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Activity of seaweed extracts and polysaccharide-enriched extracts from *Ulva lactuca* and *Padina gymnospora* as growth promoters of tomato and mung bean plants

Rosalba Mireya Hernández-Herrera [1,2] · Fernando Santacruz-Ruvalcaba [2] · Julia Zañudo-Hernández [2] · Gustavo Hernández-Carmona [1]

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Abstract Although marine seaweeds have been used as biostimulants since the beginning of modern agriculture, studies have only recently focused on the ability of seaweed extracts and their polysaccharides to enhance growth of plants. In this work, two bioassays were carried out to study the growth-promoting activity of seaweed extracts and polysaccharide-enriched extracts from *Ulva lactuca* and *Padina gymnospora*, obtained in neutral and alkaline conditions. Initially, the effect of seaweed extracts and polysaccharide-enriched extracts on seed germination and growth-promoting activity on tomato (*Solanum lycopersicum* cv. Río Grande) plants under in vitro conditions was studied. Half-strength Murashige-Skoog (MS) medium with or without sucrose was supplemented with different concentrations of seaweed extracts (2, 4, and 10 mg mL^{-1}) or polysaccharide-enriched extracts (0.2, 0.4, and 1.0 mg mL^{-1}). The parameters evaluated were germination percentage, radicle and shoot length, and dry weight. In a second experiment, polysaccharide-enriched extracts at 1.0 mg mL^{-1} and indole-3-butyric acid as the control were studied for root inducer activity in mung bean (*Vigna radiata*). The majority of seaweed extracts had an inhibitory effect on seed germination. However, a significant effect ($P \leq 0.05$) on tomato seedling growth (except for dry weight) was shown with seaweed extracts at 2 mg mL^{-1} included in half-strength MS medium with sucrose (30 g L^{-1}). Moreover, 10 mg mL^{-1} neutral and alkaline seaweed extracts had an inhibitory effect on the parameters evaluated. In contrast, polysaccharide-enriched extracts obtained from *U. lactuca* and *P. gymnospora* promoted germination and stimulated growth of tomato plants compared to the controls. Additionally, treatment of mung bean hypocotyl cuttings with polysaccharide-enriched extracts of *U. lactuca* and *P. gymnospora* induced rooting more rapidly and in greater number compared to the controls. These results provide evidence that polysaccharide-enriched extracts act as an effective growth-promoting treatment.

Keywords Seaweed extracts · Polysaccharide-enriched extracts · Growth stimulation · In vitro culture · Chlorophyta · Phaeophyta

Introduction

Seaweed extracts are an organic growth promoter and their potential remains unexploited in Mexican agriculture. Previous reports have emphasized the importance of seaweed extracts and their utilization with significant results on plants grown in greenhouse and field conditions, such as vegetables, bulb-crops (potato, carrot, beet, sweet potato), fruit crops (orange, lemon, banana, peach, pear-tree, tomato, pea, pepper, brinjal), grains (corn rice, maize), legume crop (black gram, green gram, common bean), and flowers (orchid, rose, sunflower), or under in vitro culture conditions (Arabidopsis, tomato, brinjal, finger millet). Seaweed manures or extracts

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✉ Gustavo Hernández-Carmona
gcamona@ipn.mx

[1] Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas (CICIMAR), Av. IPN S/N. Col. Playa Palo de Santa Rita, C.P. 23096 La Paz, Baja California Sur, México

[2] Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA), Universidad de Guadalajara, Calle Ramón Padilla Sánchez No. 2100, Predio Las Agujas, La Venta del Astillero, C.P. 45110 Zapopan, Jalisco, México

applied in different ways exhibit a wide range of positive responses that include increased germination, root development system, increased chlorophyll content and leaf area, fruit quality, and plant vigor and resistance to pathogens (Hong et al. 2007; Rayorath et al. 2008; Khan et al. 2009; Craigie 2011; Vinoth et al. 2012a, b, 2014; Mattner et al. 2013; González et al. 2013a, b; Briceño-Domínguez et al. 2014; Satish et al. 2015a, b; Ali et al. 2015; Singh et al. 2015). As these beneficial effects are achieved with small doses of seaweed extracts, the active constituents are thought to be growth hormones such as auxins, cytokinin, gibberellins, and low molecular weight components (polyamines and brassinosteroids) that are all effective at low concentrations. In addition, major components such as polyphenols (phloroglucinol and its derivate eckol) identified in algal extracts promote growth activity, as well as polysaccharides (alginate, fucoidan, laminaran and carrageenans or their derived oligosaccharides) which also act as plant growth promoters (Hong et al. 2007; Khan et al. 2009; Craigie 2011; González et al. 2013a; Rengasamy et al. 2015a, b).

Polysaccharides, or fractions such as oligosaccharides, increased seed germination, stimulated growth of root, higher yields, and favored resistance to diseases in various crops after spraying plants with crude extracts (Iwasaki and Matsubara 2000; Mercier et al. 2001; Laporte et al. 2007; Chandía and Matsuhiro 2008; Paulert et al. 2009; González et al. 2013a, b, 2014). Total polysaccharide concentrations in seaweed species as *Ulva lactuca* and *Padina gymnospora* ranged from 4 to 76 % dry weight (Holdt and Kraan 2011). Alginates are the major components of brown seaweed cell walls (Vera et al. 2011); in contrast, ulvan is the most important constituent of green seaweed cell walls, representing 8 to 29 % of the algal dry weight (Lahaye and Robic 2007). Alginate polysaccharides were effective in promoting plant growth (Cao et al. 2007). In particular, alginate-derived oligosaccharides (ADO), obtained by depolymerization of alginates from brown seaweeds, triggered the stimulation of growth, promotion of germination, and shoot elongation in different plants species (Yonemoto et al. 1993; Natsume et al. 1994) by enhancing nitrogen assimilation and basal metabolism (González et al. 2013b). Oligo alginates, obtained with an alginate lyase, induced germination of maize seeds (Hu et al. 2004), stimulated growth of roots in lettuce (Iwasaki and Matsubara 2000), stimulated elongation of carrot and rice, and increased biomass of tomato plants (Iwasaki and Matsubara 2000; Liu et al. 2000; Xu et al. 2003). Alginate depolymerized by treatment with γ-radiation enhanced growth of rice and peanut plants cultivated hydroponically (Hien et al. 2000). Also, tobacco plants treated with polymannuronic acid fraction (Poly-Ma) obtained by partial acid hydrolysis of sodium alginates showed an increase in height over the controls (Laporte et al. 2007). In addition, ulvans from green seaweed increase the germination of common bean (Paulert et al. 2009).

