

Artículo Original

**Germination and seedling growth responses of tomato
(*Solanum lycopersicum* L.) to seaweed extracts applied on seeds**

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Abstract

In the present study the effect of neutral and alkaline seaweed extracts was evaluated on the seed germination and tomato seedlings growth. The seeds were embedded in different dilutions (0.2, 0.4 and 1.0%) with both extracts. Seeds treated with *Ulva lactuca* and *Padina gymnospora* extracts at (0.2 %) enhanced germination parameters. However, alkaline seaweed extracts showed better results for germination parameters such as lower mean germination time and better seedling vigor index, resulting in an increase in shoot and root length of plants in response to compounds formed by the alkaline hydrolysis process. Most seaweed extracts tested showed significant effects on seedling shoot length. The significant positive effects in average radicle length was maintained in seedlings-treated with *U. lactuca* and *P. gymnospora* at 0.2 %. Similarly, the total length of the plants was increased from seeds priming with both extracts at a low concentration of 0.2%. Finally, the highest dry weight was recorded from seeds soaked with *P. gymnospora* extracts at 1.0 %. This study provides information on the use of seaweed extracts, especially *U. lactuca* and *P. gymnospora*, to be applied as a priming agent for tomato seed germination, which is potentially able to promote rapid and more uniform seed germination and plant growth.

Key words: *Extracts, Seaweed, Germination, Growth, Tomato.*

Resumen

En el presente trabajo se estudió el efecto de extractos neutros y alcalinos de algas marinas sobre la germinación y el crecimiento de plántulas de tomate. Las semillas fueron embebidas en diferentes diluciones (0.2, 0.4 y 1.0 %) de los extractos. Ambos extractos neutros y alcalinos de *Ulva lactuca* y *Padina gymnospora* al (0.2 %) incrementaron el porcentaje de germinación. Sin embargo, los extractos alcalinos mostraron mejores resultados en los parámetros de germinación, un tiempo medio de germinación menor y un alto índice de vigor de las plántulas, lo que resulta en un aumento de la longitud de los brotes y raíces en respuesta a los compuestos obtenidos durante el proceso de hidrólisis alcalina. La mayoría de los extractos algales mostraron efectos significativos sobre la longitud del brote de las plántulas. En contraste, la longitud de la radícula de plántulas tratadas con *U. lactuca* y *P. gymnospora* al 0.2 % se

incrementó significativamente. Así mismo, la longitud total de las plantas se incrementó a partir de semillas embebidas con ambos extractos a una dilución baja de 0.2%. Finalmente, el peso seco más alto se registró a partir de semillas embebidas con ambos extractos neutros y alcalinos de *P. gymnospora* al 1.0 %. Este estudio provee información de la utilización de los extractos de algas marinas especialmente de *U. lactuca* y *P. gymnospora* para aplicarse como agente de imprimación para la germinación de semillas de tomate, ya que es potencialmente capaz de promover una germinación de las semillas y un crecimiento más rápidos y uniforme de las plantas.

Palabras clave: *extractos, algas marinas, germinación, crecimiento, tomate.*

1. Introducción

Several studies have described the priming of seeds to enhance the germination rate and equal opportunity of growth thereby reducing the emergence time of many horticultural and agricultural crops (Basra *et al.*, 2002; Lee & Kim, 1999; Brocklehurst & Dearman, 1983). Seed priming is a commercially used technique to hydrate the seed to a point where germination processes begin. Mainly the priming treatments imply imbibing the seed with limited quantities of water to allow the necessary hydration and improvement of metabolic processes of germination. The correct application induces a more rapid, more uniform and higher rate of germination, resulting in a decrease of the mean germination time (which could be considered as an indicator of seed vigor) when seeds are transferred into germination conditions (Sivritepe & Dourado, 1995; Dell'Aquila, 1987).

Traditional priming procedures include hydropriming (seeds primed with water), osmopriming (soaking seeds in osmotic solutions like polyethylene glycol), halopriming (imbibing seeds in salt solutions), thermopriming (management of seed with low or high temperatures), solid matrix priming (the agent solid matrices and water are mixed completely and then seeds are added) and biopriming (hydration by biological compounds). Each treatment has benefits and complications and may have

variable effects depending upon the type of test, selection of crop, stage of plant development, method of application concentration/dose and duration of treatments (Ashraf & Foolad, 2005).

