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Original article – Thematic Issue

The influence of *Ecklonia maxima* seaweed extract on growth, photosynthetic activity and mineral composition of *Brassica rapa* L. subsp. *sylvestris* under nutrient stress conditions

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Summary

Over the past twenty years, several technological innovations have been proposed in order to enhance the sustainability of agricultural production systems through a significant reduction of chemicals. Plant biostimulants, like seaweed extracts (SWE) represent interesting tools for reducing the use of chemicals and improving crop tolerance to abiotic stresses, such as nutrient deficiency. The aim of the study was to determine growth, yield, photosynthetic rate, SPAD index, mineral composition and leaf quality of a greenhouse grown *Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort., also known as *friariello campano* either untreated or treated (via root application) with *Ecklonia maxima* SWE. For root application, 100 mL of solution containing 3 mL L^{-1} of SWE were applied to the growing medium at weekly interval. *Friariello campano* ecotype plants were supplied with three nutrient solution concentrations: full strength (EC = 2.0 dS m^{-1}), half strength (1.0 dS m^{-1}) and tap water (0.5 dS m^{-1}). Agronomical and physiological parameters were substantially reduced when the EC was 0.5 dS m^{-1}. Decreasing the EC in the nutrient solution from 2.0 to 0.5 dS m^{-1} increased the hydrophilic antioxidant activity and total phenols contents. The half-strength nutrient solution should be adopted under our conditions due to similar crop performance to the full strength solution, and the higher leaf antioxidant activity and total phenolics, whereas the total ascorbic acid was reduced. Root application of SWE increased fresh and dry yield by 13.5% and 16.6%, respectively, as well as hydrophilic antioxidant activity in comparison to untreated plants. The better crop performance of *friariello campano* ecotype plants treated with SWE was associated with an improvement of their nutritional status (higher P and K and lower Na concentrations), higher photosynthetic rate and chlorophyll content in plants independently of nutrient concentration treatment. The positive effect of SWE application could be attributed to the composition of brown seaweed extracts, especially the presence of polysaccharides and osmoprotectants as betaines and betaine-like compounds.

Keywords
antioxidant activity, *friariello campano* ecotype, gas exchange, Kelpak®, nutrient solution concentration, SPAD index

Significance of this study

What is already known on this subject?

- Extract of seaweeds have been gaining interest in sustainable agriculture due to their biostimulant effects that enhance nutrient use efficiency and stimulate plant development.

What are the new findings?

- The half-strength nutrient solution should be adopted under our conditions due to higher leaf quality and since no significant difference on yield was observed compared to the full strength solution. Improved nutritional status, higher photosynthetic rate and chlorophyll content recorded in seaweed extract (*Ecklonia maxima*) treatment may have assisted *friariello campano* ecotype plants in a more efficient translocation of minerals and assimilates to the sink irrespective of nutrient concentration treatment allowing a better plant growth.

What is the expected impact on horticulture?

- These findings might play a significant role in fertilizer management decisions, since the application of seaweed extract biostimulant can promote the performance of *friariello campano* ecotype in a sustainable way.

Introduction

The environmental impact of agricultural systems as well as the necessity to cut down the cost of production through a significant reduction of chemicals has become a primary issue in modern horticulture (Colla et al., 2015a; Rouphael et al., 2015). In order to meet these goals, growers must optimize the application of fertilizers which has been an efficient tool for enhancing crop productivity under both open-field and greenhouse conditions (Colla et al., 2015b). This is especially true in greenhouse industry where the amounts of applied fertilizers per unit area are much higher than any other agricultural systems (Rouphael and Colla, 2009).

Recently, several technological innovations have been proposed to promote the sustainability of production systems for vegetable crops through a reduction of fertilizer application (Colla et al., 2015b). An environmentally-friendly tool would be the use of natural products known as 'Plant Biostimulants' (PB) (du Jardin, 2015). PB's include several substances (chitosan, humic and fulvic acids, protein hydro-

lysates, seaweed extracts) and microorganisms (arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria, and *Trichoderma* spp.), whose functions when applied to plants and/or the soil are able to stimulate nutrient uptake, tolerance to abiotic stresses and to improve the product quality (du Jardin, 2015).

