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Enhanced Growth and Nutritional Properties of Radish Sprouts Using Extracts from *Anabaena minutissima* and *Sargassum vulgare*

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ABSTRACT: This study investigated the seed priming effects with aqueous extracts from the cyanobacterium *Anabaena minutissima* (AM) and the brown seaweed *Sargassum vulgare* (SV) on the growth and nutritional properties of China Rose (CR), Daikon (D), and Sango Red (SR) radish varieties. AM and SV biomasses were chemically analyzed. FTIR spectra of biomasses exhibited functional groups characteristic of amides I and II of proteins in AM and functional groups associated with the pyranose ring of carbohydrates in SV. The extracts differed in total proteins, phycobiliproteins, carbohydrates, chlorophylls, carotenoids, and antioxidant activity. Seed priming with AM and SV particularly increased seed germination (2% in CR), moisture (5% in D with AM), sprout weight (35% with AM), and height (12% with SV). In the elemental analysis of sprouts, Na, Ca, and Mg levels increased variably across all varieties of both extracts. Principal component analysis revealed significant separation among treatments in SR and D varieties, confirming the effectiveness of the seed priming.

KEYWORDS: *radish sprouts, Anabaena minutissima, Sargassum vulgare, seed priming, antioxidant compounds*

INTRODUCTION

Our food system is a significant contributor to global greenhouse gas emissions, in addition to consuming large amounts of natural resources and causing loss of biodiversity. This can lead to negative health impacts and reduce the economic returns and livelihoods for all users, especially for primary producers.[1] Emerging technologies, scientific findings, and growing consumer awareness concerning healthy food demand are expected to benefit all users. In this context, realizing healthy, fair, and environmentally friendly food solutions is crucial. In the last half-century, with the study of nutritional models and the advent of the Mediterranean diet, food has been given a key role in the prevention and improvement of the quality of life, and this has become even more important with the study and use of healthy foods.[2,3] These new foods are rich in bioactive compounds that would help to maintain an healthy life and reduce the progression of disabling diseases as well as reducing their incidence and severity.[4] Consumers are increasingly aware of the importance of nutrition and require a diversified range of food that contributes to well-being. Still, little known in the Mediterranean diet, vegetable sprouts have been widely used in the vegan and vegetarian diets and due to their composition, sprouts are good examples of functional and healthy foods, thanks to their number of nutrients including amino acids, dietary fiber, trace elements, and vitamins as well as phenolic compounds.[5,6] Nowadays, the market offers many seeds and ready-to-eat sprouted seeds, which meet the consumer interest for their convenience of use, quality, and taste; moreover, due to their composition, sprouts deliver beneficial bioactive compounds once incorporated into our diet. Within the current diversity of sprouts, cruciferous types are noticed because of their high content of nitrogen−sulfur compounds (glucosinolates and their derivatives, isothiocyanates), phenolic compounds (mainly phenolic acids, flavonols, and anthocyanins), protein, and micronutrients. Radishes (*Raphanus sativus* L.) are a member of the cruciferous family, available in different varieties that vary in terms of shape, size, and color.[7] Some varieties are most famous in Asia, while others are more popular in Western countries, and nowadays, many of these varieties are available in fresh market and through specialized seed vendors. According to Li and Zhu,[8] vegetable sprouts have a higher nutritional value than mature plants. The content of phenolic compounds in cruciferous sprouts is ten times higher than in mature vegetables.[9] Furthermore, phenolic compounds are important secondary plant metabolites with a wide range of biological effects. Li and Zhu[8] described their effect in reducing the risk of cancer, inhibition of plasma platelet aggregation, diabetes and heart disease, in vitro antibacterial, anti-inflammatory, and antiallergenic effects. Furthermore, among nitrogen−sulfur compounds, isothiocyanates are known for their antioxidant, anticancer, and cancer-preventive effects.[10−12]

Some authors showed that alga extracts can enhance the growth, yield, and nutritional quality of radish plants and

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improve the yield, leaf photosynthesis, and ripening time of tomato.[13,14] Seaweed extracts are a mixture of biologically active substances, such as alginates, vitamins, proteins, free amino acids, polyphenols, and micro- and macro-elements that may be involved in plant growth stimulation.[15−17] Several studies have described the use of brown alga extracts to enhance the growth, yield, and nutritional quality of horticultural plants.[10−12] Some of them regard extracts from algae belonging to the *Sargassum* genus.[13,14] Mahmoud et al.[13] reported that radish seed and leaf treatment with *Sargassum vulgare* extract increased vegetative growth as well as chlorophyll and carotenoid content. On tomato, soil treatment with an extract from *Sargassum horneri* improved yield, leaf photosynthesis, and ripening time of tomato.[14] Application of extracts from brown algae belonging to other genera, such as *Cystoseira gibraltarica*, *Fucus spiralis*, and *Bifurcaria bifurcate*, improved the plant length, dry weights, and the content of sugars, proteins, chlorophyll *a*, chlorophyll *b*, and carotenoids.[18] Hashtroudi et al.[19] showed that cyanobacteria such as *Anabaena* sp. and *Nostoc* sp. applied to the soil increased plant height, root length, dry and fresh weight, and leaf number of tomato, cucumber, and squash. On *Lupius termis*, treatments with *A. flos-aquae* and *N. muscorum* increased seed germination, chlorophyll *a* and *b*, total carbohydrates, and total nitrogen content.[20]

Here, we investigate the effect of water extracts from the cyanobacterium *Anabaena minutissima* (AM) and the brown alga *Sargassun vulgare* (SV) as presowing seed soaking on physiological and biochemical parameters of radish sprouts that are important functional healthy food for human consumption. This study aimed to characterize AM and SV biomasses and their extracts and to evaluate the effect of water extracts on sprout characteristics such as (i) growth and yield, (ii) content of proteins and some antioxidant compounds, (iii) antioxidant activity, and (iv) elemental composition. This work addresses the objectives of sustainable consumption and production of nutritious food outlined in the 2030 Agenda, which aims to promote human and environmental well-being.

MATERIALS AND METHODS

The cyanobacterium AM (Lemmermann, 1898) BEA 0300B was provided by Banco Español de Algas (BEA; Spanish Bank of Algae). The strain was isolated from Fuerteventura Island, Canary Islands, Spain (28.400° N, 14.157° W), in 2010. It was cultivated in BG11 media under an irradiation of 120 μmol photons m^{-2} s^{-1}, provided by cool-white, fluorescent light bulbs, in a 16:8 h light−dark cycle, at 24 °C. Cells were harvested during the late exponential phase, approximately 8 days of cultivation, by centrifugation at 8000 rpm for 10 min and dehydrated using a freeze-dryer (Labconco, FreeZone 6) to obtain the microalgal powder.