In principle, plants could be used as biosensors (bioassay model systems) for detecting the presence of bioactive molecules, testing and even assessing the effects of bioactivity, and could be used as a convenient system to ensure uniform bioactivity of seaweed products (Rayorath et al. 2008). Tomato is one of the most studied higher plants for genetic, molecular, and physiological studies (McCormick et al. 1986). In vitro culture protocols have been well-established for tomato using different explants such as cotyledons, hypocotyl, and leaf (Vikram et al. 2011) and recently in combination with seaweed extracts (Vinoth et al. 2012a, b). Mung bean cuttings may serve as an important experimental system to elucidate the induced root formation using indole-3-butyric acid (IBA) as a reference, (Hess 1961; Crouch and van Staden 1992; Jain et al. 2008; Sharma et al. 2012; Briceño-Domínguez et al. 2014; Rengasamy et al. 2015a; Lötze and Hoffman 2015).

Usually, in vitro culture methods depend on the use of macro and micronutrients and sucrose as an energy and carbon source as well as an osmotic agent for plant nutrition (George et al. 2008). The carbohydrates added to the culture medium supply energy for plant metabolism (Caldas et al. 1998) and are essential for in vitro growth and development, because photosynthesis is insufficient, due to the growth taking place in conditions unsuitable for photosynthesis or without photosynthesis (in darkness). Normally, green tissues are not sufficiently autotrophic under in vitro conditions and depend on the availability of carbohydrates in the growing medium (Pierik 1997).

The hypothesis of this study was that reducing the compounds of Murashige-Skoog (MS) medium at half-strength and eliminating sucrose as a carbon source, but incorporating the seaweed extracts, that contain macro/micronutrients and polysaccharide-enriched extracts, in combination with the compound present in the medium, can be used by the tomato plants to stimulate growth activity. In addition, the effects of polysaccharide-enriched extracts as a root inducer of mung bean plants were analyzed to assess whether these products can be considered and used as equivalent growth promoters.

Material and methods

Preparation of seaweed extracts and polysaccharide-enriched extracts

Two seaweeds, *Ulva lactuca* Linnaeus and *Padina gymnospora* (Kützing) Sonder, were collected from Bahía Careyitos, Jalisco, México (19° 43″ N, 105° 02″ W) during May and November 2009. The samples was then washed with seawater to remove epiphytes and sand particles and then oven-dried for 72 h at 60 °C. The dried algae were milled using an electric milling machine (IKA M 20, Sigma-Aldrich, USA) to 0.50 mm and used to prepare the seaweed

extracts and polysaccharide-enriched extracts. The neutral seaweed extracts (NSE) were prepared according to Hernández-Herrera et al. (2014) and the alkaline seaweed extracts (ASE) according to Briceño-Domínguez et al. (2014). The polysaccharide-enriched extracts (N-PEEs and A-PEEs) were obtained from both seaweed extracts NSE and ASE respectively, as previously described by Cluzet et al. (2004) (according to the experimental design) (Fig. S1). The precipitates were recovered by filtration, lyophilized and stored at −20 °C until use (dried fraction was weighed and dissolved in culture medium previously autoclaved). Furthermore, the pH and electrical conductivity (EC) of the PPEs were measured using a pH meter and conductivity meter. All determinations were performed in triplicate.

Chemical analysis of seaweed extracts and polysaccharide-enriched extracts

Proximate analyses were carried out following the procedures form the Association of Official Analytical Chemists (AOAC 2000), the moisture content (drying over at 60 °C to constant weight, method 930.36), ash (calcination at 550 °C in muffle method 942.05), crude fiber (Soxhlet, method 962.09), ether extract (Soxhlet apparatus, method 954.02), and nitrogen content by micro-Kjeldahl method (method 976.05). The protein content of a conversion factor of 6.25 (method 954.04) was applied. Indirect estimation of carbohydrates was calculated according to the following equation: % carbohydrates = 100 − (% protein + % fat) and dry matter by difference. Total carbohydrate (T-CHO) and total reducing sugars (T-RS) of N-PEE and A-PEE were extracted according to Carnal and Black (1989) and measured by the Nelson–Somogyi test (Nelson 1944; Somogyi 1952). Soluble sugars were extracted from each tissue according to adapted methodologies (Geigenberger et al. 1998; Wright et al. 1998). Briefly, 50 mg of ground vacuum-dried tissue was extracted in HEPES-KOH (50 mM; pH 7.4); 5 mM $MgCl_2$, 80 % ethanol, three times by 10 min at 80 °C. Soluble extracts were combined and assayed enzymatically for sucrose (SUC), glucose (GLC), and fructose (FRC) in a microplate format (Tiessen et al. 2002). The insoluble starch pellet was dissolved in 0.5 mL HEPES-KOH (10 mM; pH 7.4) at 99 °C and autoclaved at 0.124 MPa and 121 °C for 30 min. Starch was hydrolyzed in HEPES-KOH (50 mM; pH 7.5) at 37 °C overnight by the addition of ten units of α-amylase (Roche) and ten units of amyloglucosidase (Roche). Samples were centrifuged (13,000×*g* for 5 min), the resulting supernatant was stored at 4 °C, and the pellet was hydrolyzed again for 30 min at 37 °C. Both supernatants were combined, and an aliquot was enzymatically assayed for GLC, as above. Information reported previously by Hernández-Herrera et al. (2014) with reference to mineral composition in the experimental seaweeds was included.

Bioassays for tomato seed germinated under in vitro conditions

Seed of tomato (*Solanum lycopersicum* cv. Río Grande; Crown seed, California, USA) were disinfected superficially using a soap solution for 5 min, followed by immersion in a 4 % sodium hypochlorite (NaClO) solution for 10 min and triple-rinsed in sterilized distilled water 1 min for each under aseptic conditions.

Germination bioassays were performed according to Satish et al. (2015a). Surface-disinfected seeds (one group of 100) were grown in glass jars containing 25 mL half-strength MS basal media (Murashige and Skoog 1962) with 30 g L^{-1} of sucrose (Suc 30) or without sucrose (Suc 0) (as controls) and combined with different concentrations of SEs (at 2, 4, and 10 mg mL^{-1}) or PEEs (at 0.2, 0.4, and 1.0 mg mL^{-1}). All cultures were solidified with agar (8 g L^{-1}), adjusted to pH 5.8 ± 0.1, using 1 N HCL and 1 N NaOH prior to the addition of agar and autoclaving at 121 °C and 0.124 MPa for 15 min. In a controlled environment growth chamber, cultures were incubated at 25 ± 2 °C in the dark for 3 days and shifted to light by providing 50 μmol photons m^{-2} s^{-1} with white fluorescent tubes. Seeds were considered germinated once the radicle protruded more than 2 mm. Germinations were observed daily over a period of 8 days according to methods of the Association of Official Seed Analysts (AOSA 2005), and after 8 days of incubation, germination percentage over the control was determined. The experiment was repeated twice.

