of abiotic stress over recent years, largely due to climate change causing an unprecedented increase in extreme weather patterns and events (Battacharyya et al. 2015). Recent studies have demonstrated a negative impact of future climate on barley yield in the Mediterranean with some locations be less affected than others (Cammarano et al. 2019).

Salt accumulation leads to osmotic stress, ion toxicity, nutrient imbalances, and water deficit (Zörb et al. 2019). Limited plant growth resulting from salt stress cannot be assigned to a single physiological process; the metabolic explanations for this phenomenon include reductions in photosynthetic activity, stomatal closure, reduced water potential, deficiencies in nutrient uptake, inhibited leaf and fruit growth, and enzymatic and gene signaling changes (Escalante et al. 2015). However, salt tolerance of plant depends on species, climatic conditions, types of substrate or soil, and irrigation method (Niu et al. 2010). Niu et al. (2010) tested the response of several ornamental plants to saline irrigation water and found that shoot dry weight and growth index of ornamental pepper "Black Pearl" and vinca (*Catharanthus roseus*) "Rose" decreased linearly as salinity increased. However, the salinity thresholds of irrigation water in which growth reduction occurred were 4.0 dS m^{-1} for *Angelonia angustifolia* and ornamental pepper (*Capsicum annuum*) "Calico." Arun et al. (2016) screened petunia cultivars against sodium chloride (0–125 mM) and observed that some cultivars were found to be highly

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susceptible to NaCl treatment, but others show some resistance against salinity.

Strategies that are currently available to mitigate the abiotic stresses that adversely affect plant productivity are scarce (Battacharyya et al. 2015). Some examples are external addition of some plant growth regulators, osmoprotectants, and nitric oxide (Arun et al. 2016; El-Sayed 2019). Biostimulants are products derived from organic material that, when applied in small quantities, are able to stimulate the growth and development of various crops under both optimal and stressful conditions (Ronga et al. 2019). Biostimulants can mitigate the adverse effects of abiotic stress via induction of stress-related genes and antioxidant molecules and enhance the plant content in beneficial phytochemicals (Ertani et al. 2019).

Microalgae are attracting the interest of agrochemical industries and farmers due to their biostimulant and biofertilizer properties (Ronga et al. 2019). Microalgae can also be produced using wastewater, thus reducing the production cost (Acien et al. 2016). Microalgae are a rich source of carbohydrates, lipids, proteins, pigments, and vitamins (Coppens et al. 2016) and contain phytohormones such as indole acetic acid, cytokinin, gibberellic acid, polyamines, and abscisic acid (Plaza et al. 2018). They are known to play crucial roles in plant development and represent a potentially sustainable alternative for enhancing and protecting agricultural crops (García-González and Sommerfeld 2016). *Arthrospira* (*Spirulina*) *platensis* is one of the photosynthetic cyanobacteria (El-Sayed 2019). This cyanobacterium has a rich content of protein, essential amino acids, minerals, vitamins, and essential fatty acids (Kim 2015). Additionally, *A. platensis* exhibited IAA-like and gibberellin (GA)-like activities (Ertani et al. 2019). The effect of *A. platensis* on plant growth rate is positively evident not only in growing plants under normal conditions (Ertani et al. 2019) but also in the growing plants under salt stress conditions by its valuable components that scavenge ROS and alleviate its adverse effects through regulating the metabolic processes in the plant (El-Sayed 2019).

Although *Petunia hybrida* is a popular and economically important ornamental plant, less attention has been paid to improve salt tolerance of this species (Arun et al. 2016). The aim of this work was to study the effect of *A. platensis* on the growth of *Petunia* plants under salt stress conditions over two growing seasons (winter and spring). Application of the microalgae was performed as a strategy to alleviate salinity stress.

Material and methods

Greenhouse trial

The trials were carried out in a 170 m^2 greenhouse with zenith ventilation as well as relative humidity and temperature control. Two experiments were performed—the first was conducted over the winter season (October–January), whereas the second was carried out in the spring (February–May). During the winter season, the mother plants, which provide the cuttings, are grown. In this case, the plant does not bloom. During the spring season, the commercial plants are cultivated. The temperature and relative humidity were recorded every 15 min with a HOBO U12-013 data logger (Onset Computer Corporation, USA) placed at canopy height in the central part of the cultivation table where the plants are grown. External radiation was measured every 15 min with a Q20-B sensor. To estimate the internal radiation, the cover transmission coefficient was estimated as a ratio between the internal and external radiation using a manual quantum photoradiometer (Detal OHM, model RAD/PAR). The average temperature, humidity, and global radiation in the winter season were 16.0 °C, 55.6%, and 0.52 MJ m^2 day^{-1}, respectively; the maximum and minimum averages of the temperature and humidity were 23.9 and 9.8 °C and 69.0 and 49.2%, respectively. The average temperature, humidity, and global radiation in the spring season were 17.5 °C, 68.8%, and 1.40 MJ m^2 day^{-1}, respectively; the maximum and minimum averages of the temperature and humidity were 24.6 and 12.4 °C and 79.6 and 52.9%, respectively.

The species studied was the *Petunia* x *hybrida* cultivar Surfinia Purple. Plant cuttings were transplanted into 2.5 L pots. In each pot, one plant was grown. The substrate used was a mixture of peat and perlite 80:20 (v/v). The white peat substrate (Brill WPS medium) is a potting substrate with high air capacity (41%), high stability, a cation exchange capacity of 99 meq (100 g)$^{-1}$, pH of 5.7, and salt content of 1.0 g L^{-1}. Projar expanded perlite has a total porosity of 92–98% and a size fractions of 1–5 mm. Fertigation was applied manually until the leachate fraction reached 20%. The average dose for *Petunia* x *hybrida* was 55 mL per plant per day in winter and 75 mL per plant per day in spring.