The brown macroalga SV (Agardh, 1820) was collected from the east coast of the Gran Canary Island, Canary Islands, Spain (27.982° N, 15.375W), in June 1999. In particular, it was collected from the seaweed masses that wash ashore and form the wrack line. It was assumed that the material that arrived at the coast was in the same condition. After rinsing with distilled water, the mixture was lyophilized (Labconco, FreeZone 6) to produce the algal powder.

Chemical and ATR-FTIR Characterization of Biomass. Total carbon and nitrogen contents of AM and SV biomasses powder were determined using an elemental analyzer (CHNS-O EA 1110 Thermo Fisher Scientific, Massachusetts, USA).

The total metal content was determined on AM and SV biomasses by microwave-assisted acid digestion (Milestone, Shelton, CT, USA) with HNO_3 suprapure (Carlo Erba, Milano, Italy) and H_2O_2 (30 w/w %) as previously described by Righini et al.[21] After mineralization, the samples were diluted with Milli-Q ultrapure distilled water. Each element was determined via inductively coupled plasma-optical emission spectroscopy (Spectro Ametek Arcos II ICP-OES, Kleve, Germany). All analyses were performed in triplicate.

ATR-FTIR spectra of AM and SV lyophilized were obtained by using a Bruker TENSOR FTIR instrument (Bruker Optics, Ettlingen, Germany) equipped with an accessory for analysis in micro-attenuated total reflection [attenuated total reflection (ATR)]. The sampling device is a microdiamond crystal, a single reflection with an angle of incidence of 45° (Specac Quest ATR, Specac Ltd., Orpington, Kent, UK). Roughly 2−3 mg of fine-pulverized lyophilized biomass was placed on the surface of the crystal and then squeezed onto the head of the crystal. Spectra were recorded from 4000 to 400 cm^{-1}, at a spectral resolution of 4 cm^{-1} and 64 scans. Background against air before each sample was performed. To clean the diamond ATR between samples, ethanol was used. Spectra were processed with GRAMS/386 spectroscopic software (version 6.00, Galactic Industries Corporation, Salem, NH, USA).

Extract Preparation. For extract preparation, SV biomass was ground to obtain a fine powder with a mortar and pestle. Freeze-dried AM and SV powder were suspended in triplicate in sterile distilled water (5 mg powder/mL water) under continuous stirring at 50 °C for 12 h and then left to cool before filtration (15 min, 5000 rpm, at room temperature) (Righini et al., with modification).[22] The three extracts for AM and SV were pooled together before use. The pH of both extracts was carried out using a pH meter (Crison Instruments, Barcelona, Spain). The measurement was repeated 3 times.

UV−Vis Spectrophotometer Characterization of Extracts. For the determination of proteins, carbohydrates, chlorophylls, carotenoids, phycobiliproteins (PBP's), and antioxidant activity, extracts were frozen at −20 °C and then lyophilized. Ten milligrams of lyophilized extracts were used in triplicates for each determination at the spectrophotometer. For protein content, the Bradford[23] colorimetric method was used with bovine serum albumin (Bio-Rad Laboratories, Inc.) as a standard in a 96-well microplate (Greiner CELLSTAR). The plate was incubated in the dark for 10 min on an ice bath, and the absorbance was measured at λ = 595 nm. The protein content was expressed as μg of protein per milliliter of extracts.

Total soluble carbohydrates were determined following the colorimetric phenol-sulfuric acid method of DuBois et al.[24] Lyophilized extracts were suspended in 2 mL of trichloroacetic acid (10%) for 60 min at 90 °C and left to cool before centrifugation (20 min, 12,000 rpm, at room temperature). First, phenol (5%) was added to the supernatant (500 μL/500 μL; 1:1), and then concentrated sulfuric acid (3 mL) was added rapidly for color development. Then, absorbance was measured at 490 nm, and total carbohydrate concentration, as glucose equivalents per mL of extracts, was calculated.

The determination of chlorophylls and carotenoids was carried out by suspending the biomass in methanol (100%) and adding $MgCO_3$ (1 mg) as described by Righini et al.[25] The solution was mixed 3 times and left for 3 h in the dark. After the incubation, the solution was centrifuged and the supernatant was used for absorbance record at 665, 652, and 470 nm. The chlorophyll α, b concentration and carotenoid content were calculated using the equations of Lichtenthaler and Buschmann and Wellburn[26,27]

$$\text{chlorophyll } \alpha \text{ (Cl } \alpha\text{) } (\mu\text{g/mL}) = 16.72 \times A_{665} - 9.16 \times A_{652}$$

$$\text{chlorophyll } b \text{ (Cl } b\text{)}(\mu\text{g/mL}) = 34.09 \times A_{652} - 15.28 \times A_{665}$$

$$\text{carotenoids } (\mu\text{g/mL}) = (1000 \times A_{470} - 1.63\text{Cl } \alpha - 104.9 \text{ Cl } b)/221$$

To determine the contents in the extracts, all of the results were converted and expressed as μg/mL of extracts.

Phycobiliproteins were determined in AM extracts only since brown algae such as SV do not contain them. For their determination, AM lyophilized biomass was suspended in phosphate buffer (0.2 M, pH 7) and then stirred at room temperature in the dark. The solution

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was centrifuged for 20 min at 13 °C and 12,000 rpm, and the PBP's phycocyanin, allophycocyanin, and phycoerythrin in the supernatant were quantified at 652, 615, and 562 nm spectrophotometrically[28,29] by using the following equations

$$\text{Phycocyanin (PC) (mg/g)} = [A_{615} - (0.474 \times A_{652})]/5.34$$

$$\text{Allophycocyanin (APC) (mg/g)} = [A_{652} - (0.208 \times A_{615})]/5.09$$

$$\text{Phycoerythrin (mg/g)} = [A_{562} - (2.41 \times \text{PC}) - (0.849 \times \text{APC})]/9.62$$

The antioxidant activity of the extracts was determined by DPPH (2,2-diphenil-1-picrylhydrazyl) assay, according to Fukumoto and Mazza.[30] 50 μL of the extract (described above) was added to 1450 μL of a 0.06 mM DPPH• methanolic solution. The mixture was left to stand for 15 min in the dark, and then the reduction of the DPPH• radical was determined by measuring the absorption at 515 nm using a spectrophotometer (Beckman Coulter DU730 UV−vis spectrophotometer, Milan, Italy).