Bioassays for tomato seedling growth under in vitro conditions

To evaluate the growth of tomato plants under in vitro conditions, a total of 56 treatments were used where 26 treatments correspond to SEs and 26 to PEEs. Two treatments served as the controls in which plants were grown in ½ MS + sucrose or ½ MS without sucrose. Four factors were randomized for the other 24 treatments. The first factor was presence of sucrose (30 g L^{-1}) or not in the culture medium. The second factor was the type of seaweed species (*U. lactuca* and *P. gymnospora*) used to prepare the SEs or PEEs. The third factor was the method of extraction by neutral or alkaline conditions to produce the SEs or PEEs. The fourth factor was the concentration (SEs; 2.0, 4.0 and 10 mg mL^{-1} or PEEs; 0.2, 0.4 and 1.0 mg mL^{-1}). All cultures were solidified with agar (8 g L^{-1}), adjusted to pH 5.8 ± 0.1, using 0.1 N HCL and 0.1 N NaOH prior to the addition of agar and autoclaving at 121 °C and 0.124 MPa for 15 min. The experimental units were arranged in a completely randomized four-factorial design. Six glass jars with six seedlings grown in 25 mL medium per jar were used for each treatment ($n = 36$), and the experiment was repeated twice. Tomato seedlings were measured after 2 weeks at the same concentrations and incubation

conditions above for germination. For each treatment, length of shoot and root (cm) were measured with a vernier caliper, as well as seedlings were dried in an oven (Terlab MA H45DM) at 60 °C for 72 h. The growth parameters were recorded at 15 days of culture.

Root inducer activity bioassay

Biological response as rooting activity to the respective polysaccharide-enriched extracts (N-PEEs and A-PEEs) was evaluated with a mung bean bioassay performed according to Lötze and Hoffman (2015). Mung bean (*Vigna radiata* (L.) Wilezek) seedlings were grown in pots using peat moss (Sunshine Mix 3) and watered daily. The seedlings were maintained at a constant temperature of 26 °C, with a light intensity of 100 μmol photons m^{-2} s^{-1} photosynthetic photon flux density, photoperiod of 16 h light and 8 h dark. After 15 days, seedlings 12 cm high were cut at the base of the stem. The cotyledons were removed, and the length of the stems was adjusted to 3 cm below the cotyledons. Polysaccharide-enriched extract solutions were prepared at 1 mg mL^{-1} (*w*/*v*) by dilution with distilled water. A dose response curve for rooting ability was performed using a concentration range of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M of indole-3-butyric acid (IBA). Distilled water was used as a control. Test solutions were placed in 50 mL centrifuge tubes and covered with Parafilm, and the plant cuttings placed in holes pierced in the film with 4 cm of the cutting immersed in the test solutions. Four seedlings were placed in each tube, with five replicate tubes. The mung bean cuttings were maintained for 12 h in the solutions and then withdrawn, rinsed with purified water, and placed in vials containing distilled water. All treatments and the dose response curve were performed at the same time (12 days) to ensure equal exposure to similar environmental growth conditions for all experimental units. The average number of roots and length of adventitious roots formed was counted.

Statistical analysis

Comparison of means of multiple groups or treatments was made by analysis of variance (ANOVA), and multiple comparisons were made by the least significant difference (LSD) range test ($P \leq 0.05$). Seaweed extracts and polysaccharide-enriched extracts were analyzed for their effect in the growth of tomato by three and four-way ANOVA with type of alga, extraction conditions, concentration, and sucrose in medium MS, as factors. In all cases, the data were tested for normality and homoscedasticity. To compare the root inducer activity, the polysaccharide-enriched extracts were analyzed by one-way ANOVA, the Kruskal–Wallis test ($P \leq 0.05$). All statistical analyses were carried out with Statgraphics Centurion XV for Windows.

Results

Physicochemical properties of seaweed and polysaccharide-enriched extracts

The proximate analyses showed that the two algal species had different compositions. Protein and carbohydrate content was higher, and lipid and fiber content was lower in *U. lactuca* compared to *P. gymnospora*. Total nitrogen and sodium were higher in *U. lactuca*, but potassium and calcium were higher in *P. gymnospora*. The phosphate concentration was low in both seaweeds (Table 1).

The pH and EC were higher in the A-PEEs than in N-PEEs (Table 2). T-CHO content in PEEs showed higher values in *U. lactuca* than *P. gymnospora*. The proportions were reversed when T-RS were determined; *P. gymnospora* had higher values than *U. lactuca*. The glucose, sucrose, and fructose contents in A-PEEs were higher than in N-PEEs. Starch was detected on A-PEEs, while *P. gymnospora* had higher values than *U. lactuca* (Table 2).

Effect of seaweed extracts and polysaccharide-enriched extracts on tomato seed germination under in vitro conditions

Germination occurred after 2 days in almost all treatments. The majority of SEs had an inhibitory effect on seed germination, especially in ½ MS + Suc 30 + NSE. Germination percentage with NSE was dose-dependent with the lowest concentration (2.0 mg mL^{-1}) being similar to the control and the

Table 1 Proximate composition of seaweed species

Composition	*Ulva lactuca*	*Padina gymnospora*
Crude protein	11.78 ± 0.01a	9.83 ± 0.01b
Crude lipid	0.03 ± 0.01b	0.53 ± 0.01a
Ash	31.13 ± 0.01a	30.66 ± 0.03a
Fiber	5.63 ± 0.01b	15.86 ± 0.03a
Carbohydrates	41.25 ± 0.01a	37.90 ± 0.01b
Dry matter	90.00 ± 0.01b	94.75 ± 0.01a
Moisture	10.00 ± 0.01a	5.25 ± 0.01b
Total N	1.88 ± 0.01a	1.56 ± 0.01b
P	0.10 ± 0.08a[a]	0.10 ± 0.08a[a]
Na	5.57 ± 0.80a[a]	1.81 ± 0.50b[a]
K	1.85 ± 0.30b[a]	4.27 ± 0.60a[a]
Ca	1.88 ± 0.06b[a]	3.65 ± 0.40a[a]

Based on % dry weight (g 100 g^{-1} dry weight).Values are average ± standard error ($n = 3$). Means in the same line followed by different letters are significantly different