In the last two decades, priming seed have been intensively investigated. Several researchers have used different types of soaking treatments of various crops seeds to increase the rate and uniformity of emergence and better establishment of seedlings (Basra *et al.*, 2005; Ashraf *et al.*, 2003; Bose & Mishra, 1999).

The biopriming technique can also be carried out with macroalgae at low concentrations (parts per million) (Sharma *et al.*, 2014). Previous research was carried out on seeds primed with seaweed extracts and the effect on seed viability and the rate of germination, with promising results obtained in some species (Sivritepe & Sivritepe, 2008). Seaweed extract as organic biostimulant is fast becoming accepted practice in agriculture and horticulture due to its beneficial effects (Battacharyya *et al.*, 2015; du Jardin 2015; Khan *et al.*, 2009).

The biostimulant present in seaweed extract increase the vegetative growth (Di Filippo-Herrera *et al.*, 2018; Briceño-Domínguez *et al.*, 2014; Basher *et al.*, 2012; Demir *et al.*, 2006), the leaf chlorophyll content (Vijayakumar *et al.*, 2018; Castellanos-Barriga *et al.*, 2017; Kalaivanan *et al.*, 2012; Matysiak *et al.*, 211), the stomata density (Spinelli *et al.*, 2010), photosynthetic rate

and the fruit production of the plant (Spinelli *et al.*, 2010; Sivasankari *et al.*, 2006). Also, the seaweed extracts increased levels of plant defense enzymes (Hernandez-Herrera *et al.*, 2014; Raghavendra *et al.*, 2007), and reduction of harmful seed microflora (Moller & Smith, 1999) and faster emergence and seedling vigor in several species including wheat (Kumar & Sahoo, 2011), maize (Farooq *et al.*, 2008), pepper (Sivritepe & Sivritepe, 2008), faba bean (El-Sheekh & El-Saled, 2000), barley (Burchett *et al.*, 1998), lettuce (Moller & Smith, 1998) and table beet (Wilczek & Ng, 1982). Seaweed extracts are reported to improve seed germination). The seaweed extracts are produced with classical and novel extraction techniques (Michalak & Chojnacka, 2014) by several methods under acidic, neutral or alkaline conditions (Booth, 1969). Some researchers have compared different extraction methods for bioactivity. In this regard, products obtained from algae contain a diverse range of inorganic and organic components. The inorganic components of seaweed extracts include nitrogen, phosphorous, potassium, calcium, iron, magnesium, zinc, sodium and sulphur (Khan *et al.*, 2009). In addition, the mineral components brown seaweed extracts also contain varying amounts of organic compounds that include osmolites (e.g. betaines). Additionally, other organic compounds such as polyamines, brassinosteroids, enzymes, proteins, polyphenols (to improve seedling growth in a similar way to hormones) (Battacharyya *et al.* 2015, Rengasamy *et al.*, 2015; Zewail, 2014; Stirk & van Staden, 2014; González *et al.*, 2013; Stirk *et al.*, 2004; Rolland *et al.*, 2002; Crouch & van Staden, 1993; Challen & Hemingway, 1965). Mixtures rich in carbohydrates and amino acids (Khan *et al.* 2009) as well as bioactive secondary metabolites such as vitamins and their precursors that have the potential to increase seed germination in vegetables and fruit

crops (Arioli *et al.*, 2015; du Jardin, 2015; Lakkakula *et al.*, 2015; Latique *et al.*, 2014; Vinoth *et al.*, 2012; Hong *et al.*, 2007; Stephenson, 1974) and consequently they might be used in modern agriculture as biostimulants. The aim of this research is to assess the effect of using neutral and alkaline seaweed extract as a priming technique on the germination and early growth of tomato plants.

2. Materials and Methods

2.1. Collect and production of seaweed extracts

The green algae *Ulva lactuca* (Linnaeus) and *Caulerpa sertularioides* (Gmelin), as well as the brown algae *Padina gymnospora* (Kützting) Sonder and *Sargassum liebmannii* (J. Agardh), were collected from Bahía Tenacatita (19° 28" N, 104° 84" W) and Bahía Careyitos (19° 43" N, 105° 02" W) in the State of Jalisco, Mexico. Seaweeds were collected from the intertidal zone at low tide, in May and November 2015. Samples were transported to the laboratory in plastic bags, washed with tap water to eliminate superficial salt, oven-dried for 72 h at 60 °C, and ground in an electric mill (IKA-M 20) to 0.50 mm.