Seaweeds extracts have now gained wider importance as biostimulants in horticulture (Battacharyya et al., 2015). Seaweed extracts, particularly the brown macroalgae (e.g., *Ascophyllum nodosum*, *Ecklonia maxima*, *Durvillea potatorum* and *Macrocystis pyrifera*) are considered an important source of polysaccharides, phenolic compounds, osmolytes such as mannitol and also phytohormones including abscisic acid, auxins, brassinosteroids, cytokinins and gibberellins (Khan et al., 2009; Stirk et al., 2014; Battacharyya et al., 2015).

Several studies reported that aerial application and/or soil application of brown macroalgae is capable of inducing a broad range of physiological and biochemical responses such as improving root and shoot growth, affect flowering time, enhance crop productivity and nutritional quality as well as the macro- and micronutrients mobilization (Khan et al., 2009; Craigie, 2011; Battacharyya et al., 2015). In addition, the application of seaweed extracts (*A. nodosum* and *E. maxima*) has been reported to improve crop tolerance to unfavorable environmental and soil conditions such as thermal, drought and salt stress (Craigie, 2011; Xu and Leskovar, 2015; Rouphael et al., 2017).

Brassica rapa L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort., also known as *friariello campano*, is a cool-season leafy vegetable belonging to the family of *Brassicaceae*, widely cultivated in southern Italy. It has a significant commercial importance since consumers tend to associate traditional or *niche* product with unique taste and potential bioactive value. To our knowledge no published data in the scientific literature is available concerning the effectiveness of *E. maxima* seaweed extract to improve tolerance to nutrient deficiency of *friariello campano*.

To verify our hypothesis, we exposed SWE-treated and untreated plants to three nutrient solution concentrations (full, half strength and tap water) and investigated whether SWE application could improve tolerance to nutrient deficiency. SWE-treated and untreated plants were compared in terms of growth, leaf quality, mineral composition and photosynthetic activity.

Materials and methods

Experimental design, growth conditions and plant material

A greenhouse experiment was conducted at the experimental station of the University of Naples Federico II, located in Bellizzi (Salerno), southern Italy (lat. 43°31'N, long. 14°58'E) using a 3 × 2 factorial design and completely randomized with four replicates amounting to a total of 24 experimental units with 10 plants each (n = 240 plants). The treatments used were three nutrient solution concentrations (full-, half-strength and unfertilized control-tap water) and two biostimulant applications (control and root application).

Seeds of *friariello campano* ecotype were germinated in vermiculite on 9 February 2015. The *friariello* seedlings were transplanted 16 days after sowing (DAS; 24 February) into plastic pots (one plant per pot) containing 1.5 L of peat/perlite mixture in 2:1 volume ratio. Pots were disposed in single rows at a plant density of 27 plants m^{-2}. Plants were grown under natural light conditions. Inside the unheated greenhouse, the mean air temperature and relative humidity were 17°C and 65%, respectively.

Biostimulant application and nutrient solution management

The commercial SWE Kelpak® (Kelp Products, South Africa) was used in the current trial. Kelpak® is a commercial biostimulant produced from *E. maxima* brown seaweed, which is basically harvested from South Africa west coast (Papenfus et al., 2012). It contains phytohormones (mostly auxins 11.0 mg kg^{-1}, cytokinins 0.03 mg kg^{-1}, auxin:cytokinin ratio 367:1), carbohydrates (16.9 g kg^{-1}), amino acids (2.5 g kg^{-1}), low levels of nutrients (mostly N 3.6 g kg^{-1}, P 8.2 g kg^{-1}, K 7.2 g kg^{-1}, Ca 0.8 g kg^{-1} and Mg 0.2 g kg^{-1}) and negligible amounts of other compounds like vitamins and trace elements. For root application, 100 mL of solution containing 3 mL L^{-1} of Kelpak® was applied to the growing medium of each pot at weekly interval. Root application started on 2 March (22 DAS) and SWE was applied five times during the growing cycle.