[a] By Hernández-Herrera et al. (2014)

Table 2 The pH, electro conductivity (EC), and content of total and reduced sugars in polysaccharide-enriched extracts of *U. lactuca* and *P. gymnospora* processed in neutral (N-PEEs) and alkaline (A-PEEs) conditions

Composition/treatments	N-PEE-UL	N-PEE-PG	A-PEE-UL	A-PEE-PG
pH	7.0	7.1	8.3	8.8
EC (dS m^{-1})	0.07	0.09	0.58	0.59
Total carbohydrate[a]	291.53 ± 7.07a	154.86 ± 5.35c	293.06 ± 2.54a	266.74 ± 3.82b
Total reduced sugars[a]	65.70 ± 3.06b	84.78 ± 1.63b	70.79 ± 5.0b	197.99 ± 9.1a
Glucose[b]	0.15 ± 5.65c	0.16 ± 4.80c	0.23 ± 0.03b	0.52 ± 0.22a
Sucrose[b]	0.28 ± 0.028b	0.28 ± 0.028b	0.67 ± 0.07a	0.61 ± 0.07a
Fructose[b]	0.02 ± 0.05d	0.09 ± 1.62c	0.19 ± 0.09b	0.26 ± 0.05a
Starch[b]	nd	nd	2.14 ± 0.61b	24.52 ± 0.49a

Values are expressed as mean ± SD ($n = 3$). Means in the same line followed by different letters are significantly different. *nd* Not detected

nd not detected

[a] mg g^{-1}, dry wt

[b] μmol g^{-1}, dry wt

highest concentration being the most inhibitory. In contrast, ASE (2.0 and 4.0 mg mL^{-1}) slightly enhanced germination on ½ MS medium with or without sucrose. The ASE of *P. gymnospora* had the most stimulatory effect on germination compared to the control (Fig. 1a). Higher concentrations of SEs tested were adversely affected and also decreased the response of percentage seed germination in tomato plants.

In contrast, germination of seeds grown on ½ MS medium with or without sucrose plus PEEs exhibited an increase in germination over the control with ½ MS + Suc 30 + PEEs

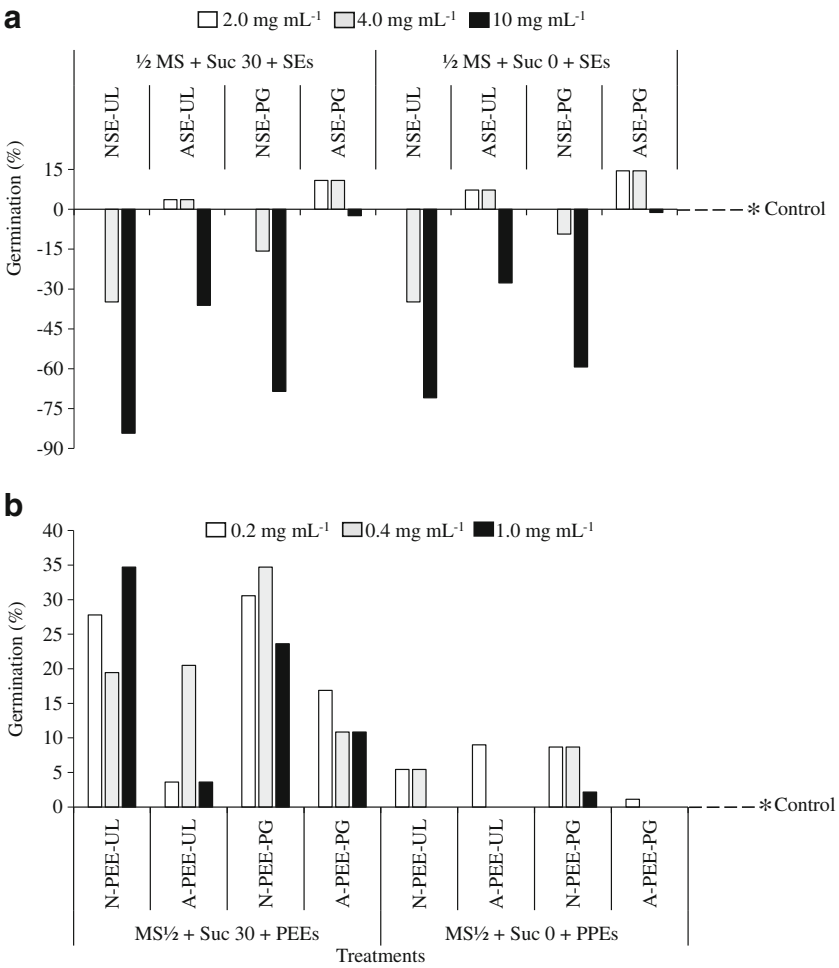

Fig. 1 Seed germination of tomato culture on in vitro condition in half-strength MS medium (½ MS) with sucrose 30 g L^{-1} (Suc 30) or without (Suc 0), and supplemented with different concentrations of **a** neutral (NSE) and alkaline (ASE) seaweed extracts or **b** with polysaccharide-enriched extracts obtained with neutral (N-PPE) or alkaline (A-PPE) conditions from *Ulva lactuca* (UL) and *Padina gymnospora* (PG). Values represent the mean of $n = 100$ seed. The mark (*asterisk*) indicates baseline of the figure which corresponds to the control

being the most effective. Also, germination of seeds grown on half-strength MS medium in presence of N-PEE had higher germination than seeds treated with A-PEEs (Fig. 1b). In particular, N-PEEs of *P. gymnospora* at 1.0 mg mL^{-1} and *U. lactuca* at 0.4 mg mL^{-1} were effective treatments. Seed germination increase over the control is illustrated in Fig. S2.

Effect of seaweed extracts and polysaccharide-enriched extracts on tomato seedling growth under in vitro conditions

The factors as algae type, extraction method, concentrations of extracts, and sucrose combined with ½ MS influenced the tomato seedling growth stimulation. SE (2 mg mL^{-1}) + ½ MS + Suc 30 g L^{-1} exhibited a significant effect ($P \leq 0.05$) on tomato seedling growth (shoot and radicle length) (Fig. 2a–d). ASE treatment was more effective in influencing the shoot length of the tomato seedling than NSE (Fig. 2a, d). Furthermore, NSE extract was more effective in promoting radicle length than the ASE extract (Fig. 2b, e). An increase in the concentration of seaweed extracts progressively reduced the dry weight when compared to controls (Fig. 2c, f). Moreover, both 10 mg L^{-1} NSE and ASE had an inhibitory effect on the parameters evaluated. In addition, the SEs + ½ MS + Suc 0 had no effect on growth of tomato plants (data not shown).