Two different techniques were used to produce liquid seaweed extracts (LSEs). The first extraction protocol was designed to obtain neutral seaweed extracts (NSE) conditions as described by (Hernandez-Herrera *et al.*, 2014), whereby 100 g of each algae sample were added to 1 L of distilled water, and stirred constantly for 15 min, followed by autoclaving at 121 °C and 1.21 kg cm⁻² for 1 h. The hot extracts were filtered through a Whatman No. 40 filter paper and stored. The pH of the extracts was not modified and ranged obtained was 6.9 to 7.5. The second extraction protocol was designed to obtain alkaline seaweed extracts (ASE) following the technique described by

(Briceno-Domínguez *et al.*, 2014), whereby 200 g of milled algae were rehydrated with 1800 mL of distilled water for 12 h at room temperature (22 °C). The next day, 1500 mL of distilled water were added to allow stirring the seaweed during the extraction step. Seaweed was placed in a water bath and the pH was adjusted with sodium carbonate to pH 10, and 80 °C. The extract was stirred with an external stirrer type and propeller for 2 h at 800 rpm. Temperature and pH were monitored and adjusted if necessary, throughout the extraction process time. The solid residue was removed with a Decanter centrifuge operated at 5,000 rpm. The final pH of the extract was adjusted to 7 with 10 % phosphoric acid. Samples of all extracts produced were coded according to the genus and species: *Ulva lactuca* (UL), *Caulerpa sertularioides* (CS), *Padina gymnospora* (PG) and *Sargassum liebmanni* (SL). Additionally, information reported previously by (Hernández-Herrera *et al.*, 2014; 2016) with reference to proximate analyses and mineral composition in the experimental seaweeds was included. Finally, the pH and electric conductivity (EC) of seaweed extracts were measured using a pH meter and conductivity meter (dS m⁻¹). All determinations were carried out in triplicate.

2.2. Seed-bioprimering treatments

The experiments were conducted with certified tomato seeds (*Solanum lycopersicum* cv. Río Grande). Tomato seeds were surface-disinfected in 4 % NaClO solution for 10 min and then triple-rinsed in sterile distilled water prior to soaking in the seaweed extracts. The seeds were divided into 13 lots; each lot comprised 400 seeds. One treatment was water-priming seed (control) and 12 were the experimental seaweed extract-priming (NSE and ASE) at different concentrations (0.2, 0.4, and 1.0 %). All priming was carried out for 24 h. The

experiment was conducted in duplicate with a randomized block design.

2.3. Seed germination

Germination was observed daily over a period of 8 d in accordance with the methods of the Association of Official Seed Analysts (AOSA 2005). Four groups of 100 seeds were tested for germination per treatment (AOSA 2005). Germination bioassays were performed according to (Hernandez-Herrera *et al.*, 2014). Tested tomato seeds were placed on a Whatman No. 5 filter paper in sterilized 90-mm Petri dishes and then treated with 5 mL-1 distilled water (control) and different concentrations (0.2, 0.4, and 1.0 %) of NSE and ASE (UL, CS, PG and SL). The plates were incubated at 25 ± 1°C and a photoperiod of 16 h light/8 h dark under cool white fluorescent light providing 50 μmol m⁻² s⁻¹ photosynthetic photon flux density. The final germination percentage was calculated on the eighth day after planting according to the methods of the Association of Official Seed Analysts (AOSA 2005) as follows:

$$GP = \left(\frac{\text{number of germinated seeds}}{\text{total number of seeds}} \right) \times 100$$

To assess the mean germination time (MGT), a total of 400 seeds in four replicates (100 seeds/replicate) were sown between two germination filter papers and incubated in a seed germinator in the dark room at constant temperature (25 °C). Germination counts were carried out every 24 h and MGT was calculated accord with Ellis & Roberts (1981):

$$\text{Mean germination time} = \frac{\sum nd}{\sum n}$$

where n = Number of seeds newly germinated at day d, and d = Number of days counted from the beginning of the germination test.

