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Enhancement of *in-vitro* antioxidant properties and growth of amaranth seed sprouts treated with seaweed extracts

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Abstract

Amaranth sprouts contain phytochemicals with antioxidant activity that can neutralize free radicals that damage cellular macromolecules and change the stability of cells. In some seeds, phytochemicals content can be enhanced when these are imbibed with an aqueous seaweed extract (ASE). This study evaluated the effect of different concentrations (0.5, 1.0, and 2.5 mg mL^{-1}) of *Padina durvillei* (TP) and *Ulva lactuca* (TU) extracts, with distilled water as a control treatment, on the growth of amaranth sprouts and content of total polyphenols (TPC), flavonoids (TFC), chlorophylls *a* and *b* (TCCa and TCCb), carotenoids (TCC), betacyanins (TBC) and, the antioxidant *in-vitro* activity of sprouts. There was a positive effect on hypocotyl and root growth of sprouts when treated with 2.5 mg mL^{-1} and 0.5 mg mL^{-1} TP and TU extracts, respectively. The highest antioxidant activity ocurred in sprouts grown in 0.5 mg mL^{-1} TP. Sprouts treated with 0.5 mg mL^{-1} ASE had a higher content of bioactive compounds compared to those treated with water, with the TP extract associated with the highest content of TPC (9.52 mg QE g^{-1}) and TFC (0.567 mg GAE g^{-1}), TCCa, and TCCb (0.09 and 0.12 mg g^{-1}, respectively), sprouts grown in 0.5 mg mL^{-1} TU accumulated a higher TCC content (0.04 mg g^{-1} dry sprout). Treatment with at 2.5 mg mL^{-1} TP and TU extracts produced sprouts that accumulated a higher TBC content in the cotyledons. These results demonstrated the biostimulant effect of ASEs when applied to amaranth seeds, and can be a suitable option for sustainable agriculture.

Keywords Antioxidant activity · Biostimulant · Carotenoids · Chlorophylls · Flavonoids · Phytochemicals · Polyphenols · Chlorophyceae · Phaeophyceae

Introduction

Current food consumption behaviors are increasingly focused on seeking alternatives that, in addition to nourishing, provide a health benefit. Thus, micro-scale vegetable production and the popularity of sprouted seeds have increased for their high nutritional value and content of bioactive phytochemicals (Rouphael et al. 2021).

According to the Commission Implementing Regulation (EU) No 208/2013, sprouts are defined as "the product obtained from the germination of seeds and their development in water or another medium, collected before the development of true leaves and to be eaten whole, including the seed and grasse" (EU 2013). Sprouts provide more nutritional benefits compared to seeds or mature plants; they are rich in essential amino acids and fatty acids, simple sugars, and phytochemicals such as polyphenols and terpenoids that exhibit antioxidant properties, and, when consumed as food, provide beneficial effects to human health (Wojdyło et al. 2020).

While many seeds of vegetables, cereals and herbs can be used to produce sprouts, the most popular being cereal and pseudo-cereal seeds. *Amaranthus* is a pseudo-cereal of high nutritional quality and a source of functional antioxidant compounds like betacyanin, lutein, quercetin, niacin, vitamin C, tocopherols, and phenolic compounds (Odongo et al. 2018; Jimenez et al. 2019; Jimoh et al. 2019; House et al. 2020). The germination process leads to nutritional changes as germination reduces non-nutritional compounds such as trypsin inhibitors, lectins, tannins, and saponins (Cornejo et al. 2019). The germination process

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of *Amaranthus caudatus* seeds increases the content of phenolic compounds, in turn producing an increase in antioxidant activity (Aguilar-Felices et al. 2019; Cornejo et al. 2019; Pilco-Quesada et al. 2020). The production and accumulation of secondary metabolites like polyphenols, carotenoids, chlorophyll, and betacyanin may also be enhanced by particular conditions and pretreatments used for germination.

Many abiotic and biotic elicitors have been evaluated as potential factors that induce foster changes in the phytochemical composition of amaranth sprouts. For instance, a direct-electric-current treatment of 500 mA applied for a short period (5 min) has been used as pretreatment of amaranth seeds to induce quantitative changes in the antioxidant enzymatic system of 6-day sprouts, achieving to increase the total content of flavonoids and phenolic compounds relative to untreated amaranth sprouts (Ozuna et al. 2018); selenium supplementation in seed germination was significantly correlated with betacyanin content (recognized for its anti-inflammatory properties) in four amaranth species, with sprouts of *Amaranthus cruentus* accumulating the highest concentration of betacyanins (19.30 ± 0.57–28.85 ± 2.23 mg amaranth per 100 g fresh weight; (Tyszka-Czochara et al. 2016). Betacyanin content in *Amaranthus mangostanus* seedlings can be upregulated by treatment with methyl jasmonate and ethylene (Cao et al. 2012). Treatment with biostimulants is a promising alternative to other conventional biological techniques used to improve the bioactive compounds and biological activities of sprouts (Liu et al. 2019).

Some algal species act as elicitors of plant germination and growth. For example, *Capsicum annuum* seeds soaked with 8% *Padina gymnospora* aqueous extract showed an improved germination process and root growth, along with the presence of phytochemicals in the plant (Thriunavakkarasu et al. 2020). Likewise, a low concentration (0.2%) of *Ulva lactuca* extracts obtained by acid hydrolysis applied to seeds of mung bean (*Vigna radiata*), significantly improved germination rates and increased the production of seedlings, and led to higher contents of protein, chlorophyll, and total and reducing sugars in sprouts, compared to controls (Castellanos-Barriga et al. 2017). Synergistic effects of the application of *Cladophora glomerata* extracts and near-infrared radiation (NIR) in soybean (*Glycine max*), improved germination and chlorophyll content in seedlings (Michalak et al. 2018).