The interaction between extraction conditions, type of alga, concentration of polysaccharide-enriched extracts, and sucrose combined with ½ MS medium demonstrates that plants treated with both N-PEEs and A-PEEs had a positive effect ($P \leq 0.05$) on shoot length, radical length, and dry weight in tomato plants under in vitro culture (Fig. S3). The PEEs of *P. gymnospora* (at 0.2 and 0.4 mg mL^{-1}) + ½ MS + Suc 30 showed a larger stimulatory effect on tomato shoot length (Fig. 3a, d). In addition, N-PEE and A-PEE of *U. lactuca* (at 0.2 and 1.0 mg mL^{-1}, respectively) as well as A-PEE of *P. gymnospora* at 1.0 mg mL^{-1} increased radicle length (Fig. 3b, e). Dry weight of seedlings treated with N-PEE of *P. gymnospora* (0.4 mg L^{-1}) + ½ MS + Suc 30 was significantly greater than the control (Fig. 3c). A-PEEs had no positive effect on dry weight (Fig. 3f).

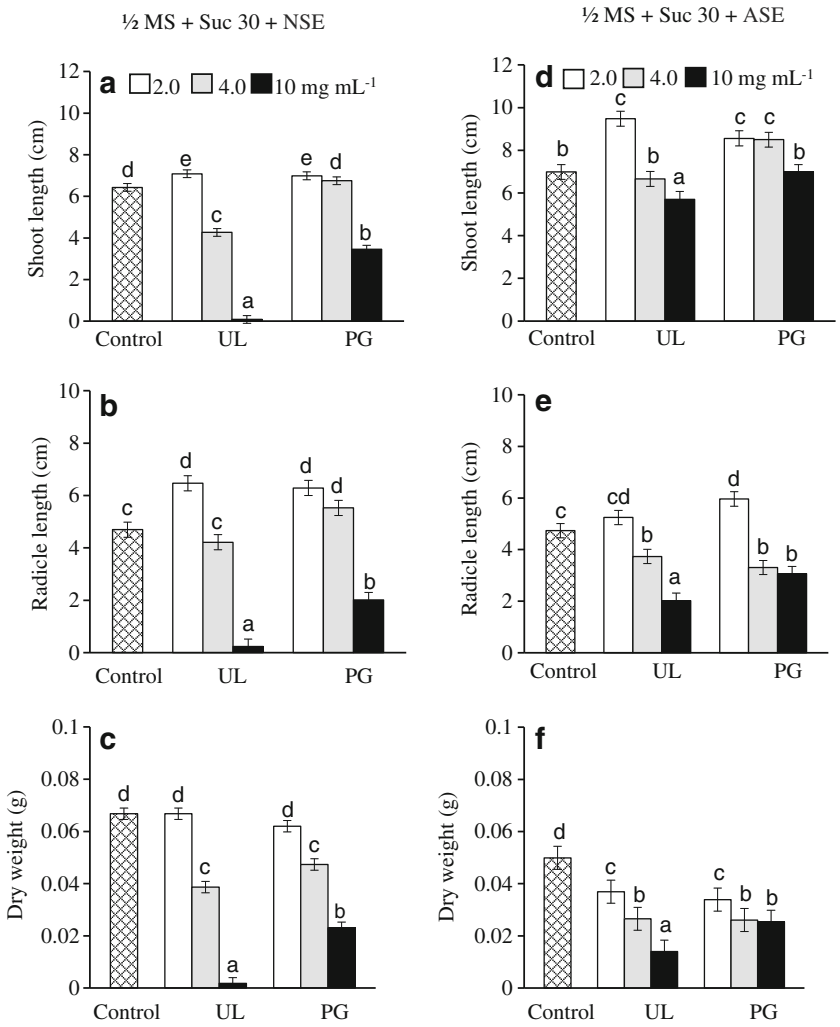

Fig. 2 Effect of neutral (NSE; **a**–**c**) and alkaline (ASE; **d**–**f**) seaweed extracts of *Ulva lactuca* (UL) and *Padina gymnospora* (PG) combined whit half-strength MS medium (½ MS) and sucrose 30 g L^{-1} (Suc 30) on shoot length and radicle length of tomato seedlings grown under culture in vitro. Control (half-strength MS with sucrose at 30 g L^{-1}). Columns denoted by a different letter are significantly different at $P \leq 0.05$. Values represent the mean of $n = 36$ seedlings; *bars* represent standard errors

In the same way, tomato seedlings cultured in ½ MS + Suc 0 + N-PEE of *P. gymnospora* at all concentrations had significantly increased shoot, radicle length, and dry weight ($P \leq 0.05$; Fig. 4a–c). There were no differences in shoot and radicle length of tomato seedlings grown with A-PEEs, except to *P. gymnospora* that showed an effect on radicle length at high concentrations (Fig. 4d, e). Dry weight was increased significantly compared to the control (Fig. 4f).

Root inducer activity bioassay

Effect of auxin IBA exhibited a significant effect on induction of roots ($P \leq 0.05$) with the number of lateral roots increasing with increasing concentrations of auxin. Linear regression analysis confirmed that in this experiment, the optimal response of rooting from cuttings is when these are subjected to immersion in an IBA solution 21.65 mg L^{-1} or higher to 10^{-3} M with each cutting having 16.25 roots on average (Fig. 5a). In this experiment, addition of auxin to the medium caused a significant decrease in root elongation (22 to 42 %) compared to the control (Fig. 6a).

Effects of polysaccharide-enriched extracts In the mung bioassay, most of the beans treated with polysaccharide-enriched extracts showed a statistically significant increase in number and length rooting compared to the control (water) when tested at a concentration of 1.0 mg mL^{-1} ($P \leq 0.05$; Fig. 5b). The greatest number of roots was achieved with polysaccharide-enriched extracts obtained with neutral rather than alkaline conditions. The N-PEE of *U. lactuca* exhibited higher number of roots than did its equivalent IBA to 10^{-3}, and N-PEE of *P. gymnospora* extract induced similar number of roots to 10^{-4} equivalent IBA. In contrast, A-PEE conditions presented a low number of roots. The number of roots recorded with A-PEE of *U. lactuca* was equivalent to 10^{-5} and A-PEE of *P. gymnospora* was similar to 10^{-6} equivalent IBA (Fig. 5b).