The radical scavenging activity was calculated as a percentage of DPPH• discoloration, by using the equation

$$[(AT_0 - AT_{15})/AT_0] \times 100$$

where AT_0 is the absorbance of the DPPH• solution; and AT_{15} is the absorbance of the solution after 15 min of incubation. Trolox (Merck KGaA, Darmstadt, Germany) was used for the standard calibration curve from 0.05 to 1 mM.

Plant Material. Radish sprout seeds of Daikon (D) and Sango Red (SR) were supplied by VIVO, Società Cooperativa Agricola, San Giorgio (Cesena, Italy), while China Rose (CR) seeds were purchased from Bavicchi Spa (Perugia, Italy) and stored in a dry and dark place at room temperature.

Seed Priming Treatment with Extracts. For seed priming, 1200 seeds for each radish variety (D, SR, and CR) were counted and divided into three groups: 400 seeds were soaked in the AM extract (30 mL), 400 in the SV extract (30 mL), and 400 in distilled water (30 mL) as the control for 12 h. Then, seeds were seeded on one dish of a germinator (model Geo TZZ0870 Geo Plus; Bavicchi, Italy). The germinators were then randomly incubated in a growth chamber at 25 °C, light exposure, 16/8 h-light/dark, and relative humidity of 70−75%, and sprayed with distilled water every day. After 7 days of incubation, the germination was recorded, and 20 g of sprouts randomly chosen was collected from each treatment and the control for each of the following measurements: moisture, nitrogen compounds, minerals, phenolic content and related antioxidant activity, anthocyanin, and peroxidase activity. Collected sprouts were immediately frozen at −20 °C until use. The experiment was repeated 3 times ($n = 3$).

Sprout Proximate Composition. *Moisture.* An amount of 5 g of sprouts was thinly ground and then dried in an oven at 110 °C until a constant weight was achieved. Moisture was expressed by % (w/w).

Total Nitrogen Compounds. One gram of the sample was digested with H_2SO_4, distilled, and titrated according to the Kjeldahl method, Norma FIL-IDF, no. 20B, Brussels, Belgium, 1993.[31]

Sprout Chemical Characterization. The mineral content of sprouts was determined by microwave-assisted acid digestion with HNO_3 suprapure (Carlo Erba, Milano, Italy) and H_2O_2 (30 w/w %), as described above for biomass chemical characterization.

Each element was determined via ICP-OES (Spectro Ametek Arcos II ICP-OES, Kleve, Germany), using the same seaweed elemental analysis protocol. All analyses were performed in triplicate.

Sprout Antioxidant Compounds and Antioxidant Activity. *Extraction Procedure.* The sample (5 g) was homogenized with 80% methanol (15 mL). Tubes were capped and stored for 12 h at 4 °C. Extracts were centrifuged at 12,000*g* for 10 min and stored at −20 °C until use.

Total Phenolic and Anthocyanin Content. Total phenolic content in radish sprouts was determined by using the Folin−Ciocalteu methods by Singleton and Rossi[32] with minor modifications. An aliquot of methanolic extract (described above) ranging from 50 to 200 μL was added to 50 μL of the Folin−Ciocalteu reagent (Merck KGaA, Darmstadt, Germany). The mixture was allowed to stand for 5 min and afterward, 2 mL of a 10% (w/v) aqueous Na_2CO_3 solution were added, and the volume was finally adjusted to 10 mL. The samples were allowed to stand for 90 min at room temperature before the absorbance at 700 nm was measured, using a spectrophotometer (Beckman Coulter DU730 UV−vis spectrophotometer, Milan, Italy). As a standard, (+)-Catechin (Merck KGaA, Darmstadt, Germany) was employed, and the total phenolic content is expressed as catechin equivalents per gram of fresh weight. The range of the linearity of the reference standard curve was 0.5−10 μg/mL.

Total anthocyanin content was analyzed by measuring the absorbance of each extract at pH 1 and pH 4.5.[33] The sample was homogenized with methanol for 12 h, followed by centrifugation at 12,000*g* for 10 min. The supernatant was collected and adjusted to pH 1 and 4.5 using KCl and CH_3COONa buffer solution, respectively. Absorbance of the extract was measured spectrophotometrically at 520 and 700 nm as follows

$$A = [(A_{520} - A_{700})_{\text{pH1}} - (A_{520} - A_{700})_{\text{pH4.5}}]$$

Anthocyanins were expressed as mg of cyanidin-3-glucoside equivalent/g of fresh weight, using a molar extinction coefficient of 26,900 M^{-1} cm^{-1} and a molecular weight of 449 g/mol. For all spectrophotometric readings, a spectrophotometer (Beckman Coulter DU730 UV−vis, Milan, Italy) was used.

DPPH Scavenging and Peroxidase Activity Assay. DPPH scavenging assay was determined as described above for the extract spectrophotometer characterization.

Peroxidase (EC 1.11.1.7) activity was assayed in triplicate, according to Caruso et al.[34] by measuring at 30 °C, the absorbance increases at 470 nm due to the formation of tetra guaiacol from 0.46% (v/v) guaiacol and 13 mM H_2O_2 in 50 mM Tris buffer (pH 7.4). Protein extract (6 mg of total protein) was added to 1.5 mL (final volume) of the reaction mixture for each sample. Peroxidase activity was calculated using an absorption coefficient for the tetra guaiacol of 26.6/mM/cm at 470 nm.

The experiment was repeated three times ($n = 3$).

Statistical Analysis. Data of the effect of seed treatment with the extracts on seed germination, sprout weight and height, root length, sprout moisture, content of sprout proteins, anthocyanins and phenols, and activity of DPPH and peroxidases were analyzed by two-way analysis of variance (ANOVA) ($p < 0.05$), and means were separated by Tukey's test ($p < 0.05$). For extract spectrophotometer characterization and sprout elemental composition from seed treated with the extracts, the *t*-test ($p < 0.05$) has been used. These analyses were performed with GraphPad Prism software, v. 4.03, 2005. Principal component analysis (PCA) was performed as a multivariate analysis on the physiological and biochemical parameters of radish sprouts collected after the incubation with AM and SV extracts. PCA analysis was performed in the R environment on RStudio version 2021.09.2 Build 382 (R Core Team, 2022) using the "prcomp" function on the correlation data matrix, with the function "scale" set to "true". Two-way ANOVA was performed on the scores obtained for the first two principal components (PC1 and PC2) after PCA analysis. The significance of tests was assessed at a *p*-value less than or equal to 0.05. Post-hoc HSD Tukey's test was performed to investigate differences between treatments when ANOVA returned a significant global test.