Despite the above evidence that seaweed extracts have the potential to be used as elicitors of seed germination and to stimulate the accumulation of antioxidant compounds in the sprouts produced, little is known about their efficacy in increasing the functionality of amaranth sprouts. Therefore, the objective of the present study was to evaluate the effect of treating amaranth seeds with extracts of *Ulva lactuca* and *Padina durvillei* on sprout production, phytochemicals content, sprout growth, and antioxidant activity.

Material and methods

Seaweed collection, seeds and reagents

Specimens of the seaweeds *Padina durvillei* Bory and *Ulva lactuca*L were collected in Mazatlan Bay, Sinaloa, Mexico (23°1′29.1"N, 106°25′29.7'W), in March 2017. The seaweeds were identified with taxonomic keys (León Álvarez et al. 2017; León Álvarez and Núñez Reséndiz, 2017; Ochoa-Izaguirre et al. 2007) and compared with the specimens of herbarium of Universidad autonoma de Sinaloa: ID 4665–2013-FACIMAR (CONABIO 2022). Fresh samples were rinsed with distilled water, lyophilized, ground with a commercial grinder, and stored at -20 °C until used. All chemicals used in this study were analytical grade from Sigma-Aldrich (USA), unless otherwise specified.

A commercial stock of amaranth seeds (*Amaranthus* spp) was purchased in a local market in Guadalajara, Mexico.

Aqueous seaweed extracts (ASE)

Aqueous seaweed extracts were obtained using distilled water according to Tierney et al. (2013) modified as follows: dried algal material was mixed with water at 21 °C (1:10 ratio, w:v) stirring for 3 h. Then, the extract was filtered through a glass fibre filter (1.2 µm pore size) and the algal residue was extracted again (twice) as described above. Filtrates were pooled and centrifuged at $12{,}000 \times g$ for 20 min at 4 °C, and the supernatant was collected. Finally, the aqueous seaweed extract (ASE) was lyophilized and stored at -20 °C until analyzed.

Amaranth sprout production

Tetrazolium viability test

Six replicates of fifty seeds per treatment and concentration were hydrated in a microtube containing 200 µL test solution: distilled water (control treatment) or ASE from *P. durvillaei* or *U. lactuca* at different concentrations (0.5, 1, and 2.5 mg mL^{-1}) for 3 h at room temperature.

After preconditioning, seeds were completely immersed in 200 µL 2, 3, 5-triphenyl tetrazolium chloride solution (1%) and incubated in the dark at 37 °C for 2 h (ISTA 2010). Afterward, the tetrazolium solution was discarded, the seeds were rinsed thoroughly and then dried at room temperature for examination.

Seeds were examined individually under a stereomicroscope at 40× and classified according to coloration. Seeds were classified into different categories based on physiological state: viable seeds with healthy and vigorous embryonic tissues were pale red-color throughout the embryo and endosperm; tissues undergoing deterioration were an intense red-color; non-viable

seeds with dead tissue were a dull white-color; and immature tissue was a green-color (Craviotto et al. 2008).

Seed pre-treatment with ASE

Before germination and pretreatment, seeds were disinfected according to Montoya González et al. (2016), through washing with distilled water followed by treatment with commercial 2% sodium hypochlorite for 15 min, then rinsed with distilled water (3 times).

Disinfected amaranth seeds were soaked in the pre-treatment solutions (5 ratio, v/w) for 30 min. The pre-treatment solutions were distilled water as negative control (*T0*), commercial biofertilizer (ByoAlga® from *Ascophyllum nodosum*: Sustainable Products Biodynamics S.A.S.) as reference control (TB), and test solutions of ASE from *Padina durvillei* (TP) and *Ulva lactuca* (TU) at 0.5, 1.0 and 2.5 mg mL^{-1}.

Germination assay

The pre-treated seeds (fifty seeds per treatment and concentration) were placed in Petri dishes on moist filter paper with 4 mL of distilled water (6 replicates per treatment). If necessary, the filter paper was moist every week to prevent the fermentation process of sprouting. Germination was monitored daily for 5 days, maintaining a temperature of 25–28 °C, constant relative humidity (25%), and under fluorescent light (50 µmol photons m-*2* s^{-1}) with a 16/8 h light/dark photoperiod. The percentage of germination was determined with the following equation:

$$\%\ Germination = \left(\frac{\#\ germinated\ seeds}{\#\ total\ seeds\ tested}\right) \times 100$$

Development of sprouts

Seeds (5 g) soaked in the pretreatment solutions were germinated for 5 days in a disinfected glass container (500 mL) capped with a mesh lid to allow oxygen exchange. Germination chambers were maintained at 25–28 °C, 25% relative humidity under fluorescent light (50 µmol photons m^{-2} s^{-1}) with a 16/8 h light/dark photoperiod cycle. Sprouts were hydrated with water. Hypocotyl height and root length were measured (in mm) using a digital vernier.

Sprout extracts

To determine potentially bioactive compounds contained in ASE-treated amaranth sprouts, these were subjected to extraction using the method described by Paśko et al. (2009). Briefly, 1 g lyophilized sprouts was mixed with 40 mL extraction solution (absolute methanol: 0.16 M HCl: distilled water, in a 8:1:1 ratio) for 2 h with constant stirring. Then, the extract was recovered by filtration (Whatman No. 2) and kept in dark at -20 °C until used.

Phytochemicals analysis

Chlorophylls and total carotenoid content Chlorophyll *a, b*, and total carotenoid content were determined according to Wellburn (1994). Briefly, sample solutions were diluted as needed with 80% methanol and the absorbance was read at the wavelength characteristic of chlorophylls and carotenoids (i.e., 470, 653, and 666 nm). Eighty percent methanol (v/v) was used as blank. Trolox was used as standard Concentration values were referred to 1 mL of extract obtained from 1 g dry sprouts.