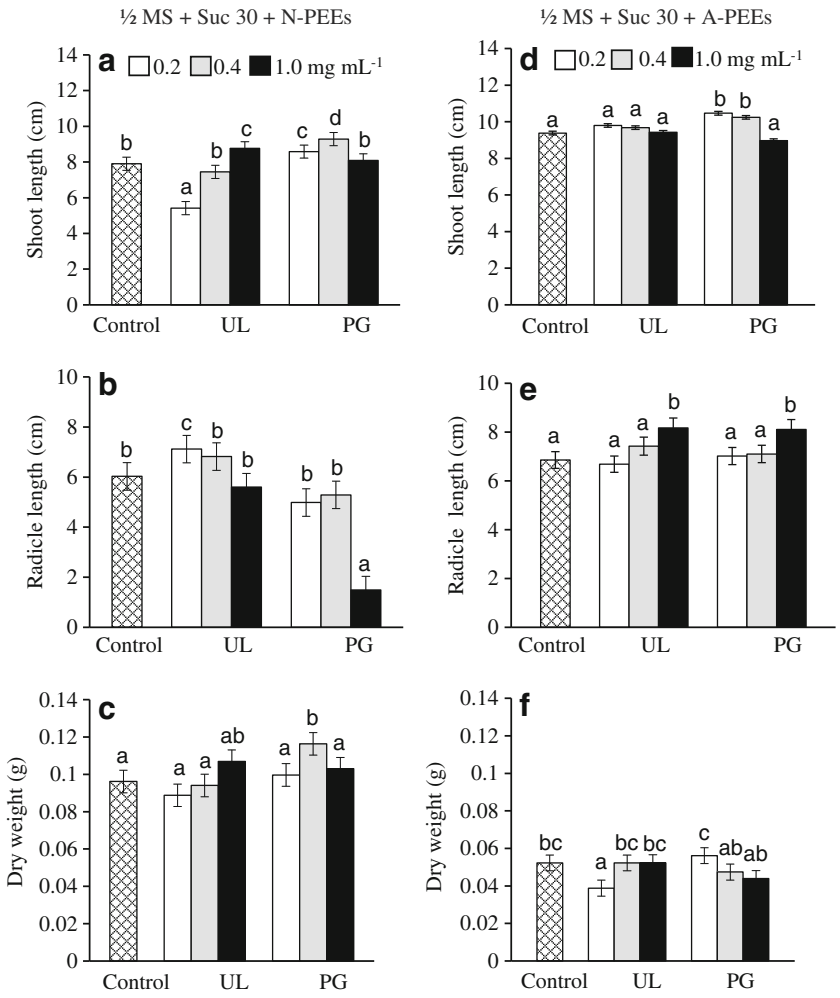

Fig. 3 Effect of polysaccharide-enriched extracts obtained with neutral (N-PEE; **a**–**c**) and alkaline (A-PEE; **d**–**e**) conditions from *Ulva lactuca* (UL) and *Padina gymnospora* (PG) combined with half-strength MS medium (½ MS) without sucrose (Suc 30), on shoot length, radicle length, and dry weight of tomato seedlings. Columns denoted by a different letter are significantly different at $P \leq 0.05$. Values represent the mean of $n = 36$ seedlings; *bars* represent standard errors

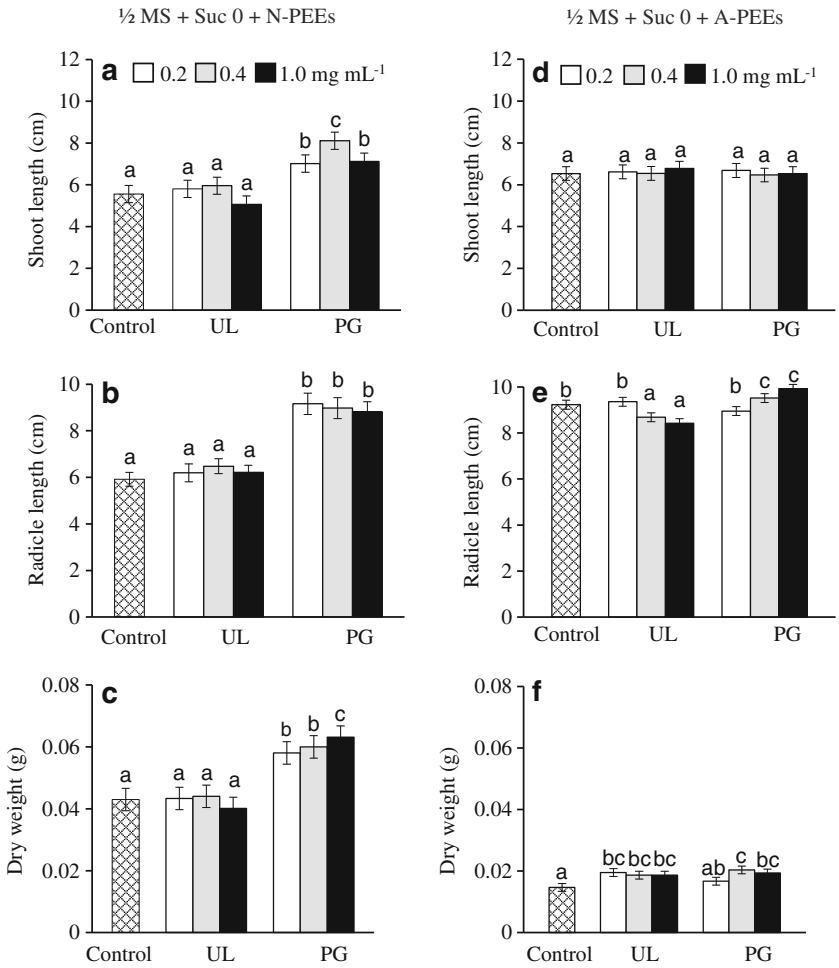

Fig. 4 Effect of polysaccharide-enriched extracts obtained with neutral (N-PEE; **a**–**c**) and alkaline (A-PEE; **d**–**e**) conditions from *Ulva lactuca* (UL) and *Padina gymnospora* (PG) combined with half-strength MS medium (½ MS) without sucrose (Suc 0), on shoot length, radicle length, and dry weight of tomato seedlings. Columns denoted by a different letter are significantly different at $P \leq 0.05$. Values represent the mean of $n = 36$ seedlings; *bars* represent standard errors

In addition, 1.0 mg L^{-1} N-PEE of *U. lactuca* and *P. gymnospora* promoted the formation of longer roots (Fig. 6b) while A-PEE had no significant effect on root length. The roots in the control were induced only on the base of the hypocotyls. In contrast, polysaccharide-enriched extracts and IBA altered their position on the hypocotyls with roots formed more extensively along the hypocotyl.