Arthrospira platensis hydrolysate preparation

Arthrospira platensis was supplied by Biorizon company (Almeria, Spain). The *A. platensis* hydrolysates were produced from a sludge containing up to 100 g L^{-1} of biomass by enzymatic hydrolysis under controlled conditions (pH = 8, temperature = 40 °C) for 4 h providing 0.2% weight biomass/weight of proteases (Alcalase and Flavourzyme from Novozymes). Enzymatic hydrolysis experiments were performed in a batch reactor of 9-cm diameter and 2:1 height to diameter ratio, equipped with temperature and pH control. Agitation was supplied by a 3-cm-diameter Rushton turbine. Nutrient content of the *A. platensis* hydrolysate was N 1.198 ± 0.005, P 0.187 ± 0.004, K 0.157 ± 0.005, Ca 0.157 ± 0.004, and Mg 0.048 ± 0.001 (%). The hormone content of the *A. platensis* hydrolysate was cytokinins 2752.1, gibberellins

56.24, indole acetic acid 10.3, ABA 1.03, salicylic acid 0.61, and jasmonic acid 0.84 ng g^{-1}.

Treatments

Six treatments were performed during the winter season: C2 (foliar application with water and fertigation EC 2.0 dS m^{-1}, control 1), Ar2 (foliar application with *A. platensis* hydrolysate and fertigation EC 2.0 dS m^{-1}), C2.5 (foliar application with water and fertigation EC 2.5 dS m^{-1}, control 2), Ar2.5 (foliar application with *A. platensis* hydrolysate and fertigation EC 2.5 dS m^{-1}), C3 (foliar application with water and fertigation EC 3.0 dS m^{-1}, control 3), and Ar3 (foliar application with *A. platensis* hydrolysate and fertigation EC 3.0 dS m^{-1}). During the summer season, two treatments were performed: C3 (foliar application with water and fertigation EC 3.0 dS m^{-1}, control 1) and Ar3 (foliar application with *A. platensis* hydrolysate and fertigation EC 3.0 dS m^{-1}). The hydrolysates *A. platensis* concentration was 5 g L^{-1}. Foliar spraying was applied weekly.

The concentrations (in mmol L^{-1}) in the C2 (standard nutrient solution) were as follows: 0.52 $H_2PO_4^-$, 7.20 NO_3^-, 0.71 NH_4^+, 1.71 SO_4^{2-}, 3.00 K^+, 2.49 Ca^{2+}, and 1.0 Mg^{2+}. Phosphoric acid, nitric acid, calcium nitrate, potassium nitrate, ammonium nitrate, and potassium sulfate were used for preparing nutrient solutions. The pH and EC of the irrigation water used to prepare the nutrient solution were 8 and 0.9 dS m^{-1}, and the pH and EC of the standard nutrient solution were 6 and 2 dS m^{-1}, respectively. After nutrient solution was prepared, NaCl was added to obtain 2.5 and 3.0 dS m^{-1} EC.

Nutrient solution analysis

The parameters determined in the nutrient solutions tested were pH, EC, NO_3^-, SO_4^{2-}, $H_2PO_4^-$, Ca^{2+}, Mg^{2+}, and K^+. The pH was measured with a Crison Micro pH 2001 pH meter and the EC with a Crison Micro CM 2200 conductivity meter. Nitrate, SO_4^{2-}, $H_2PO_4^-$, Ca^{2+}, Mg^{2+}, and K^+ were determined by HPLC (high performance liquid chromatography; Metrohm 883 Basic IC Plus). NO_3^-, SO_4^{2-}, and $H_2PO_4^-$ were quantified using a Metrosep A SUPP 4 column (with an IC conductivity detector range of 0–15,000 μS cm^{-1}). The mobile phase was prepared by mixing 190.6 mg of CO_3^{2-} and 142.8 mg of HCO_3^- and then diluting this in 1 L of deionized water, acidified with H_2SO_4 (50 mM). The Ca^{2+}, Mg^{2+}, and K^+ were quantified using a Metrosep C4 column (with an IC conductivity detector range of 0–15,000 μS cm^{-1}), and the mobile phase was prepared by mixing 117 mg of 2,6-pyridinedicarboxylic acid and 1.7 mL of nitric acid (1 M) diluted in 1 L of deionized water.

Biometric parameters

The fresh and dry weights, internode length, leaf length and width, and number of leaves and shoots were registered at the end of the cultivation, 70 and 49 days after the beginning of the trial for the winter and spring season, respectively. The number of leaves and shoots per plant was counted. The internode length and the leaf length and width were measured using a ruler. The number of flowers per plant was counted every week during the crop cycle. After removing the substrate, the plant material comprising 12 plants per treatment was separated into different vegetal fractions, absorption organs (roots), conductive organs (stems and petioles), photosynthetic organs (leaves), and flowers, which were weighed separately on a COBOS G M5-1000 scale (with a precision of 0.005 g) to determine the fresh weight (FW). All the samples were washed and dried in a Nüve EFN500 oven (with a 30 to 300 °C range) at 60 °C for 48 h to determine the dry weight (DW). The total DW was calculated as the sum of the roots, stems and petioles, leaves, and flowers. The FW and DW totals were used to calculate the water content (WC) as *(FW-DW) × 100/FW*. The leaf area was estimated by a nondestructive method, using the formula $S = a + bLW$, as proposed by Giuffrida et al. (2011), where S is the foliar surface area, L is the leaf length (cm), W is the leaf width, and the a and b coefficients are specific to each species. Leaf color was identified according to the Munsell chart for leaves. Munsell color is based on a three-dimensional model in which each color comprised three attributes of hue (color itself), value (lightness/darkness), and chroma (color saturation or brilliance). The color of the leave was visually matched.