Total chlorophyll and carotenoid contents, in µg mL^{-1}, were calculated with the following equations (Wellburn 1994), and subsequently expressed in mg of pigment per gram of dry sprouts:

$$TCCa = 15.65A_{666} - 7.34A_{653}$$

$$TCCb = 27.054A_{653} - 11.21A_{666}$$

$$TCC = \left[\left(1000A_{470} - 2.86TCCa - 129.2TCCb\right)\right]/221$$

where $TCCa$ = total content of chlorophyll *a*; $TCCb$ = total content of chlorophyll *b*; TCC = total content of carotenoids; A = absorbance at 470, 653, or 666 nm, with six replicates.

Total phenolics content (TPC) Total soluble phenolics content was determined using the Folin–Ciocalteu method (Marigo 1973). Dry samples were resuspended in acetone (1 mg mL^{-1}). Then, 100 mL of each sample was mixed with 150 mL Folin solution (previously diluted with deionized water in a 1:1 ratio), followed by the addition of 1 mL 2% sodium carbonate in 0.4% sodium hydroxide. The mixture was incubated in the dark at room temperature for 20 min. The resulting blue complex was read in a spectrophotometer at 750 nm. Phenolic content was expressed as mg of gallic acid equivalent (GAE) per g of sample (dry weight). A gallic acid standard curve was constructed for the concentration range of 0–0.25 mg mL^{-1} with six replicates for each treatment.

Total flavonoid content (TFC) Total flavonoid content was determined according to Luximon-Ramma et al. (2002). Samples of sprout extract (1 mL) were diluted in equal volumes of a 2% aluminum chloride solution (2 g of $AlCl_3 \cdot 6H_2O$ in 100 mL methanol). The mixture was incubated at room temperature for 10 min. Absorbance was read

at 367 nm. The results were expressed in mg of quercetin equivalents (QE) per gram of dry sprout. A quercetin standard curve was constructed for the concentration range of 0–0.5 mg mL^{-1} with six replicates for each treatment.

Total betacyanin content (TBC) Total betacyanin content was determined in cotyledons of *Amaranthus* sprouts according to Chávez et al. (2015) with modifications: twenty fresh 2-day cotyledons were incubated in test tubes with 10 mL 10% ethanol solution at 50 °C for 30 min. Absorbance values of the pigment solutions were read at 536 nm. Total betacyanin content (TBC) was calculated with the equation:

$$TBC\left(\frac{mg}{100}ml\right) = \left({}^{A\times M}/_{E\times b}\right) \times 1000$$

where: A is the absorbance of the pigment solution; M is the molar weight of betacyanin (550.48 g mol^{-1}); E is the molar extinction coefficient of betacyanin (1120 L mol^{-1} cm^{-1}), and b is the path length of quartz cuvettes (1 cm) with six replicates for each treatment.

In-vitro Antioxidant Capacity Assays

DPPH free-radical scavenging activity The free-radical scavenging potential of the ASE was assessed according to the method by Mensor et al. (2001), modified as follows: a 100 mL aliquot of each extract (at concentrations of 0.0015 to 1.5 mg mL^{-1}) was mixed with 900 mL 0.3 mmol L^{-1} 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in ethanol. The mixture was incubated in the dark at room temperature for 30 min and absorbance was read at 518 nm.

The percentage of DPPH scavenging was calculated with the following equation:

$$\%DPPH\ scavenging = [(1 - \text{Absorbance of sample})/\text{Absorbance of Control}] \times 100$$

Trolox was used as standard (0.0008–0.1 mg mL^{-1}), whereas the DPPH solution served as a control to calculate the degree of radical scavenging by samples and the reference standard, expressed in Trolox equivalent antioxidant activity (TEAC, mg TE g^{-1} of dry sprout) with six replicates for each treatment.

Ferric reducing antioxidant power (FRAP) assay The FRAP assay was performed according to Benzie and Strain (1996), with minor modification reported by Szőllősi and Varga (2002). The FRAP reagent was prepared from three different solutions: Solution A: 300 mM acetate buffer, pH 3.6; Solution B: 10 mmol L^{-1} TPTZ dissolved in 40 mmol L^{-1} HCl; Solution C: 20 mmol L^{-1} $FeCl_3 \cdot 6H_2O$. The work solution was prepared by mixing A, B, and C in a 10:1:1 ratio (by volume). For the assay, 100 µL sample was mixed with 1400 µL FRAP, then incubated at room temperature for 30 min in the dark. The absorbance was read at 593 nm. Trolox was used as reference standard (0.01–0.7 mg mL^{-1}) to calculate TEAC (mg TE g^{-1} of dry sprout) with six replicates for each treatment.

Statistical analyses

The results are reported as the mean ± standard deviation. The percentages of germination and viability were arcsin-transformed prior to analysis. All data were tested for homoscedasticity (Levene test) and normality (Kolmogorov–Smirnov test) assumptions. A one-way analysis of variance (ANOVA) was used to test the data for significant