RESULTS AND DISCUSSION

In this study, we selected AM and SV for their different taxa. *Anabaena* is a genus of the Cyanobacteria phylum (commonly, though inaccurately, referred to as blue-green algae) belonging to the Bacteria kingdom, whereas *Sargassum* is a genus of brown seaweed belonging to the Chromista kingdom. These

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Table 1. Macro and Micronutrient Composition (±SD, Dry Mass) Detected in Biomass of AM and SV

elements	unit	AM	SV
C	%	42.46 ± 2.20	25.52 ± 0.18
N	%	9.44 ± 0.41	1.31 ± 0.02
P	mg/kg	11.13 ± 1.04	515 ± 9.55
K	mg/kg	7.96 ± 0.30	13,941 ± 10.57
S	mg/kg	3.92 ± 0.18	9511 ± 9.65
Ca	mg/kg	4.53 ± 0.15	14,788 ± 16.32
Mg	mg/kg	5.38 ± 0.20	8420 ± 8.81
Na	mg/kg	n.d.	35,668 ± 13.23
Fe	mg/kg	1.97 ± 0.03	372 ± 2.40
Cu	mg/kg	0.01 ± 0.001	4.48 ± 0.11
Zn	mg/kg	0.04 ± 0.001	58.14 ± 1.50
Mn	mg/kg	0.26 ± 0.004	20.52 ± 1.30

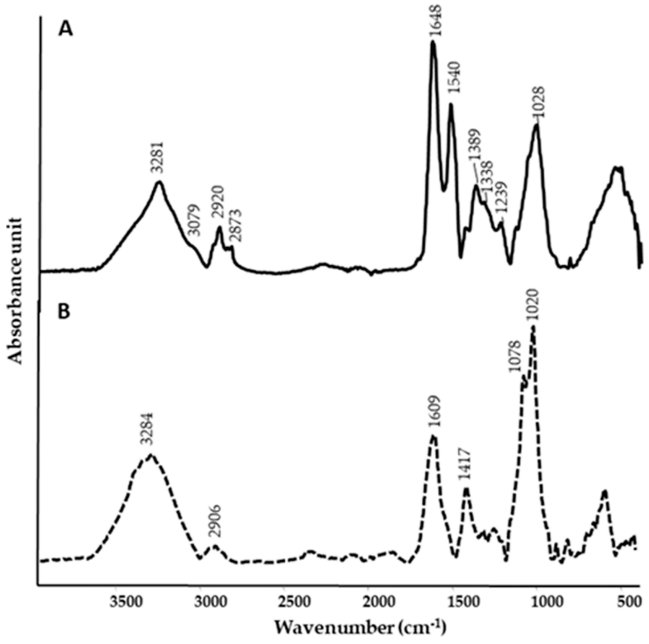

Figure 1. ATR-FTIR spectra (4000−400 cm^{-1}) of AM (A, solid line) and SV (B, dotted line) biomasses.

Table 2. Spectrophotometer Characterization of Extracts from AM and SV[a]

μg/mL extract	AM	SV
carbohydrates (μg/mL)	823.48 ± 62.73	1745.75 ± 113.26[b]
proteins (μg/mL)	196.39 ± 34.32	n.d.
chlorophyll *a* (μg/mL)	1.15 ± 0.09[b]	0.17 ± 0.00
chlorophyll *b* (μg/mL)	0.66 ± 0.02[b]	0.44 ± 0.01
chlorophyll*a* + *b* (μg/mL)	1.81 ± 0.11[b]	0.61 ± 0.01
carotenoids (μg/mL)	0.09 ± 0.02	n.d.
phycocyanin (mg/mL)	0.039 ± 0.002	n.d.
allophycocyanin (mg/mL)	0.033 ± 0.003	n.d.
phycoerythrin (mg/mL)	0.039 ± 0.002	n.d.
antioxidant activity (DPPH inhibition %)	5.46 ± 0.34	7.69 ± 0.33[b]
pH	7.0 ± 0.13	6.0 ± 0.04

[a]Values are means of 3 independent experiments ± SD. [b]Significant highest value in a line according to *t*-test ($p < 0.05$). n.d. = not detected.

Table 3. Effect of Seed Treatment with AM and SV Extracts on Seed Germination of CR, D, and SR Radish Varieties (%)

treatment[a]	variety		
	CR	D	SR
Co	97.28 ± 0.72 aA	94.93 ± 0.75 aA	95.19 ± 1.36 aA
AM	99.68 ± 0.14 bB	93.87 ± 1.11 aA	95.71 ± 0.77 aA
SV	99.23 ± 0.35 bB	95.45 ± 0.67 aA	95.99 ± 0.62 aA

[a]Treatment and variety factors and their interaction are significant according to two-way ANOVA ($p < 0.05$). F (2, 27) = 4.25, $p < 0.05$ (for treatment factor), F (2, 27) = 62.08, $p < 0.05$ (for variety), and F (4, 27) = 0.0242 (for interaction). Data are means of 3 independent experiments ($n = 3$) ± SD. Different lower-case letters in a column and different upper-case letters in a line indicate significant differences according to the Tukey test ($p < 0.05$). Co = control.

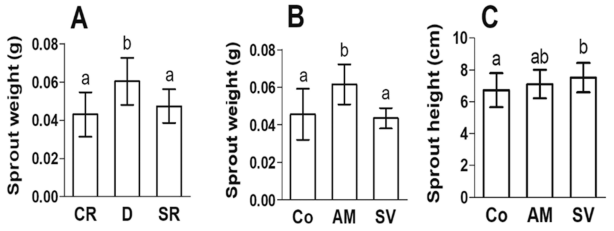

Figure 2. Effect of seed treatment with AM and SV on sprout weight (A,B) and sprout height (C). CR = China Rose; D = Daikon; SR = Sango Red; and Co = control. For both parameters, treatment × variety interaction is not significant according to two-way ANOVA ($p > 0.05$). For sprout weight, the variety (A) and treatment (B) factors are significant according to two-way ANOVA ($p < 0.05$), F (2, 27) = 16.73 and F (2, 27) = 19.98, respectively. For sprout height, the treatment factor is significant ($p < 0.05$), F (2, 27) = 6.22, while the variety factor is not significant ($p > 0.05$) according to two-way ANOVA. Columns are means of 3 independent experiments ($n = 3$) ± SD. Different letters indicate significant differences according to the Tukey test ($p < 0.05$).