Discussion

Results revealed that the two algae have different chemical compositions. Mineral content, pH, and EC of neutral and alkaline extracts also affect the bioactivity of the extracts (Booth 1969, Henry 2005). In addition, yield and quality of total carbohydrates and total reducing sugars extracted from the seaweeds were dependent on the extraction method and species. Higher values of T-CHO and T-RS were obtained with alkaline extractions than with the neutral extractions from two seaweed species with *P. gymnospora* having higher values than *U. lactuca*. Similar results were observed by Sharma et al. (2012) in the thermal profiles of polysaccharides extracted under neutral or alkaline conditions. Compared to the neutral extracts, the materials from the alkaline extractions were well degraded (fine particle size) indicating depolymerization during extraction. In the present study, the extraction protocol was designed to retain the soluble cell wall components of the seaweeds as the main active compounds could be ulvans, alginates, or fucans, all of which are known to trigger plant growth responses (Paulert et al. 2009; Craigie 2011; González et al. 2013b).

Some studies have reported positive effects of seaweed extracts on seed vigor as a result of priming (Moller and Smith 1998; Demir et al. 2006; Farooq et al. 2008; Rathore et al. 2009; Spinelli et al. 2010), but inhibition of germination has also been observed, highlighting the need for caution in the use of seaweed extracts (Aitken and Senn 1965). The present results showed that seed germination of tomato plants was negatively influenced by seaweed extracts on half-strength of MS media with sucrose. This effect could partly be attributed to the role of minerals such as Na, K, Ca, and P present in the extracts that could influence the osmotic potential. Generally, culture media high in salt and sugar content reduce germination efficacy. Increasing concentrations of

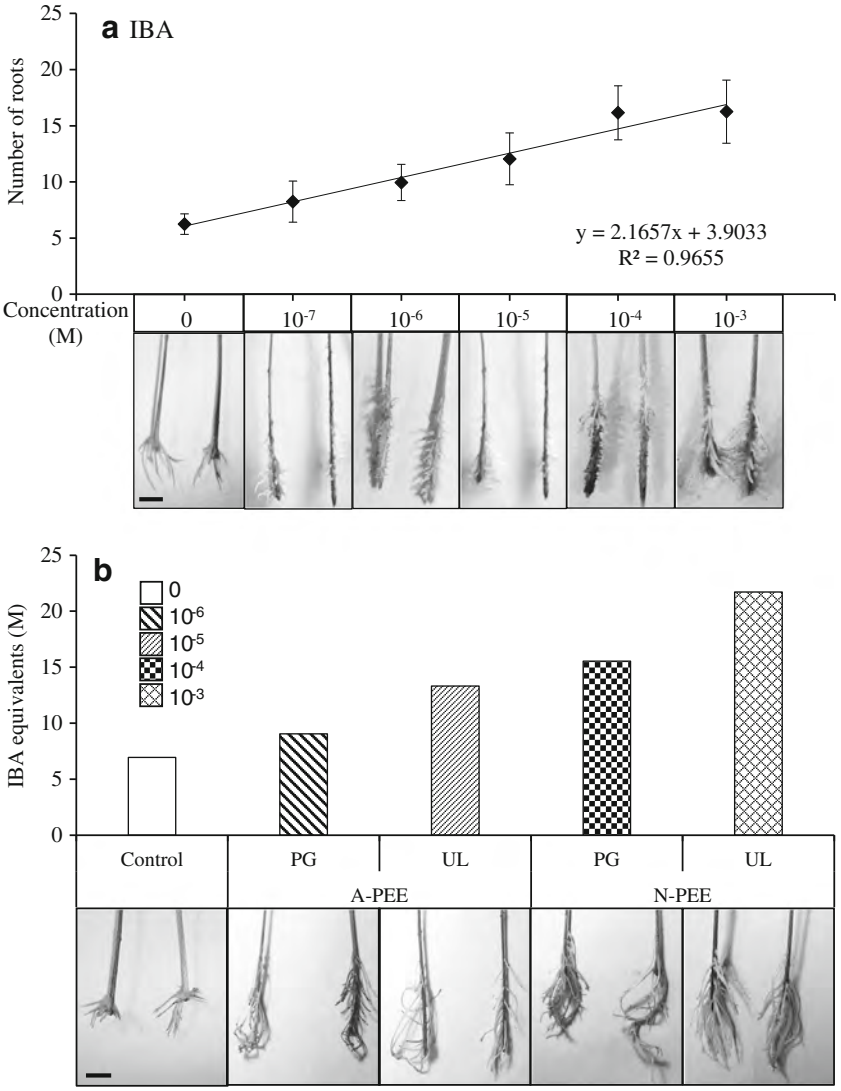

Fig. 5 Root inducer activity. **a** A standard curve for root formation in mung bean plants treated with indol-3-butyric acid (IBA) at 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} as reference. **b** IBA equivalents on root formation with polysaccharide-enriched extracts obtained with neutral (N-PEE) and alkaline (A-PEE) conditions from *Ulva lactuca* (UL) and *Padina gymnospora* (PG) at concentration of 1.0 mg mL^{-1}. Values represent the mean of $n = 20$ seedlings, *bars* represent standard errors. Bar = 1 cm

seaweed extracts not only prevent germination of tomato seeds (Vinoth et al. 2012a, b, 2014) but also extend the germination time by delaying the germination onset (Hernández-Herrera et al. 2014). Hence, in the present study, the higher germination percentage in the control could be due to the absence of salts in the medium and therefore seeds were able to imbibe water. Tomato is moderately sensitive to salinity (Basher et al. 2012). The higher seed germination compared to the control in half-strength MS with sucrose and supplemented with polysaccharide-enriched extracts of *U. lactuca* and *P. gymnospora* in combination with the absence of salts in the medium may be explained by the seeds being more efficient at absorbing water and incorporating compounds such as ulvan and alginate-derived oligosaccharide. Hu et al. (2004) showed that alginate-derived oligosaccharide increased maize seed germination from 0.5 to 5.0 % because of promotion of amylase activity and acceleration of the metabolic activity of the seed.

Similarly, extracts and/or polysaccharides from seaweeds also have a stimulatory effect on plant physiology. The presence of bioactive substances can enhance efficiency of stomatal uptake of nutrients and minerals compared to the untreated plants (Mancuso et al. 2006). In the present research, supplementation with neutral and alkaline seaweed extracts at lower concentrations in half-strength MS and sucrose improved the shoot and root growth, similar to previous reports on vegetables (Vinoth et al. 2014; Satish et al. 2015a, b). Hernández-Herrera et al. (2014) reported that seaweed extracts from *U. lactuca* and *P. gymnospora* stimulated growth of plants by supplying macronutrients and micronutrients. Seaweed extracts containing 6-benzylaninopurine and indole-3-butyric acid induced plant growth, regeneration, and development of tomato (Vinoth et al. 2014), and phloroglucinol and eckol extracted from *Ecklonia maxima* stimulate growth and development in mung bean and maize seedlings (Rengasamy et al. 2015b).