The vigor index (SVI) was considered as the product of seedling vigor (root and shoot length) according to (Orchard, 1977) by using the equation:

$$\text{SVI} = (\text{seedling length (cm)} \times \text{GP})$$

2.3. Growth parameters of tomato seedlings

The growth parameters (shoot length, radicle length, total plant height and dry weight) were measured by the effects of LSEs on 12 days old tomato seedlings. Parameters were calculated according to Hernández-Herrera *et al.* (2014).

2.4. Statistical analysis

In all cases, the data were tested for normality and homoscedasticity. For comparison of means of multiple groups or treatments, analysis of variance (one-way ANOVA) and the multiple comparison test of least significant difference (LSD) ($p = 0.05$) were used. All statistical analyses were performed with the statistical package STATGRAPHICS® Centurion XV for Windows.

3. Results

3.1. Physicochemical properties of seaweed and neutral and alkaline seaweed extracts

The proximate analyses showed that the four algal species had different compositions. Protein and carbohydrate content were higher, and lipid and fiber content were lower, except in to *C. sertularioides*. Total nitrogen and sodium were higher in green seaweeds, but potassium was higher in brown seaweeds. The phosphate concentration was low in all seaweeds (Table 1).

The values of pH of alkaline seaweed extracts (ASE) were neutral and slightly acidic than those for neutral seaweed extracts (NSE) made from dark green colored extract of *C. sertularioides* and brown colored

extract of *S. liebmannii*. The value of EC increases in all LSEs with an increase in the concentrations. In ASE produced with the brown algae *P. gymnospora* and *S. liebmannii*, a brownish red and brown liquid viscous product was formed. In contrast, lower viscosity was recorded for green seaweed extracts at 121 °C (Table 2).

3.2. Germination and growth parameters of tomato seedlings

Germination occurred in all treatments in the 2nd day. The effect of the neutral seaweed extracts (NSE) showed significantly highest seed germination than alkaline seaweed extracts (ASE) treatments (Table 3). Seed primed in treatments of NSE and ASE from *U. lactuca* and *P. gymnospora* showed significantly highest seed germination than control ($p < 0.05$) (Table 3). In contrast, seeds treated with both (NSE and ASE) treatments of *C. sertularioides* had an inhibitory effect on seed germination (Table 3).

Notably, rapid and harmonized germination was observed in the seeds that was primed with the seaweed extracts prepared with *U. lactuca* and *P. gymnospora* at 0.2 %. The SNE and ASE of *U. lactuca* exhibited an increase in germination (75 and 66 %) compared to the control. Similarly, the SNE and ASE of *P. gymnospora* showed an enhanced germination (76 and 75 %), being the most effective. Also, the higher germination was as associated with lower mean germination time (TMG). The SNE and ASE at 0.2 % of *U. lactuca* and *P. gymnospora* reduced the TMG (5.6 and 5.3 days) in both cases, and consequently greater seedling vigor (SVI) to SNE and ANE of *U. lactuca* (1,027 and 1,415 respectively) and SNE and ANE of *P. gymnospora* (1,262 and 1,265, respectively) showed significantly highest values than control ($p < 0.05$) (Table 3).

Table 1. Proximate composition of seaweed species.

| | Green seaweeds | | Brown seaweed | |
|------------------|---------------------|--------------------------------|-----------------------------|--------------------------|
| | <i>Ulva lactuca</i> | <i>Caulerpa sertularioides</i> | <i>Sargassum liebmannii</i> | <i>Padina gymnospora</i> |
| Crude protein ** | 11.78±0.01 | 24.02±0.03 | 8.39±0.03 | 9.83±0.01 |
| Crude lipid** | 0.03±0.01 | 0.51±0.03 | 0.56±0.03 | 0.53±0.01 |
| Ash** | 31.13±0.01 | 31.55±0.03 | 30.52±0.03 | 30.66±0.03 |
| Fiber** | 5.63±0.01 | 25.11±0.03 | 7.85±0.03 | 15.86±0.03 |
| Carbohydrates** | 41.25±0.01 | 12.57±0.03 | 45.68±0.03 | 37.90±0.01 |
| Dry matter** | 90.00±0.01 | 93.76±0.03 | 93.0±0.03 | 94.75±0.01 |
| Moisture** | 10.00±0.01 | 6.24±0.03 | 7.0 ±0.03 | 5.25±0.01 |
| Total N* | 1.88±0.01 | 3.95±0.01 | 1.33±0.03 | 1.56±0.01 |
| P* | 0.10±0.08 | 0.20±0.08 | 0.17±0.05 | 0.10±0.08 |
| Na* | 5.57±0.80 | 4.42±0.40 | 1.56±0.40 | 1.81±0.50 |
| K* | 1.85±0.30 | 0.47±0.40 | 4.54±1.00 | 4.27±0.60 |
| Ca* | 1.88±0.06 | 3.10±0.50 | 1.85±0.30 | 3.65±0.40 |

Based on % dry weight (g 100 g⁻¹ dry weight).