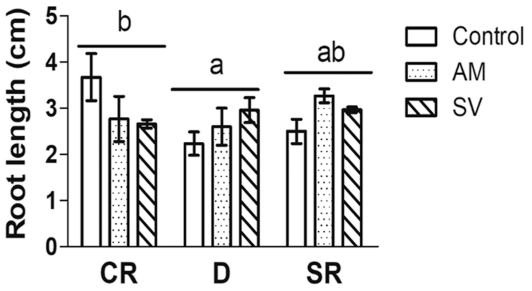

Figure 3. Effect of seed treatment with AM and SV on the root length (RL). CR = China Rose; D = Daikon; SR = Sango Red; and Co = control. Variety factor and treatment × variety interaction are significant according to two-way ANOVA ($p < 0.05$). F (2, 27) = 4.56, $p < 0.05$ (for variety factor), and F (4, 27) = 8.75 (for interaction). The treatment factor is not significant ($p > 0.05$). Columns are means of 3 independent experiments ($n = 3$) ± SD. Different letters indicate significant differences according to the Tukey test ($p < 0.05$).

two organisms are known for their ability to biostimulate plant growth and enhance plant defense responses against phytopathogens. Additionally, cyanobacteria and algae represent an interesting source of natural compounds with a large spectrum of biological activities, such as antimicrobial, antifungal, and antioxidant.[35−37] For example, researchers suggested that treatments with species of *Anabaena* and

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Table 4. Effect of Seed Treatment with AM and SV on Sprout Moisture (%) of CR, D, and SR Radish Varieties

treatment[a]	variety		
	CR	D	SR
Co	84.54 ± 0.92 aA	86.56 ± 1.22 aB	83.67 ± 0.81 aA
AM	86.87 ± 2.82 aA	91.02 ± 1.26 bA	86.47 ± 0.87 aA
SV	86.64 ± 0.89 aA	87.80 ± 0.69 aA	88.59 ± 1.32 aA

[a]Treatment and variety factors and their interaction are significant according to two-way ANOVA ($p < 0.05$). F (2, 27) = 14.77, $p < 0.05$ (for treatment factor), F (2, 27) = 9.07, $p < 0.05$ (for variety factor), and F (4, 27) = 3.34 (for interaction). Data are means of 3 independent experiments ($n = 3$) ± SD. Different lower-case letters in a column and different upper-case letters in a line indicate significant differences according to the Tukey test ($p < 0.05$). Co = control.

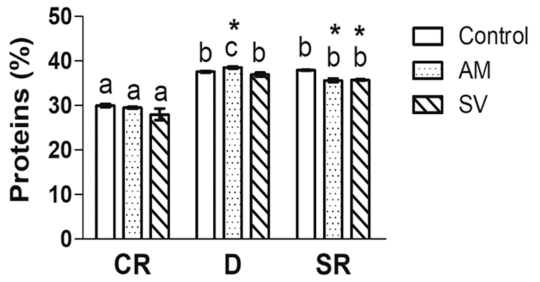

Figure 4. Effect of seed treatment with AM and SV on the sprout protein content of CR, D, and SR radish varieties. Co = control. Treatment, variety, and their interaction are significant according to two-way ANOVA ($p < 0.05$). F (2, 27) = 22.36 (for treatment factor), F (2, 27) = 721.45 (for variety factor), and F (4, 27) = 8.21 (for interaction). Columns are means of 3 independent experiments ($n = 3$) ± SD. Different letters indicate significant differences within each treatment and the asterisk indicates significant differences between each treatment and the corresponding control within each variety, according to the Tukey test ($p < 0.05$).

Table 5. Elemental Composition (μg/g) ± SD of Radish Sprouts of CR, SR, and D Varieties upon Seed Treatment with AM Aqueous Extract in Comparison to the Control (Co)

		elements (μg/g)		
		CR	SR	D
Na	Co	7.0 ± 0.2	63.0 ± 2.0	72.0 ± 2.0
	AM	150.0 ± 5.0[a]	214.0 ± 7.0[a]	212.0 ± 6.0[a]
Ca	Co	1051.0 ± 25.0	1297.0 ± 95.0	1015.0 ± 19.0
	AM	1045.0 ± 31.0	1569.0 ± 79.0[a]	1107.0 ± 38.0[a]
Mg	Co	2337.5 ± 7.5	2777.0 ± 81.0	2965.5 ± 18.5
	AM	2367.0 ± 99.0	3449.5 ± 81.5[a]	3528.5 ± 74.5[a]
K	Co	5292.0 ± 74.0	6864.0 ± 69.0	7440.0 ± 47.0
	AM	4931.0 ± 33.0[a]	6894.0 ± 78.0	9001.0 ± 35.0[a]
Fe	Co	24.5 ± 0.9	13.1 ± 0.1	16.3 ± 0.2
	AM	30.0 ± 0.4[a]	22.2 ± 0.2[a]	5.3 ± 0.0[a]
Mn	Co	15.0 ± 0.1	16.7 ± 0.1	21.5 ± 0.1
	AM	14.4 ± 0.4	17.8 ± 0.0[a]	18.1 ± 0.1[a]
Cu	Co	3.9 ± 0.0	2.8 ± 0.0	7.2 ± 0.1
	AM	2.0 ± 0.0[a]	1.2 ± 0.0[a]	2.2 ± 0.0[a]
Zn	Co	11.7 ± 0.1	11.1 ± 0.1	11.9 ± 0.0
	AM	11.1 ± 0.0[a]	11.1 ± 0.0	11.5 ± 0.1[a]

[a]Values are significantly different from the control (t-test, $p < 0.05$).

Sargassum were able to reduce disease symptoms of powdery mildew caused by *Podosphaera xanthii* on cucurbit plants.[22,35] Moreover, an increase in the germination of tomato seeds with AM treatment was reported.[21] On the other hand, only one study has examined the effect of*Sargassum* spp. aqueous extract on germination and chemical composition of radish sprouts, a vegetable with high nutritional values.[13] The seed priming obtained with a presowing seed soak treatment in AM and SV aqueous extract has been explored in this context.

Elemental Analysis and ATR-FTIR Characterization of Biomass. Algae and microalgae are generally rich in macro- and micronutrients depending on the species, classes, culture growth conditions, stage, and harvest time.[38,39] Therefore, these parameters affect the chemical composition of the extracts and their potential effects.[40] As expected, AM and SV showed significant differences in elemental composition due to their different taxa (Table 1). In particular, AM revealed considerable amounts of C (42.46%) and N (9.44%), presumably due to its N fixation activity. On the contrary, the N amount was greatly lower in SV (1.31%). Furthermore, SV exhibited a noteworthy quantity of macro-elements, specifically including P (515 mg/kg), K (13,941 mg/kg), S (9511 mg/kg), and Ca (14,788 mg/kg). These findings are consistent with the previous research on *Sargassum* spp.[41,42] In addition, the sample contained a high level of microelements, particularly iron (372 mg/kg).