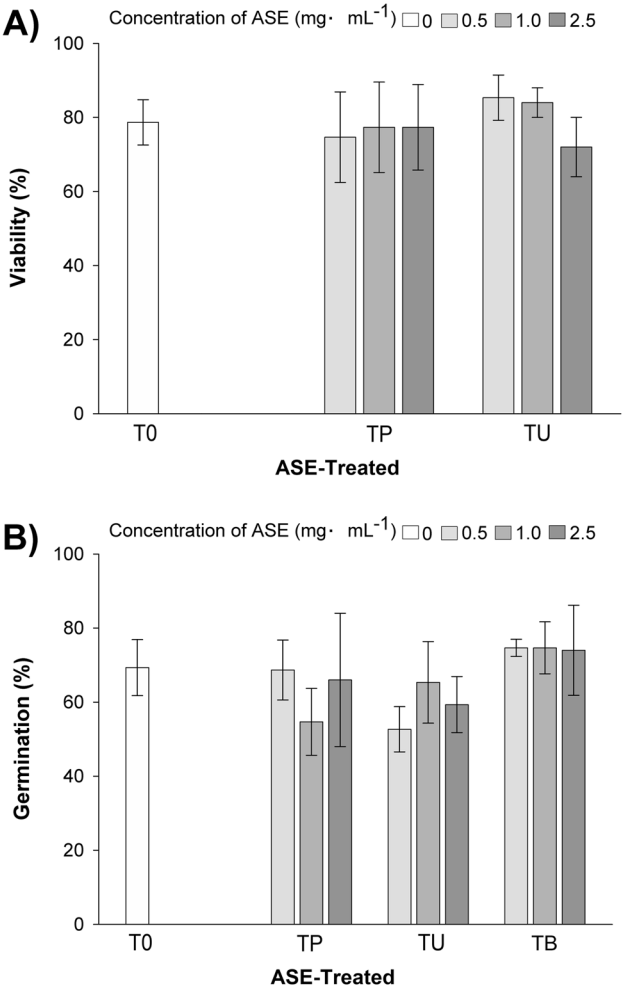

Fig. 1 Effect of concentration of aqueous seaweed extracts (mg · mL^{-1}) on percentage viability (**A**) and germination (**B**). Data are mean ± S.D. ($n = 3$), 50 seeds by replicate were used. Distilled water as a negative control (*T0*); ByoAlga® from *Ascophyllum nodosum* (*TB*), ASE from *Padina durvillei* (*TP*) and *Ulva lactuca* (*TU*)

differences among treatments, followed by Tukey's post hoc test. The results of the biochemical assays were analyzed statistically using the Student´s *t*-test. Differences were significant at $P < 0.05$. All statistical analyses were performed using SigmaPlot version 11.0 (Systat Software, Inc.; Germany).

Results

Viability and germination test

The viability test with tetrazolium salts showed that amaranth seeds treated with algal extracts show non-significant differences ($P < 0.05$) in mean percent viability between treatments TP, TU, TB, and T0. Percent viability ranged between 72 and 85% (Fig. 1A). In all treatments, percent viability exceeded the 70% germination threshold established officially for marketing, (Bauer et al. 2003).

Treatments with TP and TU did not reduce the viability of amaranth seeds, indicating that seeds remain viable at the different concentrations tested (Fig. 2).

The percent germination was greater than 50% in all treatments (Fig. 1B), with non-significant differences ($P < 0.05$) between treatments; however, the highest percentages were observed in treatments TB and TU at 0.5 mg mL^{-1} and 1.0 mg mL^{-1} compared with the T0 treatment and other algae extracts; the treatments with 0.5 mg mL^{-1} TP and 2.5 mg mL^{-1} TU yielded the lowest percent germination.

Growth of sprouts by seaweed extract

Treatments with the different ASE concentrations tested showed significant differences ($P < 0.05$) in the hypocotyl and root growth of amaranth sprouts compared to the control treatment (T0; Fig. 3). The ASE at 1.0 mg mL^{-1} and 2.5 mg mL^{-1} TU and 2.5 mg mL^{-1} TP produced significantly greater hypocotyl growth in sprouts, with 2.5 mg mL^{-1} TU and TB treatments having the greatest statistical difference relative to the other TP and TU concentrations tested, as well as the T0 and the commercial fertilizer based on *A. nodosum* applied at 0.5 mg mL^{-1} TB (Fig. 3A). treatments TP and TU promoted significantly greater root growth relative to the control treatment (T0). Treatmet with 2.5 mg mL^{-1} TP produced similar results to 0.5 mg mL^{-1} TB. Both treatments showed significant differences ($P < 0.05$) compared to 2.5 mg mL^{-1} TU and the ASE treatments at low concentrations; by contrast, treatments with 1.0 and 2.5 mg mL^{-1} TB elicited greater root growth compared to TP and TU (Fig. 3B).