The quality of the irrigation water was typical of the area, which is characterized by high bicarbonate concentration (5.5 mM). The concentrations of ions expressed as mM were: Ca (1.6), Mg (0.7), K (0.1), Na (0.4) and Cl (0.3). Values of pH and electrical conductivity (EC) were 7.35 and 0.52 dS m^{-1}, respectively.

The full strength nutrient solution was a modified Hoagland formulation with a composition of: 13.0 mM NO_3-N, 1.0 mM NH_4-N, 1.75 mM S, 1.5 mM P, 5.0 mM K, 4.5 mM Ca, 2 mM Mg, 1.0 mM Na, 1.0 mM Cl, 20 µM Fe, 9 µM Mn, 0.3 µM Cu, 1.6 µM Zn, 20 µM B, and 0.3 µM Mo. The half-strength nutrient solution had 50% of the macronutrient and the same micronutrient concentrations of the full-strength nutrient solution. In all tanks, the EC was 2 dS m^{-1}, 1 dS m^{-1} and 0.5 dS m^{-1} for the full-, half-strength and tap water treatments. In all treatments, the pH of the nutrient solution was 6.0 ± 0.2. The nutrient solution was pumped from independent tanks through drip-irrigation system, with one emitter per plant at a flow rate of 2 L h^{-1}. One to three fertigation events per day of 1 to 3 minutes was adopted. The nutrient solution was not recirculated to avoid any change in the nutrient concentration during the growing cycle.

Marketable yield and growth measurements

At final harvest (53 DAS), all plants were separated into leaves and stems. Both plant tissues were dried in forced-air oven at 80°C for 72 h for dried biomass determination. Shoot biomass was equal to the sum of aerial vegetative plant tissues (leaves + stems). The number of leaves and the total leaf area were also recorded. Leaf area was measured with an electronic area meter Li-Cor 3000 area meter (Li-Cor, Lincoln, NE, USA).

Soil plant analysis development (SPAD) index and color measurements

At the end of the growing cycle, the SPAD index was measured on fully expanded leaves by means of portable chlorophyll meter SPAD-502 (Konica-Minolta, Chiyoda, Tokyo, Japan). Fifteen leaves per experimental unit were randomly measured and averaged to a single SPAD value for each replicate. At the same date the Commission Internationale de l'Eclairage (CIE) color space parameters: L* (lightness), a* (-a* greenness) and b* (+b* yellowness) were recorded at the center of the upper leaf surface using a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd., Tokyo, Japan).

Net photosynthetic rate

Two days before the final harvest, the net CO_2 assimilation rate (A_{CO2}) was measured with a portable gas exchange analyzer (LCA-4; ADC BioScientific Ltd., Hoddesdon, UK) equipped with a broadleaf chamber. The measurement of A_{CO2} was carried out between 11:00 and 13:00 h on the youngest fully expanded leaves, using eight replicates for each treatment. Leaves were left for two minutes in the broadleaf chamber in order to reach a constant value. Photosynthetically active radiation, relative humidity and carbon dioxide concentrations were set at ambient values: 550 ± 50 µmol photons m^{-2} s^{-1}, 60 ± 5 % and 365 ± 5 ppm, respectively and the flow rate of air was 400 mL s^{-1}.

Leaf quality measurements

For the determination of nitrate content, antioxidant activity, ascorbic acid and total phenolic contents, fresh leaves were frozen in liquid nitrogen and stored at -80°C until used.

Nitrate content was determined on aqueous extracts of the dried leaf samples, based on the cadmium reduction method (Sah, 1994) using a Hach DR 2000 spectrophotometer (Hach Company, Loveland, Colorado, USA). The absorbance of the solution was determined at 550 nm wavelength, and the final result was expressed as fresh weight based on the dry matter content.