In Figure 1, the ATR-FTIR spectra of SV and AM biomasses are shown. A broader and more intense band appeared in both samples, around 3280 cm^{-1}, which is assigned to the O−H stretching vibrations. AM sample, rather than SV, presented the typical shoulder, around 3070 cm^{-1}, related to the stretching of NH bonds in secondary amides. In the region between 3000 and 2800 cm^{-1}, there were several peaks in each spectrum, attributed to the stretching vibrations of C−H groups, possibly as part of aliphatic chains of lipids. The largest differences were in the region between 1800 and 1200 cm^{-1}.

The brown alga (SV) displayed absorptions at 1609 and 1417 cm^{-1}, suggesting the presence of carboxylate groups. Furthermore, the presence of two strong peaks at 1078 and 1020 cm^{-1} is assigned to C−O−C and C−C stretching vibration of the pyranose ring.[21,43,44] These bands, together with those previously described, can be attributed to different polysaccharides, such as alginates and fucoidan, which are commonly found in *Sargassum* species.[45] This finding was also supported by the extract analysis, which revealed a significant quantity of carbohydrates (Table 2). In contrast, AM showed two prominent peaks, the first one at 1648 cm^{-1}, assigned to the amide I band, and the second one at 1540 cm^{-1}, referred to the amide II band.[21] The above finding is consistent with the chemical analysis, which highlighted the great nitrogen and protein content in AM. Thus, the different chemical compositions observed in AM and SV are due to their different origins.

Spectrophotometer Characterization of Extracts. The extraction method used in this study gave a yield of 2.99 ± 0.36 and 2.71 ± 0.11 mg of dried biomass per milliliter of the extract from AM and SV, respectively. Both extract yields did not differ significantly ($p = 0.073$, t-test). Conversely, the extract pH of AM (7.00 ± 0.13) was significantly higher than that of SV (6.04 ± 0.04; $p = 0.0003$, t-test). Cyanobacterium and brown alga extracts are characterized by high levels of compounds, even though their concentration varied considerably between them (Table 2). The highest amount of carbohydrates was found in the SV extract (1745.75 g/mL) compared to that of AM (823.48 g/mL). Proteins, carotenoids,

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Table 6. Elemental Composition (μg/g) ± SD of Radish Sprouts of CR, SR, and D Varieties upon Seed Treatment with SV Aqueous Extract in Comparison to the Control (Co)

		elements (μg/g)		
		CR	SR	D
Na	control	7.0 ± 0.2	63.0 ± 2.0	72.0 ± 2.0
	SV	184.0 ± 4.0[a]	173.0 ± 4.0[a]	325.0 ± 15.0[a]
Ca	control	1051.0 ± 25.0	1297.0 ± 95.0	1015.0 ± 19.0
	SV	1153.0 ± 53.0[a]	1526.0 ± 93.0[a]	1016.0 ± 9.0
Mg	control	2337.5 ± 7.5	2777.0 ± 81.0	2965.5 ± 18.5
	SV	2593.0 ± 101.0[a]	3154.0 ± 66.0[a]	3373.5 ± 55.5[a]
K	control	5292.0 ± 74.0	6864.0 ± 69.0	7440.0 ± 47.0
	SV	5187.0 ± 55.0	7451.0 ± 89.0[a]	8376.5 ± 77.5[a]
Fe	control	24.5 ± 0.9	13.1 ± 0.1	16.3 ± 0.2
	SV	32.7 ± 0.3[a]	13.0 ± 0.0	11.9 ± 0.1[a]
Mn	control	15.0 ± 0.1	16.7 ± 0.1	21.5 ± 0.1
	SV	14.8 ± 0.2	20.8 ± 0.0[a]	21.1 ± 0.2[a]
Cu	control	3.9 ± 0.0	2.8 ± 0.0	7.2 ± 0.1
	SV	5.6 ± 0.1[a]	7.5 ± 0.3[a]	6.0 ± 0.0[a]
Zn	control	11.7 ± 0.1	11.1 ± 0.1	11.9 ± 0.0
	SV	10.4 ± 0.1[a]	11.0 ± 0.1	12.7 ± 0.2[a]

[a]Values are significantly different from the control (t-test, $p < 0.05$).

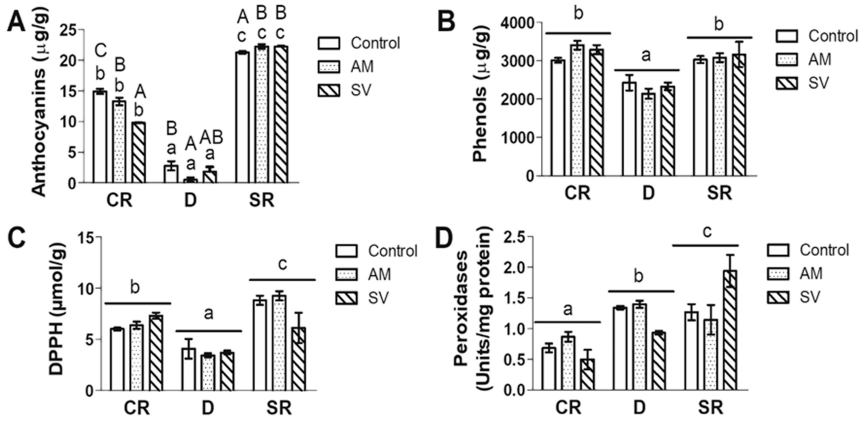

Figure 5. Effect of seed treatment with AM and SV and water as control (Co) on anthocyanin and phenol content (A,B), antioxidant capacity (C), and peroxidase activity (D) of the sprout of CR, D, and SR radish varieties. (A) Treatment, variety, and their interaction are significant according to two-way ANOVA ($p < 0.05$). F (2, 27) = 28.21 (for treatment factor), F (2, 27) = 3890.58 (for variety factor), and F (4, 27) = 39.30 (for interaction). (B,C,D) Treatment factor is not significant ($p > 0.05$), and variety factor and variety × treatment interaction are significant ($p < 0.05$) according to two-way ANOVA. (B) F (2, 27) = 89.43 (for variety factor) and F (4, 27) = 3.46 (for interaction); (C) F (2, 27) = 101.63 (for variety factor) and F (4, 27) = 10.77 (for interaction); (D) F (2, 27) = 67.44 (for variety factor) and F (4, 27) = 20.3 (for interaction). Columns are means of 3 independent experiments (n = 3) ± SD. (A) Different lower-case letters indicate significant differences within each treatment and different upper-case letters indicate significant differences between each treatment and the corresponding control within each variety, according to the Tukey test ($p < 0.05$). (B,C,D) Different lower-case letters indicate significant differences among varieties, according to the Tukey test ($p < 0.05$).