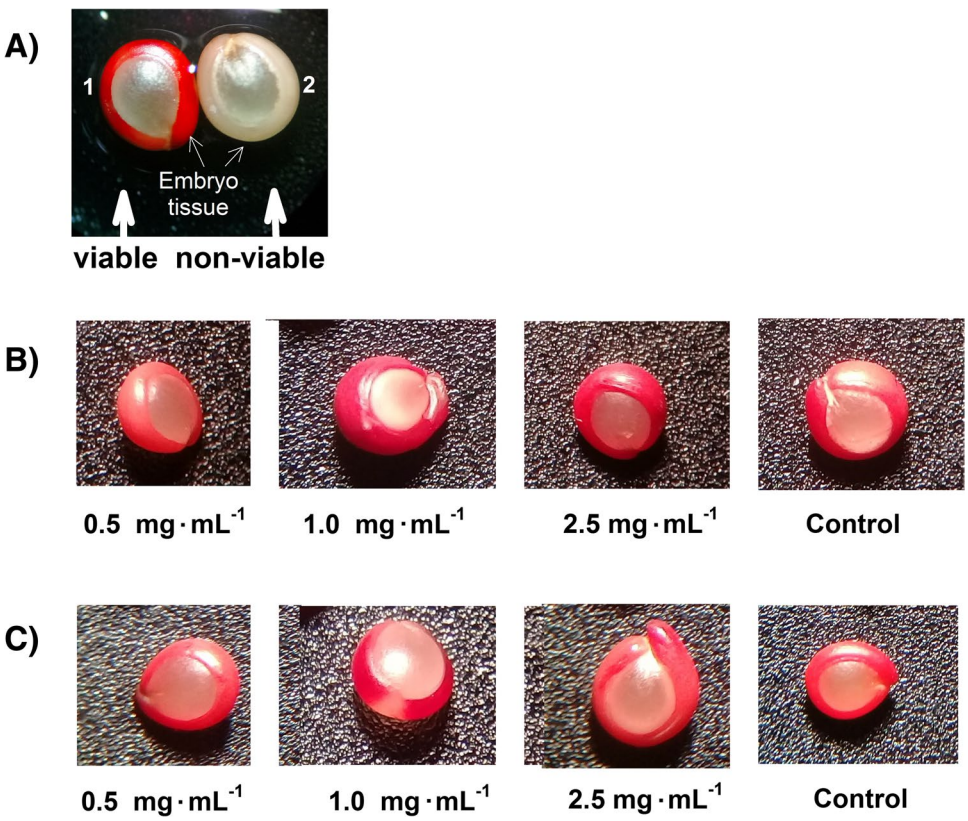

Fig. 2 Tetrazolium test of amaranth seeds after treatment with different concentrations of aqueous seaweed extract (ASE): (**A**) color differences between seeds with viable and non-viable embryos; viable seeds after ASE treatment of (**B**) *Padina durvillei* and (**C**) *Ulva lactuca*. Distilled water as a negative control

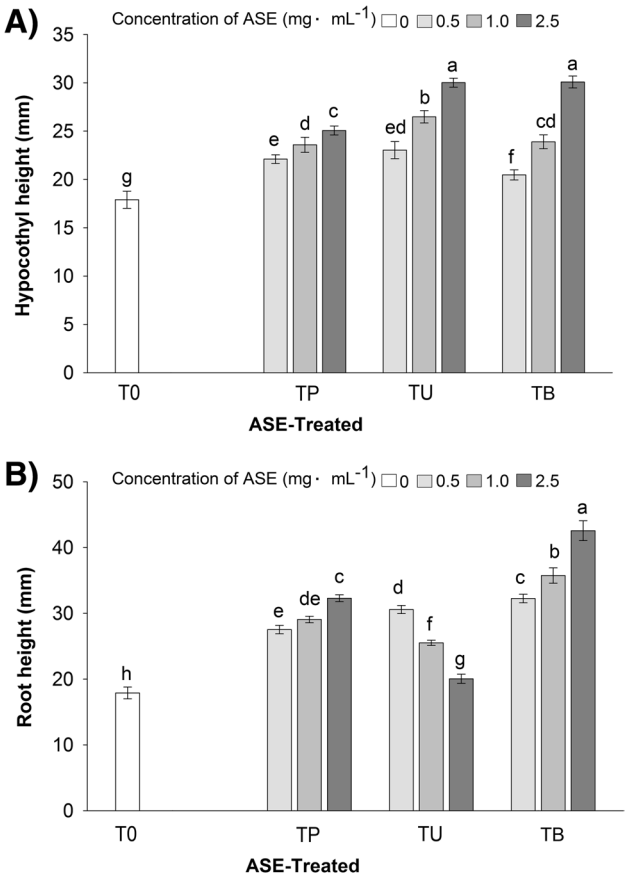

Fig. 3 Physical characteristics of amaranth sprouts after pre-treatments with different concentrations (mg mL^{-1}) of aqueous seaweed extract (ASE): (**A**) Hypocotyl height; and (**B**) root length. The values are mean of representative sample of seedlings from each pre-treatment ($n = 12$). Means with different lettersare significantly different ($P < 0.05$). Distilled water as a negative control (T0); ByoAlga® from *Ascophyllum nodosum* (TB), ASE from *Padina durvillei* (TP) and *Ulva lactuca* (TU)

Sprouts treated with algal extracts of *P. durvillei* and *U. lactuca* were larger in size and more intensely pigmented (red–purple) than sprouts treated with water (Fig. 4). the betacyanin content of sprouts treated with the algal extracts had a significantly observing a higher total betacyanin content when treated with 2.5 mg mL^{-1} TP, TU, and TB (Fig. 5).

In-vitro antioxidant activity on amaranth sprouts

Statistically significant differences (Tukey, $P < 0.05$) were observed in the antioxidant activity of sprouts grown with the algal extract treatments. Sprouts grown in 0.5 mg mL^{-1} exhibited the highest antioxidant activity in vitro sprouts imbibed with the TB algal product (ByoAlga®) showed a proportional dependence between the increase in the concentration used and TEAC values for both measures of *in-vitro* antioxidant activity (DPPH and FRAP) (Fig. 6).

Phytochemical content in amaranth sprout

To the total polyphenols content (TPC), showed an significant increase in the low (0.5 mg mL^{-1}) PT and TU treatments, ASE content decreased with higher ASE concentrations in both treatments, in contrast to treatment with a commercial product based on *A. nodosum* (TB), where TPC increased in parallel with higher product concentration (Table 1). Total flavonoid content (TFC) significantly increased in amaranth sprouts treated with 0.5 mg mL^{-1} TP relative to the control (T0) and all other concentrations of TP, flavonoid content significantly increased in sprots treated with the highest concentration of TU, sprouts treated with TB showed the significant lowest flavonoid levels (Table 1). The total content of chlorophyll *a* and *b* (TCCa and TCCb, respectively) significantly increased in sprouts pretreated with 0.5 mg mL^{-1} TP, while TU pretreatments produced a positive effect in TCCa in sprouts at concentrations below 2.5 mg mL^{-1}. In contrast, TB did not yield increases in TCCa and TCCb (Table 1). Producing pretreatments sprouts in cultures with TU allowed a higher accumulation of total carotenoids (TCC) compared with all other treatments (Table 1).