The hydrophilic antioxidant activity (HAA) from freeze dried leaves (200 mg) was extracted with distilled water and their antioxidant activity was measured with the N,N-dimethyl-p-phenylenediamine ($DMPD^+$) method (Fogliano et al., 1999) and expressed as mmol of ascorbic acid kg^{-1} dry weight. The principle of the assay is that in the presence of suitable oxidant solution DMPD can form a stable and colored radical cation ($DMPD^+$) Antioxidant compounds (AO) which are able to transfer a hydrogen atom to $DMPD^+$ quench the color and produce a discoloration of the solution proportional to their amount, hence a linear inhibition of color formation can be observed in the presence of antioxidant compounds extracted from leaf samples. HAA was determined by UV–Vis spectrophotometry based on extracts absorbance at 505 nm.

The total ascorbic acid was assessed by spectrophotometric detection on fresh plant tissues. The assay is based on the reduction of Fe^{3+} to Fe^{2+} by ASA and the spectrophotometric detection of Fe^{2+} complexes with 2,2-dipyridyl (Kampfenkel et al., 1995). Dehydroascorbate is reduced to ascorbic acid by pre-incubation of the sample with dithiothreitol (DTT). The absorbance of the solution was measured at 525 nm, and data were expressed as mg ascorbic acid on 100 g fresh weight.

Total phenolics were quantified in methanol extracts using the Folin-Ciocalteu procedure (Singleton et al., 1999) and gallic acid as an external standard. Briefly, 100 µl aliquot of the supernatant was combined with 500 µl of Folin-Ciocalteu's reagent (Sigma Aldrich Inc., St. Louis, MO, USA) and 400 µl of 7.5% sodium carbonate/water (w/v). The assay mixture was obtained inside centrifuge tubes, shaken in an IKA® Vortex Genius 3 (IKA®-Werke GmbH & Co. KG, Staufen im Breisgau, Germany) for 15 s and then allowed to stand for 30 min at room temperature. Absorption was measured after 30 min at 765 nm using a UV-Vis spectrophotometer, and the result was expressed as mg gallic acid (Sigma Aldrich Inc., St. Louis, MO, USA) per 100 g dry weight using an external standard calibration curve.

Mineral analysis

The dried leaf tissues were finely ground in a mill to pass through a 0.5-mm sieve. Then 1-g samples were used for the macronutrients (N, P, K, Ca and Mg) and Na analysis. Total N concentration was determined after mineralization with sulfuric acid in the presence of potassium sulfate and a low concentration of copper according to the Kjeldahl procedure (Bremner, 1965). Phosphorus, K, Mg, Ca and Na were extracted from 0.25 g samples with deionized water at 80°C in a shaking bath for 10 min. Then the solution was filtered, diluted and analyzed by ion Chromatography (ICS-3000, Dionex, Sunnyvale, California, USA) as described by Rouphael et al. (2017).

Statistical analysis

All data were statistically analyzed using the analysis of variance (ANOVA) in the SPSS 10 software package for Windows (www.ibm.com/software/analytics/spss). Duncan's multiple-range test was performed at $P \leq 0.05$ on each of the significant variables measured.

Results

Biomass production and net photosynthetic rate

The final leaf area, fresh and dry yield were influenced by nutrient solution concentration (C) and biostimulant treatment (B) with no significant C × B interaction, whereas the total leaf number per plant and the dry matter percentage were only affected by nutrient concentration (Table 1). Irrespective of biostimulant treatment, the final leaf area, fresh and dry yield were significantly higher using full and half-nutrient solution concentration, whereas the lowest values were recorded in plants irrigated only with tap water. Contrarily to the biometric parameters, dry matter percentage was positively affected when only water was used, with the highest value recorded at 0.5 dS m^{-1} (12.2%) compared to full and half-strength nutrient solution (avg. 7.8%; Table 1). Averaged over nutrient concentration levels, the final leaf area, fresh and dry yield in SWE-treated plants were higher