Values are average ± standard error (n=3). By Hernández-Herrera *et al.*, (2014*; 2016**).

Table 2. Physicochemical content of algae extracts of *Ulva lactuca* (UL), *Caulerpa sertularioides* (CS), *Padina gymnospora* (PG) and *Sargassum liebmannii* (SL) at different concentrations.

| Treatments (%) | Neutral seaweed extracts NSE | | | Alkaline seaweed extracts ASE | | |
|----------------|------------------------------|----------|--------------------------|-------------------------------|----------|--------------------------|
| | Color/consistency | pH | EC (ds m ⁻¹) | Color/consistency | pH | EC (ds m ⁻¹) |
| Control | | 7.00 | 0 | | 7.0 | 0 |
| UL 0.2 | Greenish- | 7.30±0.5 | 0.99±0.11 | Greenish | 7.07±0.1 | 1.17±0.57 |
| UL 0.4 | yellow | 7.36±0.5 | 1.74±0.11 | yellow | 7.06±0.1 | 1.86±0.57 |
| UL 1.0 | Liquid-aqueous | 7.41±0.5 | 3.57±0.11 | Liquid-aqueous | 7.06±0.1 | 3.99±0.00 |
| CS 0.2 | Dark green | 6.96±0.5 | 1.01±0.20 | Greenish | 7.04±0.1 | 1.40±0.58 |
| CS 0.4 | Liquid- | 7.19±0.5 | 1.85±0.20 | yellow | 7.04±0.1 | 2.68±0.58 |
| CS 1.0 | aqueous | 7.20±0.2 | 3.93±0.20 | Liquid-aqueous | 7.03±0.1 | 3.99±0.00 |
| PG 0.2 | Brownish | 7.50±0.2 | 0.77±0.10 | Dark | 7.04±0.1 | 1.10±0.58 |
| PG 0.4 | red | 7.50±0.2 | 1.43±0.10 | brownish | 7.06±0.1 | 2.00±0.58 |
| PG 1.0 | Liquid-aqueous | 7.50±0.5 | 2.98±0.10 | Liquid-viscose | 7.04±0.1 | 3.45±0.58 |
| SL 0.2 | Brown | 6.60±0.5 | 1.01±0.15 | Dark | 7.04±0.1 | 1.41±0.58 |
| SL 0.4 | Liquid- | 6.70±0.5 | 2.61±0.15 | brownish | 7.05±0.1 | 2.30±0.58 |
| SL 1.0 | aqueous | 7.00±0.5 | 3.99±0.15 | Liquid-viscous | 7.04±0.1 | 4.00±0.58 |

Values are average ± standard error (n=3).

Table 3. Effect of neutral seaweed extracts (NES) and alkaline seaweed extracts (ASE) of *Ulva lactuca* (UL), *Caulerpa sertularioides* (CS), *Padina gymnospora* (PG) and *Sargassum liebmannii* (SL) on germination, mean germination time and seedling vigor index of tomato seedlings.