Discussion

Viability and germination test

The viability test of *Amaranthus* seeds treated with ASE showed that it is not toxic to the seed at the concentrations tested with the coloration observed in *Amaranth* seeds treated with algal extracts corresponding to a viable physiological state: red (healthy and vigorous tissue) and matte white (dead tissue) (Fig. 2A) (Carvalho et al. 2014). Seaweed extracts act as natural priming agents to improve seed germination in different crop seeds. For example, according to Dutta et al. (2019), seed of red pepper (*Capsicum frutescens*) pretreated prior to germination with *Kappaphycus alvarezii* and *Gracilaria edulis* extract significantly improved germination percentage and seedling weight; *Acanthophora spicifera*, *Gelidium robustum*, *Gracilaria parvispora*, *Macrocystis pyrifera*, *Sargassum horridum*, and *Ecklonia arborea* improved germination in mung bean (*V. radiata*) (Filippo-Herrera et al. 2019). The effect on the percentage of germination of amaranth seeds treated with biostimulants based on plant extracts (*Trianthema portulacastrum*) and algal extracts (*Ascophyllum nodosum*) is similar to the effect of water imbibition (Al Sherif and Gharieb 2011; Hossain and und Niemsdorff 2018). This is consistent with the present results where no increase in percent germination occurred in seeds treated with algal extracts.

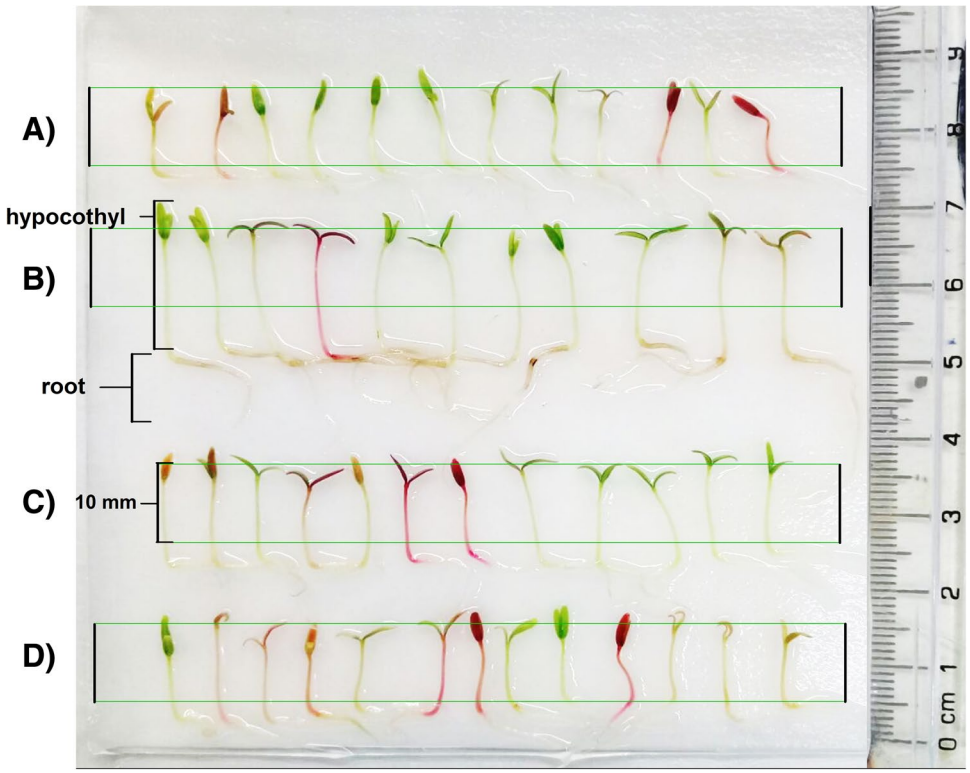

Fig. 4 Amaranth sprouts after treatment with: (**A**) T0; (**B**) TB, (**C**) TP and (**D**) TU. Scale bars indicated 10 mm. The concentration used for each treatment was 2.5 mg mL^{-1}. Distilled water as a negative control (T0); ByoAlga® from *Ascophyllum nodosum* (TB), ASE from *Padina durvillei* (TP) and *Ulva lactuca* (TU)

Growth of sprouts by seaweed extract

Different seaweed extracts, especially those from kelps like *A. nodosum*, have been utilized to increase biomass yield and boost the quality of plant products (Filippo-Herrera et al. 2019). Algal extracts improve germination, vegetative growth, and root growth, as well as promoting changes in phytochemicals such as polyphenols, carotenoids, and chlorophylls (Ali et al. 2021). For example, extracts of *A. nodosum* promoted the growth of leaves and roots of *Amaranthus tricolor* (Hossain and und Niemsdorff (2018). Extracts of *U. lactuca* had a positive effect as a root-growth promoter, enhancing germination, and promoting growth in tomato (Hernández-Herrera et al. 2018) and mung bean (Castellanos-Barriga et al. 2017). However, there is no documented evidence on the effect of extracts of *P. durvillei*, a species widely distributed on the coasts of the Mexican Pacific. This study showed that extracts of *U. lactuca* and *P. durvillei* have biostimulant activity, similar to other algal extracts (EL Boukhari et al. 2020; Ali et al. 2021).

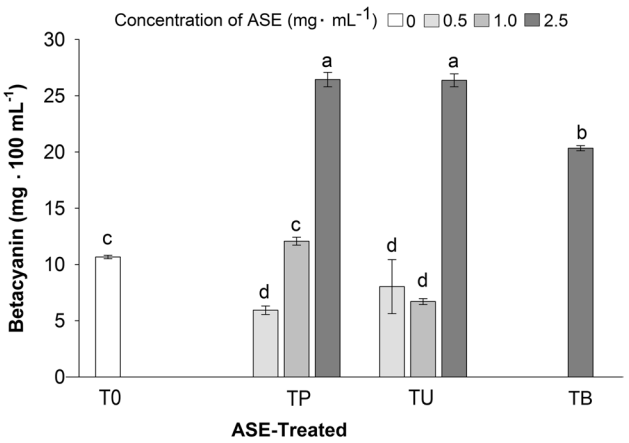

Fig. 5 Betacyanin content in amaranth sprouts after pre-treatments with different concentrations (mg mL^{-1}) of aqueous seaweed extract (ASE): The values are mean of representative sample of seedlings from each pre-treatment ($n=12$). Means with different latters are significantly different ($P<0.05$). Distilled water as a negative control (T0); ByoAlga® from *Ascophyllum nodosum* (TB), Extract from *Padina durvillei* (TP) and *Ulva lactuca* (TU)