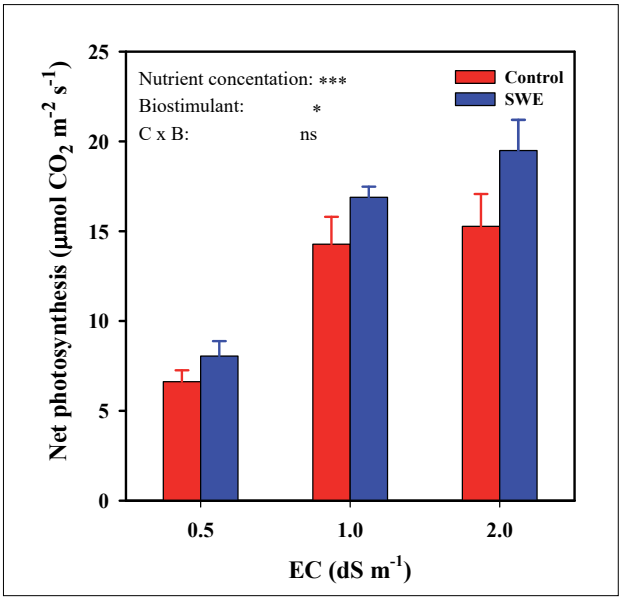


Figure 1. Effects of *Ecklonia maxima* seaweed extract (SWE) application and nutrient solution concentration on leaf net CO_2 assimilation rate. The values are means of four replicates. Vertical bars indicate ± SE of means.

Table 1. Effects of *Ecklonia maxima* seaweed extract (SWE) application and nutrient solution concentration on leaf number (LN), total leaf area (LA), marketable yield (fresh weight, fw), shoot dry weight (dw) and dry matter percentage in leaves of potted *friariello campano* ecotype.

Treatments	LN (n plant^{-1})	LA (cm^2 plant^{-1})	Yield (g fw plant^{-1})	Shoot (g dw plant^{-1})	DM (%)
Nutrient conc. (dS m^{-1})					
2.0	14.0 a	982.0 a	59.5 a	4.9 a	8.1 b
1.0	12.8 b	876.6 a	54.4 a	4.2 a	7.6 b
0.5	7.3 c	93.8 b	6.5 b	0.8 b	12.2 a
Biostimulant					
Control	11.3 a	608.3 b	37.6 b	3.0 b	9.2 a
SWE	11.4 a	693.4 a	42.7 a	3.5 a	9.4 a
Significance					
Nutrient concentration (C)	***	***	***	***	***
Biostimulant (B)	NS	*	*	*	NS
C × B	NS	NS	NS	NS	NS

Values are means of four replicates.
Different letters within each column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).
NS: non-significant; * $P < 0.05$, and *** 0.001, indicate level of significance.

Table 2. Effects of *Ecklonia maxima* seaweed extract (SWE) application and nutrient solution concentration on macronutrients and sodium concentrations of leaves of potted *friariello campano* ecotype.

Treatments	Mineral composition (g kg^{-1} dw)					
	N	P	K	Ca	Mg	Na
Nutrient conc. (dS m^{-1})						
2.0	55.0 a	11.4 a	65.6 a	30.2 a	5.2 a	2.4 a
1.0	53.5 a	10.3 a	51.3 b	28.7 a	4.7 ab	2.2 a
0.5	27.3 b	6.6 b	41.7 c	26.1 a	3.9 b	1.1 b
Biostimulant						
Control	44.8 a	8.2 b	47.9 b	27.7 a	4.4 a	2.4 a
SWE	45.5 a	10.7 a	57.8 a	29.0 a	4.8 a	1.4 b
Significance						
Nutrient concentration (C)	***	***	***	NS	***	*
Biostimulant (B)	NS	*	*	NS	NS	*
C × B	NS	NS	NS	NS	NS	NS

Values are means of four replicates.
Different letters within each column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).
NS: non-significant; * $P < 0.05$, and *** 0.001, indicate level of significance.

than those in untreated plants by 14.0%, 13.6% and 16.7%, respectively (Table 1).