Plant analysis

A subsample of the dry matter (0.2 g of dry weight) was ground up in a Wiley mill and digested (in 96% H_2SO_4) in the presence of hydrogen peroxide (H_2O_2, 30% (w/v)) at 300 °C in a sand bath to analyze the organic N, P, K, Ca, and Mg. Each treatment was replicated four times. The total Na and K was directly measured by flame spectrophotometry using an Evans Electro Selenium LTB Flame Photometer (Halstead, England). The total Ca and Mg were analyzed by atomic absorption spectrophotometry using a Perkin Elmer Atomic Absorption Spectrometer 3300. P was analyzed using the method proposed by Hogue et al. (1970) and nitrogen was analyzed using the method proposed by Krom (1980). Nitrogen was quantified by colorimetry at 630 nm using reagent A (sodium salicylate and sodium nitroprusside) and reagent B (sodium hydroxide and

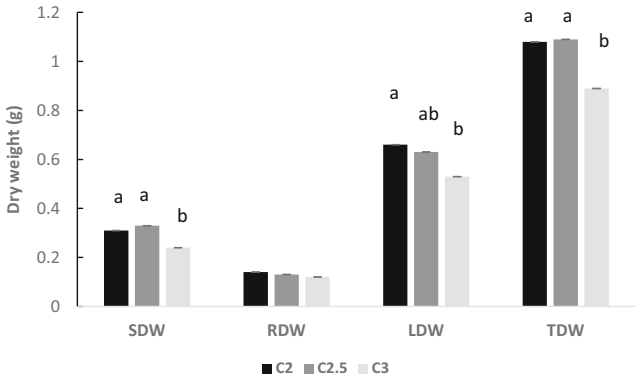


Fig. 1 Dry weight (g) of S (stems and petioles, conductive organs), R (roots), L (leaves), and T (total) at the end of the trial for the C2 (foliar application with water and fertigation EC 2.0 dS m^{-1}), C2.5 (foliar application with water and fertigation EC 2.5 dS m^{-1}), and C3 (foliar application with water and fertigation EC 3.0 dS m^{-1}) treatments. Different letters indicate significant differences between treatments at the $P < 0.05$ level using the LSD test. Values are the means of 12 plants. Error bars show standard deviations

sodium dichloroisocyanurate) and P at 430 by the molybdo-vanadate method.

Experimental design and statistical analysis

The experimental design comprised a completely randomized block with 6 (winter season) and 2 (spring season) treatments. There were 4 replicates per treatment and 3 plants (pots) per replicate (12 plants per treatments).

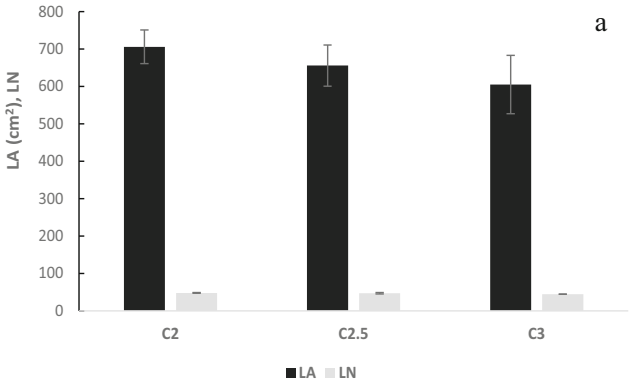
The treatment effect significance was examined using the standard analysis of variance (one-way ANOVA) and Fisher's least significant difference (LSD) test, performed using Statgraphics Centurion XVI.II software (Statpoint Technologies, Inc., USA). Differences were considered significant at $P < 0.05$.

Results

Biometric parameters

No differences in stems, root, leaves, and total dry weight between the C2 and C2.5 dS m^{-1} treatments were found (Fig. 1). However, petunias had a negative response when the salinity level rose from 2 to 3 dS m^{-1} (Fig. 1). Differences in the stems, leaves, and total dry weight were observed. The treatments with an EC of 3.0 dS m^{-1} resulted in a reduction of more than 22, 19, and 17% in the stems, leaves, and total weight, respectively. Moreover, the length of the leaves reduced when the EC increased from 2 to 3 dS m^{-1}; however, this reduction had no effect on the leaf area (Fig. 2). No differences in the root dry weight, leaf area, internode length, or number of leaves were observed between treatments C2 and C3 (Fig. 2a and b). Regarding the root/shoot dry weight, there were fewer differences between treatments. The shoot/root ratios for the C2, C2.5, and C3 treatments were 0.14, 0.14, and 0.16 g g^{-1}, respectively. The Munsell chart was used to define color for all the treatments at the end of the assay, and the leaves were identified as 5GY 5/8 for color, value, and chroma, respectively.