phycocyanin, allophycocyanin, and phycoerythrin were observed only in the AM extract while chlorophyll *a* and chlorophyll *b* were found in both AM and SV with the highest content in AM. These results align with a previous study showing that an aqueous extract from the brown alga *Ecklonia maxima*, which belongs to the same class (Phaeophyceae) of SV, contained a low level of chlorophylls.[22] On the other hand, the SV extract demonstrated significantly higher levels of antioxidant activity (DPPH inhibition %) than AM. A high level of scavenging activity in the algal extract is relevant to obtaining functional food.[40] Overall, the chemical composition of the extracts is consistent with that of the biomass composition.

Effect of Seed Priming with Extracts on Radish Development. The effect of treatment with AM and SV extracts on the percentage of seed germination of CR, D, and SR radish varieties is reported in Table 3. Two-way ANOVA indicated a significant interaction between seed treatment and variety. No significant difference was obtained among the three untreated varieties (Co). Within variety, AM and SV significantly increased the percentage of seed germination (average 2.2%) only for CR in comparison to the Co. Within the extract, the treatments significantly increased the percentage of seed germination in CR with respect to D (AM, 6.2%; SV, 3.9%) and SR (AM, 4.1%; SV, 3.3%). Similarly, aqueous extract from AM and the brown alga *E. maxima* increased the seed germination of tomato in an in vitro experiment.[21] Even the black-eyed pea germination improved when seeds were soaked with the brown algae *Sargassum wightii* and *Ascophyllum nodosum*.[46] The beneficial effects of extracts on radish sprouts were observed also for sprout weight and height (Figure 2). Sprout weight (g) was

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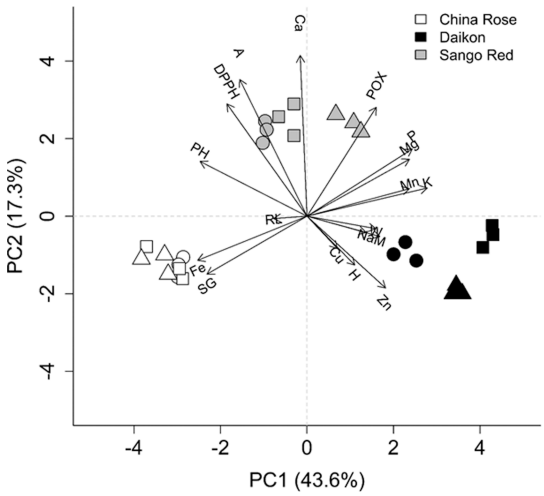

Figure 6. PCA performed on seed germination (SG), weight (W), height (H), RL, moisture (M), proteins (P), phenols (PH), anthocyanins (A), DPPH activity (DPPH), peroxidase activity (POX), and Na, Ca, Mg, K, Fe, Mn, Cu, and Zn content of sprout seed treated with extracts from AM and SV. Circles: control; squares: AM; and triangles: SV.

significantly higher in D (0.060 ± 0.012) than in CR (0.043 ± 0.012) and SR (0.047 ± 0.009). Concerning the effect of the extracts, AM increased sprout weight by 34.8% compared with Co, while SV was like Co. On the contrary, sprout weight was increased by SV (11.6% vs Co). Similarly, the cyanobacterium *Anabaena variabilis*, when applied to *Hordeum vulgare* and *Trigonella foenum-graecum* seeds, increased the seedlings' shoot length, fresh weight, and dry weight.[47] Stimulation of plant growth was also shown by Kumar and Kaur and Essa et al.[48,49] on wheat, sorghum, and sunflower after seed treatment with *A. variabilis* and *Anabaena oryzae*. The effect of the extracts on the root length is shown in Figure 3. Two-way ANOVA indicated a significant interaction between extract and variety factors, but no statistical differences among treatments were found. The root length of CR was higher than D, but both varieties did not differ from SR. Essa et al.,[49] using another cyanobacterial strain, *A. oryzae*, showed promotion of root development and length on sorghum. Overall, seed priming likely enhanced sprout growth and development due to the absorption of various active compounds with diverse structures and functions, as shown in Table 2. We may speculate that other substances may be contained in the extracts from brown algae and cyanobacteria, such as plant hormones, vitamins, amino acids, and phenols that have a role in plant growth.[15,45−47] As later discussed, even the high content of certain elements in the seed-primed sprouts may be linked to plant development.

Effect of Seed Priming with *A. minutissima* and *S. vulgare* Extracts on Radish Moisture, Protein Content, and Elemental Composition. The effect of seed priming with AM and SV extracts on the moisture and protein contents of sprouts is reported in Table 4 and Figure 4, respectively. Two-way ANOVA indicated interaction between varieties and treatments for both parameters. Moisture significantly increased only in D with AM by 5% with respect to Co (Table 4). The protein content (Figure 4) within varieties was significantly increased only by AM in D (2.6%). Within treatments, AM and SV increased protein content in D and SR compared with CR. In particular, the highest protein content was found for the D variety, which produced giant radishes. The protein increase observed with AM can be attributed to the high protein content in the AM aqueous extract, as previously discussed in Table 2 and already shown, with the majority of these proteins identified as phycobiliproteins.[22]

Cyanobacteria produce a plethora of proteins and bioactive compounds with biostimulant activity, which are known to enhance primary and secondary metabolic pathways.[50−52] The characteristics of these sprouts are especially relevant today due to the rising popularity of vegan diets.[53] Generally, sprouts offer a higher quantity of protein compared to seeds.[54] With the increased consumption of plant-based proteins, their quality has become a subject of significant discussion.[53] Therefore, incorporating protein-rich sprouts into the diet is a promising choice for maintaining a healthy lifestyle.