The A_{CO2} was significantly influenced by biostimulant and nutrient concentration, with no C × B interaction (Figure 1). Increasing the EC in the nutrient solution from 0.5 to 2.0 dS m^{-1} increased the photosynthetic rate, with no significant difference observed between the full and half-strength nutrient solution (Figure 1). Root application of SWE induced higher values of A_{CO2} in comparison to untreated plants (Figure 1).

Mineral composition of leaves

Neither nutrient solution concentration nor biostimulant treatment had a significant effect on leaf Ca concentration (average 28.3 g kg^{-1} dw). The N, P, K and Mg concentrations in leaf tissue were adversely affected by the severe reduction of the nutrient solution EC (i.e., 0.5 dS m^{-1}; Table 2). The concentrations of N, P and Mg were similar in both full and half-strength solution. The SWE biostimulant treatment, averaged over all the nutrient concentrations, affected P, K and Na concentrations in leaf tissue which were higher by 31% and 21% (for P and K, respectively) and lower by 42% (for Na) than in untreated plants (Table 2).

Color parameters

Both nutrient solution concentration and biostimulant application significantly influenced SPAD index, a* and b* parameters, whereas the leaf lightness (L*) was affected by nutrient concentration only (Table 3). Decreasing the EC of the nutrient solution from 2.0 to 0.5 dS m^{-1} reduced the SPAD index, whereas an opposite trend was observed for the b* component with the highest values recorded with 0.5 dS m^{-1}. Moreover, half and full-strength nutrient solution intensified greener color of the leaf surface and yielded lighter leaves, expressed by decreasing L* values (Table 3). Moreover, irrespective of nutrient solution concentration, the SPAD index

Table 3. Effects of *Ecklonia maxima* seaweed extract (SWE) application and nutrient solution concentration on SPAD index and Hunter color parameters L* (brightness), a* (+a* = red; -a* = green) and b* (+b* = yellow; -b* = blue) potted *friariello campano* ecotype.

Treatments	SPAD index	L*	a*	b*
Nutrient conc. (dS m^{-1})				
2.0	33.8 a	43.8 b	-8.0 a	18.5 b
1.0	30.8 b	43.0 b	-7.9 a	17.5 b
0.5	25.1 c	49.1 a	-7.2 b	21.9 a
Biostimulant				
Control	27.9 b	45.5 a	-7.5 b	20.1 a
SWE	32.0 a	45.1 a	-7.8 a	18.5 b
Significance				
Nutrient concentration (C)	***	***	**	***
Biostimulant (B)	*	NS	*	*
C × B	NS	NS	NS	NS

Values are means of four replicates.
Different letters within each column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).
NS: non-significant; * $P < 0.05$, ** 0.01 and *** 0.001, indicate level of significance.

in SWE-treated plants was higher than the one observed in the control treatment by 14.7%, whereas an opposite trend was recorded on the Hunter color parameter b* with higher values observed in untreated plants (Table 3).

Nitrate, antioxidant activity, total ascorbic acid and phenolic contents

Hydrophilic antioxidant activity (HAA), total ascorbic acid (AA) and phenolic compounds (TP) were significantly affected by nutrient concentration and biostimulant, with insignificant C × B interaction, whereas the nitrate content was only influenced by nutrient solution concentration (Table 4). Decreasing the EC of the nutrient solution from 2 to 0.5 dS m^{-1} reduced the nitrate content, while it increased the HAA, AA and TP contents of *friariello campano* ecotype. The SWE biostimulant treatment averaged over nutrient concentration affected the HAA in leaf tissue which was higher by 38.1% than untreated *friariello campano* ecotype, whereas AA and TP contents decreased by 11.5% and 12.8%, respectively when the root application of the commercial SWE Kelpak®was delivered (Table 4).