During the winter season, the *A. platensis* hydrolysate foliar application was effective at inducing salt stress tolerance in the treatments with an EC of 3.0 dS m^{-1} (Fig. 3). The number of leaves and the root and total dry weight were higher in the treatments with an EC of 3.0 dS m^{-1} when *A. platensis* hydrolysate was applied compared with the control (an EC of 3.0 dS m^{-1} and a water foliar application). The microalgae foliar application increased the number of shoots in all the treatments compared with the control. The *A. platensis* hydrolysate application enhanced the number of leaves by 16% for the treatments with 3 dS m^{-1}; enhanced the number of shoots by 27, 15, and 27% for the treatments with 3.0, 2.5, and 2.0 dS m^{-1}, respectively; and enhanced the leaf lengths by 7,



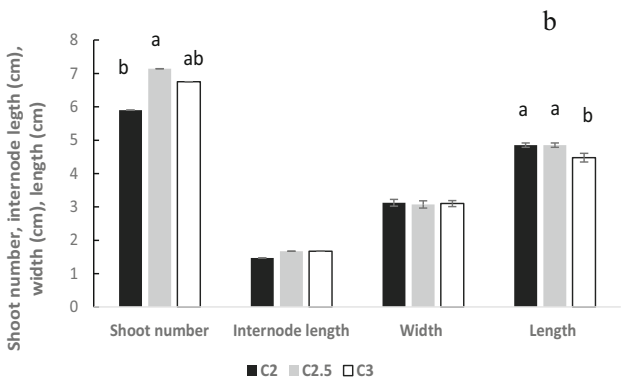


Fig. 2 Leaf area (LA) and leaf number (LN) (a) and shoot number, internode length and width, and leaf length (b) for the C2 (foliar application with water and fertigation EC 2.0 dS m^{-1}), C2.5 (foliar application with water and fertigation EC 2.5 dS m^{-1}), and C3 (foliar application with water and fertigation EC 3.0 dS m^{-1}) treatments. Different letters indicate significant differences between treatments at the $P < 0.05$ level using the LSD test. Values are the means of 12 plants. Error bars show standard deviations

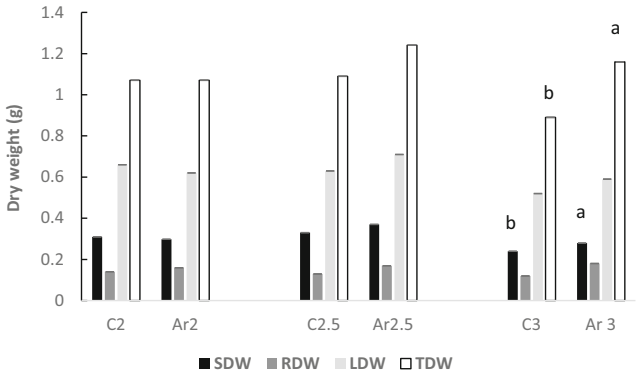


Fig. 3 Dry weight (g) of S (stems and petioles, conductive organs), R (roots), L (leaves), and T (total) at the end of the trial for the C2 (foliar application with water and fertigation EC 2.0 dS m^{-1}), Ar2 (foliar application with *Arthrospira platensis* and fertigation EC 2.0 dS m^{-1}), C2.5 (foliar application with water and fertigation EC 2.5 dS m^{-1}), Ar2.5 (foliar application with *Arthrospira platensis* and fertigation EC 2.5 dS m^{-1}), C3 (foliar application with water and fertigation EC 3.0 dS m^{-1}), and Ar3 (foliar application with *Arthrospira platensis* and fertigation EC 3.0 dS m^{-1}) treatments.. Different letters indicate significant differences between treatments at the $P < 0.05$ level using the LSD test. Values are the means of 12 plants. Error bars show standard deviations

9, and 7% for the treatments with 3.0, 2.5, and 2.0 dS m^{-1}, respectively, when compared with the control (Fig. 4 a and b). However, no differences in leaf area, internode length, leaf width, or shoot and leaf dry weight were found. Furthermore, no differences in water content were observed. The values for C2, Ar2, C2.5, Ar2.5, C3, and A3 were 93.61 ± 0.78, 93.68 ± 0.46, 98.87 ± 0.27, 93.64 ± 0.56, 94.8 ± 0.29, and 94.28 ± 1.29%, respectively.

In the spring season, the *A. platensis* hydrolysate application had a positive effect on the number of flowers, the dry weight of the flowers, and the number of leaves when the plants were irrigated with an EC of 3.0 dS m^{-1} (Figs. 5, 6a, b, and 7 a and b). However, no differences were found in terms of the shoot numbers, total dry weight, leaf area, internode length, width and length of the leaves, or water content. The water content values for C3 and Ar3 were 91.5 ± 0.01 and 91.5 ± 0.01, respectively.

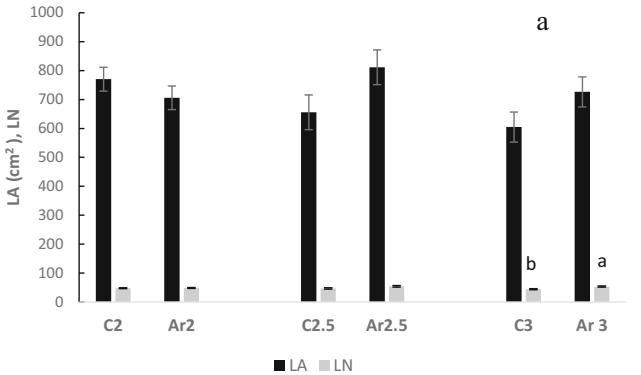
Nutritional status

The Cl^- and Na^+ levels were significantly higher in the leaves of plants that were given the salinity treatments (Table 1). However, N and P were not affected by the salinity treatments. Ca, Mg, K, and S decreased as the salinity increased. Moreover, the salinity treatment reduced Mg (by 20 and 16%), S (by 29 and 19%), Ca (by 18 and 24%), and K (by 18 and 28%) in the C2.5 and C3 treatments, respectively, compared with C2.0.