Seed priming with AM and SV influenced the mineral content of the three radish sprout varieties (Table 5). The AM treatment increased Ca (average 1.1-fold) and Mg (average 1.2-fold) in SR and D and Fe (average 1.4-fold) in SR and CR. Concerning K, the level raised (1.2-fold) in D while the Mn

Table 7. Results of Two-Way ANOVA Performed on PC1 and PC2 PCA Scores[a]

PC1				PC2			
	df	*F* value	*P* value		df	*F* value	P value
variety	2	1528.95	<0.001	variety	2	444.1715	<0.001
treatment	2	33.22	<0.001	treatment	2	6.3756	0.008
variety/treatment	4	33.05	<0.001	variety/treatment	4	6.6233	0.002
residuals	18			residuals	18		
var, trt	adj. mean	SE	Tukey	var, trt	adj. mean	SE	Tukey
Daikon, AM	4.2154	0.1441	c	Daikon, AM	−0.5077	0.1688	b
Daikon, SV	3.47	0.1441	b	Daikon, SV	−1.9265	0.1688	a
Daikon, Co	2.2676	0.1441	a	Daikon, Co	−0.9336	0.1688	b
Sango Red, AM	−0.4176	0.1441	b	Sango Red, AM	2.514	0.1688	a
Sango Red, SV	0.9971	0.1441	c	Sango Red, SV	2.4071	0.1688	a
Sango Red, Co	−0.9748	0.1441	a	Sango Red, Co	2.1911	0.1688	a
China Rose, AM	−3.1776	0.1441	a	China Rose, AM	−1.2513	0.1688	a
China Rose, SV	−3.4424	0.1441	a	China Rose, SV	−1.201	0.1688	a
China Rose, Co	−2.9376	0.1441	a	China Rose, Co	−1.292	0.1688	a

[a]Factors "variety" and "treatment" and their interaction were considered after Tukey's test with *p*-value < 0.05. AM = *Anabaena minutissima*, SV = *Sargassum vulgare*, and Co = Control. Different letters indicate significant differences according to the Tukey test ($p < 0.05$).

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level increased in SR (1.1-fold). Conversely, Cu and Zn decreased or were presented in the same amounts as for the control.

Concerning seed primed with SV, the elemental composition of the sprouts is reported in Table 6. The SV treatment increased the content of some elements in all sprout varieties. Most importantly, Na increased by 26.3, 4.5, and 2.7-fold in CR, D, and SR, respectively, Ca increased by an average of 1.2-fold in CR and SR, and Mg increased by an average of 1.1-fold in all varieties. Cu increased in CR and SR (1.4 and 2.7-fold, respectively) and K increased in SR and D (average 1.1-fold), while Fe, Mn, and Zn increased by 1.3, 1.2, and 1.1-fold in CR, SR, and D, respectively. Therefore, seeds priming with AM and SV improved the ability of the sprouts to assimilate essential minerals. Specifically, the increase in Ca is crucial for shoot growth and cell wall and membrane stability[55] and Mg is involved in a wide range of primary metabolic pathways such as the synthesis of chlorophylls and proteins.[56] A notable observation was the Na content. While Na is not essential for most plants, it can prove beneficial to plants under a multitude of circumstances, particularly when K is deficient.[57] Additionally, the presence of small amounts of Na in the growing medium has been shown to enhance the flavor of many crops.[57] Concerning K, it is of significant importance in ensuring optimal plant growth. It is an activator of several important enzymes, including those involved in protein synthesis, sugar transport, and N and C metabolism. It also regulates the opening and closing of stomata, as well as cell elongation.[58] These elements are crucial for maintaining the human body's metabolic and physiological functions. Indeed, as described by Dobrowolska-Iwanek et al.,[59] sprouts are important sources of essential minerals and trace elements due to their high availability for human organisms.

Effect of Seed Priming on Sprout Antioxidant Compounds and Antioxidant Activity. Seed priming with AM and SV and the variety factor significantly influenced the content of bioactive compounds in radish sprouts (Figure 5). Concerning anthocyanins (Figure 5A), the two-way ANOVA indicated a significant interaction between the extract and variety, but no statistical differences were found between the treatments. As expected, the content of anthocyanins depended on the radish color. Indeed, SR (the red variety) presented the highest amounts of total anthocyanins, followed by CR (pink) and D (white) in a decreasing manner. Anthocyanins are important plant pigments responsible for coloring plant tissues and organs and are also involved in response to biotic and abiotic stresses.[60] Moreover, SR had a slightly increasing effect (1.04-fold) with both treatments compared with the Co. Seed priming negatively affected anthocyanins in CR, and it did not affect the content in D.

Total phenols, peroxidase, and DPPH scavenging activities were affected only by radish varieties but not by the treatment (Figure 5B,C,D). The phenolic content increased similarly in CR and SR by 1.4-fold with respect to D. The lowest level of phenols found in D was in accordance with Li and Zhu,[8] that showed a lower amount of phenolic acid in D radish than other cruciferous types.

Concerning the DPPH scavenging activity, SR displayed on average the highest scavenging activity (8.1 μmol/g), followed by CR (6.6 μmol/g) and D (3.8 μmol/g) (Figure 5C). As demonstrated by Bors et al.,[61] the DPPH scavenging activity varied among the radish sprout varieties, with the red radish sprouts exhibiting higher activity compared to the white ones. The peroxidase activity reported in Figure 5D indicates that SR exhibited the highest average activity (1.4 U/mg protein), followed by D (1.2 U/mg protein) and CR (0.7 U/mg protein). The variability of peroxidase activity among varieties was also reported by Yue et al.,[62] but in the case of medicinal plants. The presence of peroxidase activity is significant because it is involved in counteracting biotic and abiotic stresses and other important physiological processes such as seed germination.[57]

Finally, a rational and statistical analysis of all of the results was conducted using PCA. PCA revealed that the three radish varieties (CR, D, and SR) clustered along the two principal components and therefore the "variety" factor had the highest impact on the data set, as confirmed by two-way ANOVA performed on PCA scores (Figure 6 and Table 7). However, both "variety" and "treatment" factors and their interactions were significant for PC1 and PC2 which accounted for 43.6 and 17.3% of the total explained variance, respectively. CR PCA scores did not show significant differences in PCA among the control and treatments with AM or SV, whereas all of the treatments of SR clustered and showed significant differences in PC1 but not in PC2. D showed the highest clusterization between the treatments, with significant differences between the control and treatments either in PC1 or in PC2. PCA confirmed that AM and SV treatments produced different effects on radish sprouts depending on the variety.

In conclusion, brown algae and cyanobacteria have numerous benefits for agriculture due to their fertilizing properties. However, it is important to test their individual effects and potential synergies on crops. Therefore, we evaluated the effects of two aqueous extracts of *Anabaena* and *Sargassum* species to improve the nutritional properties of radish sprouts. This study demonstrated that aqueous extracts could enhance the nutritional properties of different varieties of radish sprouts. This approach could improve crop quality, particularly in achieving sustainable consumption and production of nutritious food, as outlined in the 2030 Agenda.

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Notes

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