Discussion

Horticultural crops grown commercially, especially those cultivated in hydroponics, are focused on increasing yields through high application of fertilizers to maximize growers' income, a fact that often results in a high environmental impact. Hence, limiting the use of chemical fertilizers without affecting crop performance is a priority for the vegetable industry (Rouphael and Colla, 2009). In the present study, the lowest nutrient solution concentration (0.5 dS m^{-1}) had negative influence on yield and plant growth parameters. Our findings also demonstrated that the nutrient solution concentration could be reduced by 50% (1.0 dS m^{-1}) without any detrimental effect on leaf area, fresh and dry yield (Table 1). The lack of significance between the full- and half-

Table 4. Effects of *Ecklonia maxima* seaweed extract (SWE) application and nutrient solution concentration on nitrate content (NO_3-N, mg kg^{-1} fw), hydrophilic (HAA, mmol ascorbic acid 100 g^{-1} dw) and lipophilic (LAA, mmol Trolox 100 g^{-1} dw) antioxidant activities, total ascorbic acid (AA, mg 100 g^{-1} fw), and total phenols (TP, mg gallic acid 100 g^{-1} dw) contents of potted *friariello campano* ecotype.

Treatments	NO_3-N	HAA	AA	TP
Nutrient conc. (dS m^{-1})				
2.0	3848.7 a	1.6 c	30.6 b	48.6 c
1.0	3835.6 a	2.6 b	14.6 c	58.3 b
0.5	366.0 b	3.2 a	50.3 a	63.8 a
Biostimulant				
Control	2635.2 a	2.1 b	33.8 a	60.8 a
SWE	2725.8 a	2.9 a	29.9 b	53.0 b
Significance				
Nutrient concentration (C)	***	***	***	*
Biostimulant (B)	NS	*	*	*
C × B	NS	NS	NS	NS

Values are means of four replicates.
Different letters within each column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).
NS: non-significant; * $P < 0.05$, ** 0.01 and *** 0.001, indicate level of significance.

strength solution on plant growth parameters, as well as on biomass production could be attributed to the similar nutritional status (N, P, Ca and Mg; Table 2). Our results are in line with studies carried out on leafy vegetables such as lettuce (Chen et al., 1997) and spinach (Maruo et al., 2001) in which the nutrient solution concentration was reduced by at least 50% without any differences on yield, biomass production and mineral composition. The lack of significant changes between full and half-strength solution on agronomical aspects, indicate that under moderate nutrient deficiency *friariello campano* ecotype plants were able to store and remobilize the mineral elements (Del Amor and Marcelis, 2004). This acclimation could be associated to the up regulation of nutrient uptake by plant root and may also depend of the crop species, since *friariello campano* ecotypes are characterized by low nutrient uptake rates.

However, the root application of SWE biostimulant, increased the fresh and dry biomass in comparison with those recorded in untreated plants. A possible explanation of the biostimulant effects of *E. maxima* SWE application might be a significant accumulation of P and K and a reduction of Na in leaf tissue. Our results are consistent with the findings of several authors (Nelson and Van Staden, 1984; Crouch et al., 1990; Beckett et al., 1994; Dobromilska et al., 2008) who observed that root or foliar application of *E. maxima* SWE on nutrient stressed cucumber, lettuce, tepary bean and tomato enhanced root growth as well as the uptake and translocation of macro- and micro nutrients. The beneficial effects of brown seaweed to nutrient deficiency have been associated to the synergetic effect of specific phytohormone-like activity (i.e., auxin) and polyamines (putrescine and spermine) present in Kelpak® (Papenfus et al., 2012). The combination of these compounds might stimulate rooting, increase absorption capacity of available nutrients and consequently the growth in nutrient-stressed crops (Papenfus et al., 2013).