The *A. platensis* hydrolysate application increased the P content in the leaves by about 23% (Fig. 8a). However, the nitrogen, potassium, calcium, magnesium, sulfur, sodium, and chloride contents were not affected by the microalgae treatment (Fig. 8 a and b).

Discussion

It has been estimated that abiotic stresses lower production yield to less than 50% (Battacharyya et al. 2015). Under non-stressed conditions, the plants use most of the energy in processes necessary for maintenance and vegetative and generative growth. However, resource allocation changes with increasing levels of salinity since more resources are invested to mitigate the stress (Zörb et al. 2019). Petunias showed moderate tolerance to saline irrigation water when applied continuously, given that no difference was found in treatment



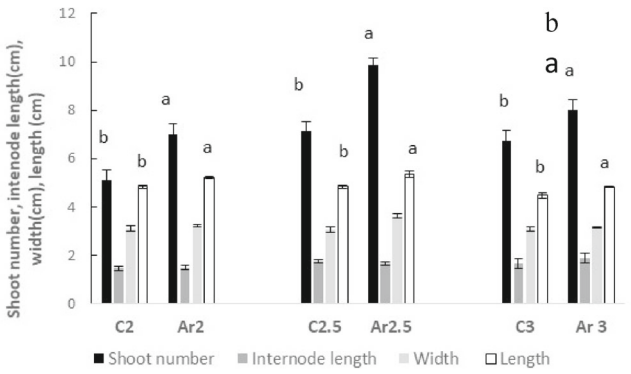


Fig. 4 Leaf area (LA) and leaf number (LN) (a) and shoot number, internode length and width, and leaf length (b) for the C2 (foliar application with water and fertigation EC 2.0 dS m^{-1}), Ar2 (foliar application with *Arthrospira platensis* and fertigation EC 2.0 dS m^{-1}), C2.5 (foliar application with water and fertigation EC 2.5 dS m^{-1}), Ar2.5 (foliar application with *Arthrospira platensis* and fertigation EC 2.5 dS m^{-1}), C3 (foliar application with water and fertigation EC 3.0 dS m^{-1}), and Ar3 (foliar application with *Arthrospira platensis* and fertigation EC 3.0 dS m^{-1}) treatments.. Different letters indicate significant differences between treatments at the $P < 0.05$ level using the LSD test. Values are the means of 12 plants. Error bars show standard deviations

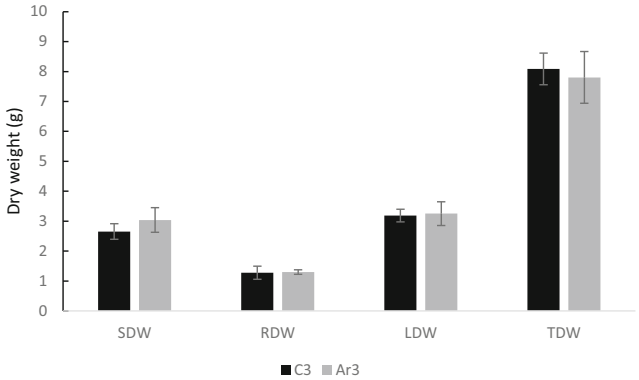
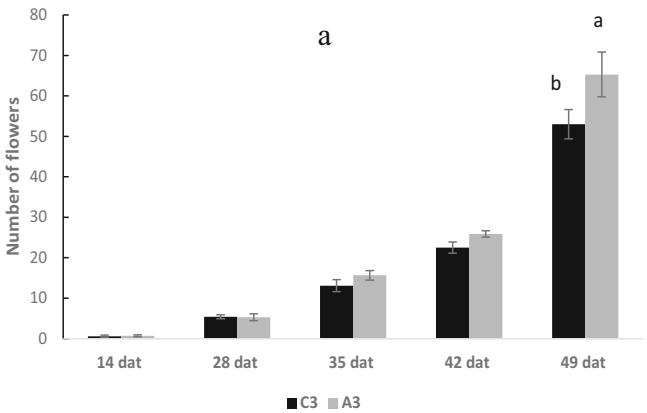


Fig. 5 Dry weight (g) of S (stems and petioles, conductive organs), R (roots), L (leaves), and T (total) for C3 (foliar application with water and fertigation EC 3.0 dS m^{-1}) and Ar3 (foliar application with *Arthrospira platensis* and fertigation EC 3.0 dS m^{-1}) treatments. Different letters indicate significant differences between treatments at the $P < 0.05$ level using the LSD test. Values are the means of 12 plants. Error bars show standard deviations