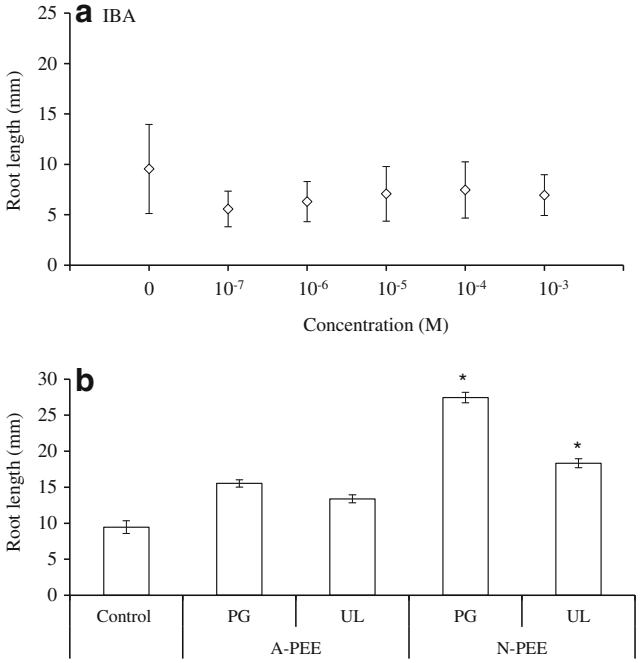

Fig. 6 Root length in mung bean plants treated with **a** indol-3-butyric acid (IBA) as reference, **b** length root formation in mung bean plants treated with polysaccharide-enriched extracts obtained with neutral (N-PEE) and alkaline (A-PEE) conditions from *Ulva lactuca* (UL) and *Padina gymnospora* (PG) at concentration of 1.0 mg mL^{-1}. The mark (*asterisk*) indicates statistically significant differences ($P \leq 0.05$) according to the nonparametric Kruskal–Wallis test. Values represent the mean of $n = 20$ seedlings, *bars* represent standard errors

Another possibility is the presence of polysaccharides in seaweed extracts (Sharma et al. 2012), as sugars are known to improve plant growth in a similar way to hormones (Rolland et al. 2002). In the present study, the presence of polysaccharides resulted in a significant increase of dry matter yield and root numbers of treated mung bean.

The low photosynthetic activity and the small leaf area of explants in in vitro conditions do not provide sufficient carbohydrates for growth and development of plants, so sucrose, minerals, and organic compounds in the medium are very important (Zimmerman 1995; Ruzic et al. 2000). In the present study, using half-strength MS medium, sucrose concentration influenced growth and accumulation of biomass (dry weight) of in vitro tomato seedlings. The presence of 30 g L^{-1} sucrose in the culture medium was the most efficient treatment for increasing shoot length and dry weight. When sucrose was not added to the culture medium, the growth was reduced. However, in the presence of polysaccharide-enriched extracts of *P. gymnospora*, the radicle length and dry weight of the tomato seedlings were increased significantly in comparison to the control. A similar effect was exhibited in a previous study where the activity of the saccharide in the agar bed was examined with sucrose and glucose instead of an oligosaccharide mixture. The activity was scarcely affected by these saccharides (Iwasaki and Matsubara, 2000). In the present research, the results confirmed that polysaccharide-enriched extracts have strong root growth-promoting activity. This positive effect may possibly be due to the presence of oligosaccharides such as glucose, sucrose, and fructose formed by hydrolysis and contained in PEEs, that can be recognized in the plant cell wall and act as signaling molecules, inducing production of phytochemical compounds (Klarzynski et al. 2003; Chandía et al. 2004; Pardee et al. 2004). Likewise, plant cell wall oligosaccharides are known to be active in induction and root growth process (Iwasaki and Matsubara, 2000, Kollárová et al. 2005). Additionally, in the present study, treatment of mung bean hypocotyl cuttings with PEE compounds induced rhizogenesis very rapidly within 5 days and better formation of roots compared to the controls. Polysaccharide-enriched extracts promoted the formation of longer roots compared with the control and IBA. These results are in concordance with Kollárová et al. (2005) who reported a similar effect for galactoglucomannan oligosaccharides (GGMOs) which exhibited root growth-promoting activity in mung bean (*V. radiata*). Roots on mung bean induced by polysaccharide-enriched extracts were formed from the central region of the hypocotyl to its base. Also, IBA stimulated the formation of roots more extensively along the hypocotyl compared with the control and PEEs. IBA at all concentrations was responsible for the formation of shorter roots compared with the control. The root elongation phase is very sensitive to auxin concentration, and it is inhibited by high concentration of auxin in the rooting medium (Kollmeier et al. 2000). With the root length being reduced with higher than optimum IBA concentrations (Ansar et al. 2009). In the present study, root length was affected differently by PEEs compared with their effect on root induction. Under the conditions used for the growth assays, the pH and EC change induced by PEEs had a slightly higher magnitude from A-PEE of *U. lactuca* and A-PEE of *P. gymnospora* (pH 8.3 to 8.8) and EC (0.58–0.59 dS m^{-1}). This increase in values of chemical conditions of A-PEEs could result in an alkalinization in the culture medium, accompanied by a rapid and sustained reduction in their root growth. Treatments with a concentration of 0.1 mg mL^{-1} from N-PEE of *U. lactuca* and *P. gymnospora* resulted with pH 7 to 7.1 and EC (0.07 to 0.09 dS m^{-1}) less than the A-PEEs.

Stimulation or inhibition of elongation growth in roots of plants provides evidence of uptake of minerals and carbohydrates leading to greater growth on both tomato and mung bean plants. The plants growing in neutral NSE and N-PEEs showed better response than plants in ASE and A-PEEs.

In conclusion, this research showed that seaweed extracts and polysaccharide-enriched extracts produced from the same seaweed source but with neutral or alkaline conditions of extraction may vary significantly in composition and thus in efficacy to induce specific plant responses following application. The results from this research have provided evidence

that the polysaccharide-enriched extracts act as an effective growth-promoting treatment that can be used as source algae at low-cost on organic agriculture of tomato and mung bean.

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