In-vitro antioxidant activity on amaranth sprouts

The present results suggest that it is possible to obtain amaranth sprouts with antioxidant properties in a shorter time (5 days after post-seedling emergence) by stimulation with ASE treatment, In commercial amaranth microgreens (> 7 days post-seedling emergence), the *in-vitro* antioxidant capacity evaluated by ORAC, FRAP, and ABTS, with values of 1.4, 0.1, and 0.6 mmoles Trolox $(100\ g)^{-1}$ fresh weight, respectively (Wojdyło et al. 2020).

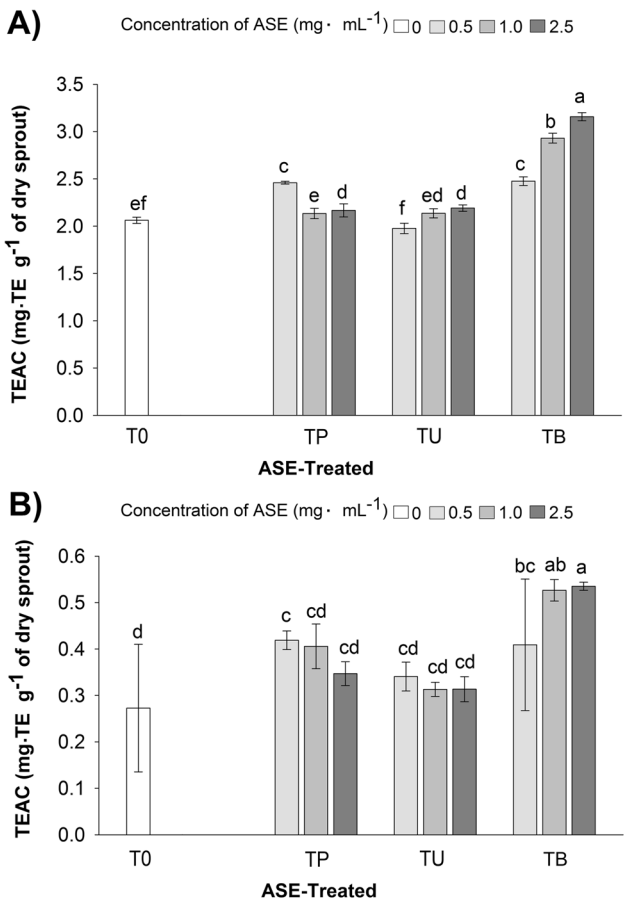

Fig. 6 Antioxidant activity of amaranth sprouts produced by treatment with different concentrations of seaweed extract (SE), assessed by (**A**) DPPH free-radical scavenging activity and (**B**) ferric reducing antioxidant power (FRAP) assay. The values are mean ± S.D. ($n = 6$). Means with different latters are significantly different ($P < 0.05$). Distilled water as a negative control (T0); ByoAlga® from *Ascophyllum nodosum* (TB), Extract from *Padina durvillei* (TP) and *Ulva lactuca* (TU)

Antioxidant activity in plants is part of the defense mechanisms that provide resistance to oxidative stress caused by free radicals. In this sense, the results showed an antioxidant effect of the sprouts treated with the algal extracts. Some plant hormones like SA are related to induces gene expression involved in enhance biosynthesis of phenolic compounds with antioxidant capacity (Ali et al. 2021).

Phytochemical content in amaranth sprout

In the present study, the growth stages evaluated included the early vegetative phase on 5 days post-seeding, so that the seed had started its stage of higher accumulation of TPC and other metabolites with antioxidant potential (Karamać et al. 2019; Martínez-Núñez et al. 2019); this facilitated the accumulation of these compounds as a stimulation effect in seeds treated with ASE, as suggested by the results obtained herein (Table 1).

According to Cao et al. (2012), inducers such as cytokines, jasmonates, and salicylates increase the production of betacyanins in amaranth sprouts. Our results showed that treatment with ASE of *P. durvillei* and *U. lactuca* prior to germination significantly increased the betacyanin levels in *Amaranthus* sprouts compared to water controls.

The results of the phytochemical analysis performed on sprouts were similar to other studies using ASEs. For example, there was an increase in chlorophyll content and phenolic compounds in the medicinal plant *Achillea millefolium* treated with algal extracts of *A. nodosum* (Pacheco et al. 2019). Extracts of *U. lactuca* produced higher contents of chlorophylls *a* and *b* in *Amaranthus roxburghinus* and *Amaranthus tricolor* (Sridhar and Rengasamy 2011), and in sprouts of bean *V. faba* (Yahmi et al. 2021). A shorter germination process of amaranth seeds (24 h) increases the accumulation of phenolic compounds by about threefold-relative to levels in ungerminated seeds, i.e., from 17.61 and 11.71 mg GAE $(100\ g)^{-1}$ sample, up to 58.55 and 36.65 mg GAE $(100\ g)^{-1}$ sample in germinated seeds of *A. caudatus* and *A. quitensis*, respectively (Cornejo et al. 2019).