C2.5 when compared with C2; nonetheless, a negative response was observed when the salinity level rose to 3 dS m^{-1}. Salt tolerance is defined as the ability of a plant to withstand the effects of high or increasing salinity without significant adverse effects such as growth and yield reduction or foliar salt damage (Niu and Sun 2019). Our results are in line with those of Cardarelli et al. (2012), who found that the highest ECs recorded (2.0 ± 0.5 dS m^{-1}) using subirrigation with a full strength nutrient solution on petunias had no effect on the plant growth parameters (the shoot dry weight, the root-to-shoot ratio, and the final leaf area) or the number of flowers. At a lower stress intensity, tolerant species maintain homeostasis (by efficient compartmentation and nutrient uptake and by not upregulating defense mechanisms), while sensitive species may already encounter disturbances in homeostasis and activate general defense responses (Zörb et al. 2019). As a rule of thumb, tolerant species often tune their antioxidant systems specifically (Zörb et al. 2019). However, when plants are subjected to high levels of salinity, all parts of the plant, including the leaves, stems, roots, and flowers, may be reduced in size (Niu and Sun 2019). According our result, the ECs in the lixiviation solution for treatments with an EC of 3.0 and 5 dS m^{-1} (data not presented) were clearly supraoptimal for a soilless petunia culture (> 2.25 dS m^{-1}) (Cardarelli et al. 2012). Both the salt tolerance and sensitivity of a specific crop depend on its ability to extract water and nutrients from saline soils and to avoid excessive tissue accumulation of salt ions (Ahmad et al. 2017). Some species tolerate salt stress by avoiding uptake of certain ions or by tolerating high ion concentration in the tissue. Sensitivity or tolerance to high internal Na or Cl levels varies among species. The bedding plants tested in this study have moderate tolerance to high internal Na and Cl concentration. Increasing salinity to 2.5 and 3.0 dS m^{-1} significantly increases the leaf concentration of Na^+ and Cl^- by about 1.4 and 1.2 and 1.7 and 1.4 times, respectively, compared with the control. Moreover, the change in the K:Na ratio seems to affect the bioenergetic processes of photosynthesis (Sudhir and Murthy 2004); the K:Na ratio in this assay changed from 3.18 for C2 to 1.84 and 1.31 for the C2.5 and C3 treatments, respectively. Sodium toxicity is based mainly on its competition with K; therefore, the cytosolic K:Na ratio, rather than the Na concentration alone, causes deleterious effects from elevated Na uptake in the plant (Marschner 2012). A salinity-induced decrease in anion uptake, in response to competition with Cl^- for uptake sites, may occur in some plant species (Marschner 2012). In this assay, only S was reduced by salinity. The inhibition of NO_3^- uptake by Cl^- depends on the plant species and the concentrations of both NO_3^- and Cl^- in the uptake medium (Xu et al. 2000). In petunia plants, the N concentration in the leaves showed no significant differences between treatments, which suggests that there are no uptake problems. A decrease in the uptake of cations in response to salinity is a common observation; hence, Mg^{2+}, Ca^{2+}, and K^+ decrease with salinity. In our study,



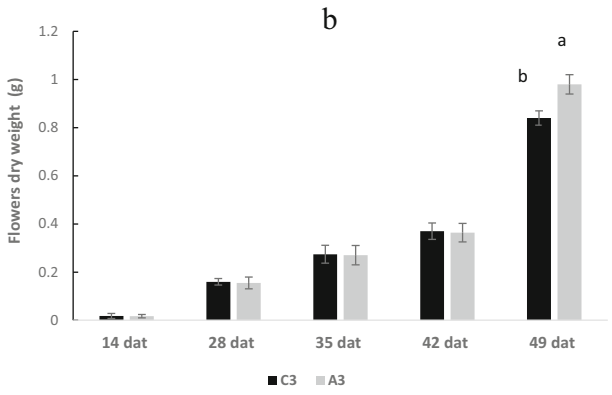


Fig. 6 Number of flowers (a) and dry weight (g) of flowers (b) for the C3 (foliar application with water and fertigation EC 3.0 dS m^{-1}) and Ar3 (foliar application with *Arthrospira platensis* and fertigation EC 3.0 dS m^{-1}) treatments Different letters indicate significant differences between treatments at the $P < 0.05$ level using the LSD test. Values are the means of 12 plants. Error bars show standard deviations

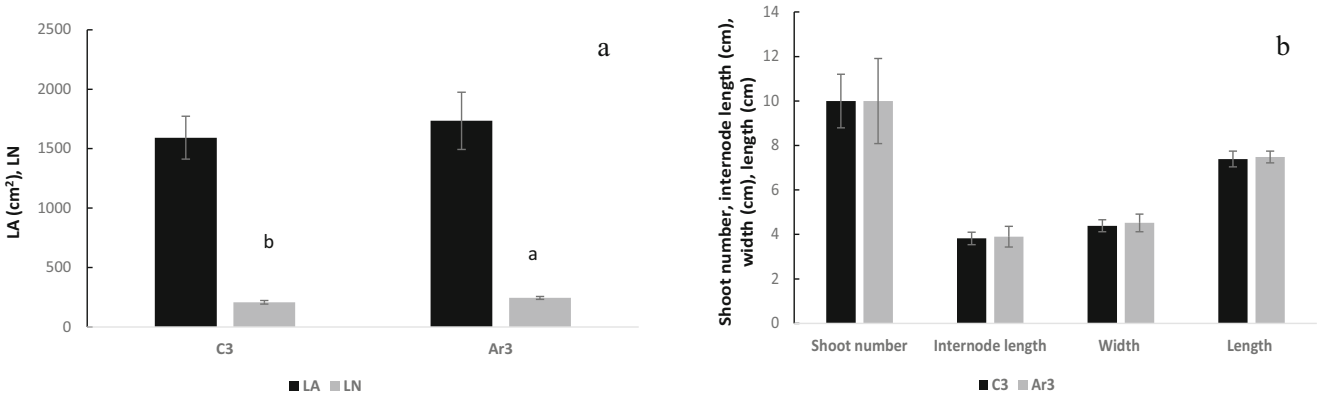


Fig. 7 Leaf area (LA) and leaf number (LN) (a) and shoot number, internode length and width, and leaf length (b) for C3 (foliar application with water and fertigation EC 3.0 dS m^{-1}) and Ar3 (foliar application with *Arthrospira platensis* and fertigation EC 3.0 dS m^{-1}) Different letters indicate significant differences between treatments at the $P < 0.05$ level using the LSD test. Values are the means of 12 plants. Error bars show standard deviations