It is well established, that nutrient stress conditions interferes with several aspects of plant physiology including photosynthesis and chlorophyll biosynthesis (Savvas et al., 2010). Irrespective of SWE application, the net CO_2 assimilation rate (A_{CO2}) sharply dropped at EC 0.5 dS m^{-1}, whereas no significant differences between the full- and half-strength nutrient solution were observed for A_{CO2} (Figure 1). The improved crop performance in terms of fresh and dry yield, which persisted throughout the three levels of nutrient solution EC, might be associated to increased A_{CO2}, which improves plant growth and yield. In fact, when averaged over nutrient solution concentration, the leaf A_{CO2} was significantly higher by 20.2% in Kelpak-treated (avg. 14.8 mmol CO_2 m^{-2} s^{-1}) than in untreated plants (avg. 12.0 mmol CO_2 m^{-2} s^{-1}). Similarly, Rouphael et al. (2016) found that under abiotic stress conditions (i.e., salinity); the foliar application of *E. maxima* SWE (3 ml L^{-1} at two weeks interval) could improve net photosynthesis mainly by reducing stomatal closure. In addition, the increased leaf area when root application of the commercial SWE was delivered, could also contribute to more photosynthetic products, leading to enhanced growth. Another important physiological parameter is the SPAD index, widely used as a non-destructive measurement of chlorophyll content (Colla et al., 2013a). When averaged over nutrient solution concentration, the SPAD index was increased by 14.7% in plants treated with SWE, suggesting that the macroalgal biostimulant was able to stimulate pigment biosynthesis and/or protecting pigment degradation, thus delaying early senescence. The increase in chlorophyll content in plants treated with SWE has been recorded previously on several vegetables including tomato, spinach, and zucchini squash (Kumari et al., 2011; Fan et al., 2013; Rouphael et al., 2017). Moreover, the beneficial effect of brown seaweed application might be associated in part to the presence of cytokinins and cytokinin-like compounds in Kelpak®, which are known to possess protective effects on chloroplasts (Zhang and Ervin, 2008; Khan et al., 2009; Battacharyya et al., 2015).

Significant variation in terms of leaf quality attributes in response to nutrient solution concentration and biostimulant root application has been recorded in the present study (Tables 3 and 4). For instance, when averaged over SWE application, the highest HAA, AA and TP and the lowest nitrate content were recorded with 0.5 dS m^{-1}. The half-strength nutrient solution should be preferred for *friariello campano* ecotype under our conditions due to similar biomass production of full-strength solution, and higher leaf quality (HAA and TP content; Table 4). Irrespective of SWE application, the accumulation of antioxidant molecules such as AA and TP was significantly lower in SWE-treated than in untreated plants (Table 4). These molecules are important to human health and have also a critical role in ensuring plant growth under environmental stresses (Atkinson and Urwin, 2012; Colla et al., 2013b). The lower content of antioxidant molecules in leaves treated with SWE, in comparison to untreated plants could be the consequence of reduced need to cope with oxidative stress. Therefore, in untreated plants the antioxidant system might not efficiently support Reactive Oxygen Species (ROS) scavenging in relation to nutrient deficiency, as demonstrated by the sharp decrease in photosynthesis activity and plant growth.

Conclusions

To summarize, it can be concluded that in both treated and untreated plants, fresh and dry yield, photosynthetic rate and macronutrient concentrations decreased sharply with nutrient solution having an EC of 0.5 dS m^{-1}. The half-strength nutrient solution should be preferred for *friariello campano* ecotype under our conditions due to similar crop performance of the full strength solution and the higher leaf nutrition value. The root application of SWE improved biomass production and the hydrophilic antioxidant activity. Improved nutritional status (higher P and K and lower Na), higher photosynthetic rate and chlorophyll content recorded in SWE treatment may have assisted *friariello campano* ecotype plants in a more efficient translocation of minerals and assimilates to the sink independently of nutrient concentration treatment allowing a better plant growth.

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