There was a significant differences in TPC in different growth stages of *A. caudatus*, where the vegetative stage (34 to 55 days post-seedling emergence) showed the highest accumulation (nearly 1 mg GAE g^{-1} fresh weight) (Karamać et al. (2019). In commercial amaranth microgreens of more than 7 days post-seedling emergence, the total content of compounds with antioxidant potential (fresh weight) was 132.9 mg $(100\ g)^{-1}$ for polyphenols and 680.5 and 4073 µg g^{-1} for carotenoids and chlorophylls, respectively (Wojdyło et al. 2020). Changes in the phytochemical content of amaranth sprouts at low concentration of algal extracts may be due to bioactive compounds (such as unique polysaccharides, plant growth promoting hormones, fatty acids, sterols, carotenoids, oxylipins, minerals, peptides, amino acids and proteins) (Filippo-Herrera et al. 2019), these substances in the extracts vary differently according to the class and species of seaweed, as well as the type of extraction method used. In this work we used aqueous seaweed extracts previously characterized by Benítez García et al. (2020) where plant growth promoting hormones (SA, and JA) were identified; these bioactive compounds at low concentrations have positive effects on the accumulation of phytochemicals in plants (Ali et al. 2021; Wang et al. 2022); this behavior, highlight potential of algae to act as a biostimulant on plants, as described by Ali et al (2021).

The presence of salicylic acid (SA) and jasmonic acid (JA) in ASEs are related to the systemic response of plants and induce the biosynthesis of secondary metabolites such as phenolic compounds, chlorophyll, and carotenoids

Table 1 Total polyphenols (TPC), flavonoids (TFC), chlorophylls *a* (TCCa), *b* (TCCb), and carotenoid (TCC) content in amaranth sprouts produced by treatment with different concentrations (mg mL^{-1}) of aqueous seaweed extract (ASE)

Concentration of ASE (mg mL^{-1})		TPC (mg QE g^{-1})	TFC (mg GAE g^{-1})	TCCa (mg g^{-1})	TCCb (mg g^{-1})	TCC (mg g^{-1})
T0	0	6.157 ± 0.305^{d}	0.565 ± 0.014^{b}	0.056 ± 0.002^{d}	0.065 ± 0.004^{c}	0.021 ± 0.001^{bc}
TP	0.5	9.520 ± 0.317^{b}	0.567 ± 0.070^{ab}	0.090 ± 0.002^{a}	0.121 ± 0.006^{a}	0.010 ± 0.001^{e}
	1.0	7.654 ± 0.128^{c}	0.456 ± 0.004^{cd}	0.054 ± 0.002^{de}	0.078 ± 0.004^{b}	0.009 ± 0.001^{d}
	2.5	6.460 ± 0.227^{d}	0.502 ± 0.009^{c}	0.051 ± 0.002^{ef}	0.041 ± 0.004^{f}	0.026 ± 0.001^{b}
TU	0.5	6.499 ± 0.776^{d}	0.519 ± 0.038^{bc}	0.078 ± 0.002^{b}	0.052 ± 0.004^{e}	0.042 ± 0.002^{a}
	1.0	6.238 ± 0.108^{d}	0.585 ± 0.055^{ab}	0.069 ± 0.002^{c}	0.056 ± 0.005^{d}	0.039 ± 0.002^{a}
	2.5	5.101 ± 0.141^{e}	0.625 ± 0.013^{a}	0.052 ± 0.002^{de}	0.036 ± 0.004^{f}	0.037 ± 0.002^{ab}
TB	0.5	6.681 ± 0.264^{d}	0.476 ± 0.004^{cd}	0.048 ± 0.002^{f}	0.054 ± 0.004^{de}	0.014 ± 0.003^{c}
	1.0	9.861 ± 0.327^{b}	0.446 ± 0.009^{d}	0.051 ± 0.002^{e}	0.063 ± 0.004^{cd}	0.012 ± 0.001^{d}
	2.5	10.976 ± 0.389^{a}	0.557 ± 0.008^{b}	0.048 ± 0.003^{f}	0.062 ± 0.006^{cd}	0.013 ± 0.002^{cd}

Data are the mean ± S.D. ($n = 6$), different letters in the values of the same column have statistically significant differences ($P < 0.05$)

All compound contents are expressed in mg per g of dry amaranth sprout used to obtain 1 mL of sprout extract: mg QE, mg of quercetin equivalent; mg GAE, mg of gallic acid equivalent

Distilled water as a negative control (T0); ByoAlga® from *Ascophyllum nodosum* (TB), aqueous seaweed extract(ASE) from *Padina durvillei* (TP) and *Ulva lactuca* (TU)

(Benítez García et al. 2020; Wang et al. 2022). For example, Jirakiattikul et al. (2021) studied the effect of salicylic acid (SA) on the accumulation of secondary metabolite and antioxidant activity in cultures of sprouts of *Musa acuminata* cv. 'Gros Michel'; their results showed that SA was effective in improving the production of total phenols (105.26 ± 6.43 mg CE g^{-1} dry extract). Separately, Wang et al. (2022) found that the application of AS and AJ to seedlings of *Allium tuberosum* Rottler ex Spreng significantly increased total chlorophyll, phenols, flavonoids, and vitamin C contents. Hence, phytohormones in *U. lactuca* and *P. durvillei* ASEs are likely to induce changes in the profile of secondary metabolites of amaranth sprouts.

Although biostimulants based on algae extracts have gained popularity in the development of green agriculture, studies with seaweed species from tropical waters are scarce. The information that can be generated from species with constant blooms on the coast of Mazatlan Sinaloa, such as *P. durvillei* and *U. lactuca*, whose extracts showed that they are capable of improving the content of antioxidant compounds in amaranth sprouts. This new information may contribute to increasing the attractiveness of the study and/or development of products based on these tropical marine algae with potential use in different crops.

Conclusion

In conclusion, aqueous seaweed extracts from *P. durvillei* and *U. lactuca* possess biostimulant activity when applied to amaranth seeds; in addition, ASEs improved the growth, yield, and functional quality of amaranth sprouts. These results suggest that extracts from these algae species applied at 2.5 mg mL^{-1} and 0.5 mg mL^{-1} are suitable as an alternative to synthetic biostimulants used in the production of sprouts.

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Data availability The data that support the findings of this study are available from the corresponding autor upon reasonable request.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

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