however, no visual nutrient deficiency symptom was seen; we observed no differences in leaf color between treatments. Potassium loss causes diminished plant growth and development (Khalid et al. 2015) and has a key role in the plant-water relationship. Lower concentrations of K^+ were observed in treatments C2.5 and C3 compared with the control although no significant differences in the water content were recorded between C2, C2.5, and C3. Decreases in the Mg^{2+} content of the leaves have also been reported in relation to salt accumulation in mangrove (*Bruguiera parviflora*), suggesting increasing membrane stability and decreased chlorophyll content, respectively (Parida et al. 2004). A decreased Ca uptake could be the result of Ca moving through the apoplasm of root tissues and being inhibited by the presence of positively charged Na. Both ions may also compete for plant uptake via non-selective cation channels (Marschner 2012).

Several studies have reported a positive effect of microalgae on plant growth. But less attention has been paid to ornamental crops. Regarding ornamental plants, Plaza et al. (2018) showed that a foliar application of *A. platensis* hydrolysate improved the root dry matter and the number of flowers per plant. In our assay, the *A. platensis* hydrolysate application enhanced plant growth in high salinity treatments both in the winter and spring. This result is supported by several studies, such as Guzmán-Murillo et al. (2013), which showed that the adverse effects of salt stress were decreased by adding microalgae extracts to the seeds. The plant stress tolerance mechanism coming from seaweed is not well studied, but research suggests that some of its bioactive components, especially betaines and cytokinins, are involved in stress management (Mukherjee and Patei 2019). Cytokinin is the hormone that is most present in *A. platensis* hydrolysate (2.752 ng g^{-1}) (Plaza et al. 2018). Targeted elevation of cytokinin levels was found to increase the tolerance of plants to abiotic stress, at least in part by diminishing the negative stress effects on photosynthesis (Vankova 2014). Furthermore, cytokinins are involved in many aspects of plant growth, development, and floral transition (Maria D'Aloia et al. 2011) and can promote shoot development, delay leaf senescence, and contribute to stress and pathogen responses (Novák et al. 2013). Moreover, foliar supplement with *A. platensis* in salinity treatment decreases the activity of the antioxidant enzymes, malondialdehyde, free amino acid, proline, and total phenol (El-Sayed 2019). In addition, the high molecular weight molecules (e.g., the polysaccharides and polyphenols) found in some microalgae, such as *Arthrospira*, work as biostimulants and impart resistance against biotic and abiotic stress (Arroussi et al. 2018; Mukherjee and Patei 2019). Arroussi et al. (2018) showed that exopolysaccharide treatment increased plant growth and balanced the K^+/Na^+ ratios. Arroussi et al. (2018) also demonstrated that the salt tolerance mechanisms were related to the jasmonic acid pathway trigger.

Table 1 Concentration in leaves (%) for the C2, C2.5, and C3 treatments

	N	P	K	Ca	Mg	S	Na	Cl
C2	4.95 ± 0.06	0.27 ± 0.04	2.99 ± 0.14a	0.92 ± 0.02a	0.25 ± 0.02a	0.31 ± 0.02a	0.94 ± 0.06c	1.31 ± 0.04c
C2.5	4.97 ± 0.20	0.26 ± 0.04	2.47 ± 0.09b	0.75 ± 0.03b	0.20 ± 0.01b	0.24 ± 0.02b	1.34 ± 0.06b	1.55 ± 0.06b
C3	5.08 ± 0.03	0.28 ± 0.03	2.15 ± 0.07c	0.70 ± 0.03c	0.20 ± 0.01b	0.26 ± 0.01b	1.63 ± 0.06a	1.86 ± 0.05a

Different letters indicate significant differences between treatments at the $P < 0.05$ level using the LDS test. Values are the means of 12 plants

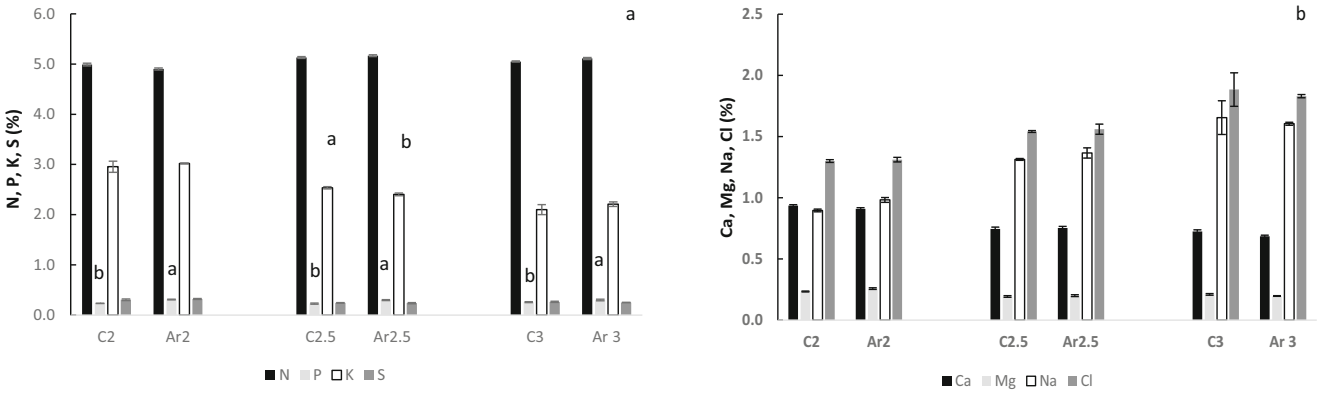


Fig. 8 Concentration in the leaves (%) for the C2 (foliar application with water and fertigation EC 2.0 dS m^{-1}), Ar2 (foliar application with *Arthrospira platensis* and fertigation EC 2.0 dS m^{-1}), C2.5 (foliar application with water and fertigation EC 2.5 dS m^{-1}), Ar2.5 (foliar application with *Arthrospira platensis* and fertigation EC 2.5 dS m^{-1}), C3 (foliar application with water and fertigation EC 3.0 dS m^{-1}), and Ar3 (foliar application with *Arthrospira platensis* and fertigation EC 3.0 dS m^{-1}). Different letters indicate significant differences between treatments at the $P < 0.05$ level using the LSD test. Values are the means of 12 plants. Error bars show standard deviations

Biostimulants, even those containing some mineral fertilizers, are not able to supply all the essential nutrients in the quantities the plant needs, their main function being to enhance plant tolerance to environmental stress (Mancuso et al. 2006). In addition to proper mineral fertilization, biostimulants can enhance the effectiveness of conventional fertilizers and promote greater nutrient translocation to the other organs (Mancuso et al. 2006). The analysis of *Arthrospir*a dry biomass revealed that it contains 6.70, 2.47, and 1.14% dry base of N, P, and K, respectively (Ronga et al. 2019), whereas the calcium content in this microalga is relatively low compared with the other minerals. Moreover, foliar applied *A. platensis* ameliorated adverse effects of salinity by enhancing N, P and K, total protein level, and photosynthetic activity (El-Sayed 2019). Greater accumulation of macro (Ca, Mg, and K) and micro (Cu, Zn) elements was also evident in plants supplied with this microalga (Ertani et al. 2019). In this assay, applying *Arthrospira* enhanced the P content in the leaves. One reason for increasing P uptake is to increase root growth. The *A. platensis* hydrolysate treatment significantly increased the root and shoot ratio. These values were 0.14 and 0.17 g g^{-1} for treatments C2 and Ar2, 0.14 and 0.16 g g^{-1} for C2.5 and Ar2.5, and 0.16 and 0.21 g g^{-1} for C3.0 and Ar3.0, respectively. A high root length to shoot dry matter ratio favors high macronutrient concentration in the shoots (Wang et al. 2016). In this regard, the presence of microalgae could benefit the absorption of nutrients and water, thus reducing the negative effects associated with a high NaCl concentration in the medium (Guzmán-Murillo et al. 2013). Another mechanism activated by seaweed extract is to significantly reduce the sodium ion accumulation in the plant; grass treated with seaweed extract had less sodium in the tissue compared with untreated grass (Battacharyya et al. 2015). Some plant rhizobacteria growth-promoting substances can produce polysaccharides, binding Na^+ in the root zone, and hence alleviate salt stress on the plant (Escalante et al. 2015). In our trial, no difference was found in the Na and Cl contents in the leaves between the control and the microalgae application. Nevertheless, the microalgae application increased the K^+/Na^+ relationship in treatments with an EC of 3.0 dS m^{-1} by 8%. The K^+/Na^+ values for C3 and Ar3.0 were 1.27 ± 0.02 and 1.38 ± 0.02 g g^{-1}, respectively. This increase was not observed in treatments with an EC of 2.0 and 2.5 dS m^{-1}, for which the values were 3.30 ± 0.17 and 3.18 ± 0.14 g g^{-1} for C2 and Ar2 and 1.93 ± 0.05 and 1.86 ± 0.12 g g^{-1} for C2.5 and Ar2.5, respectively. The application of sulfated exopolysaccharides on tomato plants alleviated the salt stress and mitigated the decrease in length and dry weight of the plant's shoot and root systems, as well as the K^+/Na^+ ratio (Arroussi et al., 2018).

Conclusions

The bedding plants tested in this study are moderately tolerant to salt stress and may be irrigated using EC 2.5 dS m^{-1} with no reduction in plant growth. A reduction in total dry weight was observed in petunias treated with the highest salt concentration (an EC of 3 dS m^{-1}). Ion toxicity resulting from excessive Cl and Na uptake and nutrient imbalance might be the cause of this reduction. The foliar application of *A. platensis* hydrolysate increased the P foliar concentration. There was increment in the number of leaves and the shoot and leaf length in the winter season and in the number of flowers, the flower dry weight, and the number of leaves in the spring season—all confirming the effectiveness of the microalgae. The foliar application of *A. platensis* hydrolysate mitigates the negative effect of NaCl on *Petunia* x *hybrida* crops at an EC of 3.0 dS m^{-1}.

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Compliance with ethical standards

Competing interest The authors declare that they have no competing interest.

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