| Germination (%) | Germination (%) | | Mean germination time (days) | | Seedling vigor index (SVI) | |
|-----------------|-----------------|------------|------------------------------|-------------|----------------------------|--------------|
| | NSE | ASE | NSE | ASE | NSE | ASE |
| Control | 30±1.4 c | 42±0.76 ef | 5.9±0.08 ef | 5.8±0.10 b | 763±40.08 b | 926±16.1 e |
| UL 0.2 | 75±2.0 g | 66±0.76 gh | 5.6±0.06 a | 5.3±0.10 a | 1027±40.08 ef | 1415±16.1 k |
| UL 0.4 | 55±1.8 e | 36±0.76 cd | 5.8±0.08 b | 5.8±0.10 ab | 947±40.08 de | 1007±16.1 f |
| UL 1.0 | 50±1.6 de | 43±0.76 de | 5.8±0.1 bc | 5.9±1.30 ab | 1097±40.08 fg | 214±16.1 b |
| CS 0.2 | 10±0.6 a | 27±0.76 bc | 6.3±0.09 h | 5.9±0.10 ab | 806±40.08 bc | 1055±16.1 gh |
| CS 0.4 | 12±3.9 b | 25±0.76 b | 6.2±0.20 g | 6.0±0.10 ab | 892±40.08 cd | 435±16.1 d |
| CS 1.0 | 10±0.9 a | 10±0.76 a | 6.5±0.10 i | 6.9±2.20 c | 623±40.08 a | 72±21.0 a |
| PG 0.2 | 76±1.1 g | 75±0.76 h | 5.6±0.06 a | 5.3±0.20 a | 1262±40.08 h | 1265±16.7 j |
| PG 0.4 | 66±1.6 f | 69±0.76 gh | 5.7±0.50 a | 5.3±0.20 a | 1230±40.08 h | 1019±16.3 fg |
| PG 1.0 | 46±2.1 d | 59±0.76 fg | 5.8±0.1 bcd | 6.3±2.9 bc | 889±40.08 cd | 350±17.5 c |
| SL 0.2 | 10±0.4 a | 60±0.7 6g | 5.9±0.1 def | 5.4±0.20 a | 1161±40.08 gh | 1190±14.7 i |
| SL 0.4 | 20±0.5 a | 59±0.76 fg | 5.9±0.1 cde | 5.5±0.30 a | 873±40.08 bcd | 937±15.7 e |
| SL 1.0 | 10±0.3 a | 47±0.76 e | 6.0±0.1 f | 6.4±2.60 bc | 635±40.08 a | 414±17.7 d |

Values are average ± standard error (n=400).

Average followed by the same letter within columns is not significantly different, according to LSD multiple range test ($P \leq 0.05$).

Similarly, ASE from *S. liebmannii* at 0.2 % showed higher germination (60 %) associated with lower mean germination time (5.4 days) and higher seedling vigor SVI (1,190). In contrast, seeds treated with *C. sertularioides* at 1.0 % were the last to germinate (6.5 and 6.9 d), and therefore required the longest germination time (Table 3).

Most of seaweed extracts had a significant effect ($p \leq 0.05$) on tomato seedlings growth. The majority of the NSE treatments tested in this study showed significant effects on seedling shoot length (6.06 to 8.34 cm) than control treatment (4.54 cm) ($p < 0.05$; Fig. 1a). Also, the ASE promoted shoot length but using a low concentration (0.2 %). The highest average shoot length was found in plants that received ASE at 0.2 % (6.27 to

7.91 cm) in comparison with control (5.23 cm) ($p < 0.05$; Fig. 1a).

Moreover, these results indicate that NSE and ASE of *U. lactuca* and *P. gymnospora* promote radicle length of tomato seedling. The highest average radicle length was found in plants that received NSE of *U. lactuca* (4.13 cm) and *P. gymnospora* (5.84 cm) and ASE of *U. lactuca* (7.34 cm) and *P. gymnospora* (6.26 cm) at 0.2 % respectively in comparison to the control ($p < 0.05$; Fig. 1b). Furthermore, the majority *C. sertularioides* and *S. liebmannii* treatments had an inhibitory effect on radicle length (Fig. 1b). In addition, all ASE priming treatments at 1.0 % had an inhibitory effect on shoot and radicle growth parameters. Application of ASE was found to be more effective in influencing the total height of the plant (up to 15.25 cm) than NSE application

(13.32 cm). All treatments at 0.2 % had a positive effect on total height, except to *C. sertularioides* ($p < 0.05$, Fig. 2a). Most of the priming treatments in the present study did not show statistically significant differences in the average of dry weight except for both seaweed extracts (NSE and ASE) of *P.*

gymnospora at 1.0 % (0.036 and 0.028 g, respectively) and *S. liebmannii* at 0.2 % (0.036 and 0.025 g, respectively). Also, NSE from *U. lactuca* at 1.0 %, and *C. sertularioides* at 0.4 % (reaching weights of 0.035 to 0.036 g) ($p < 0.05$, Fig. 2b).

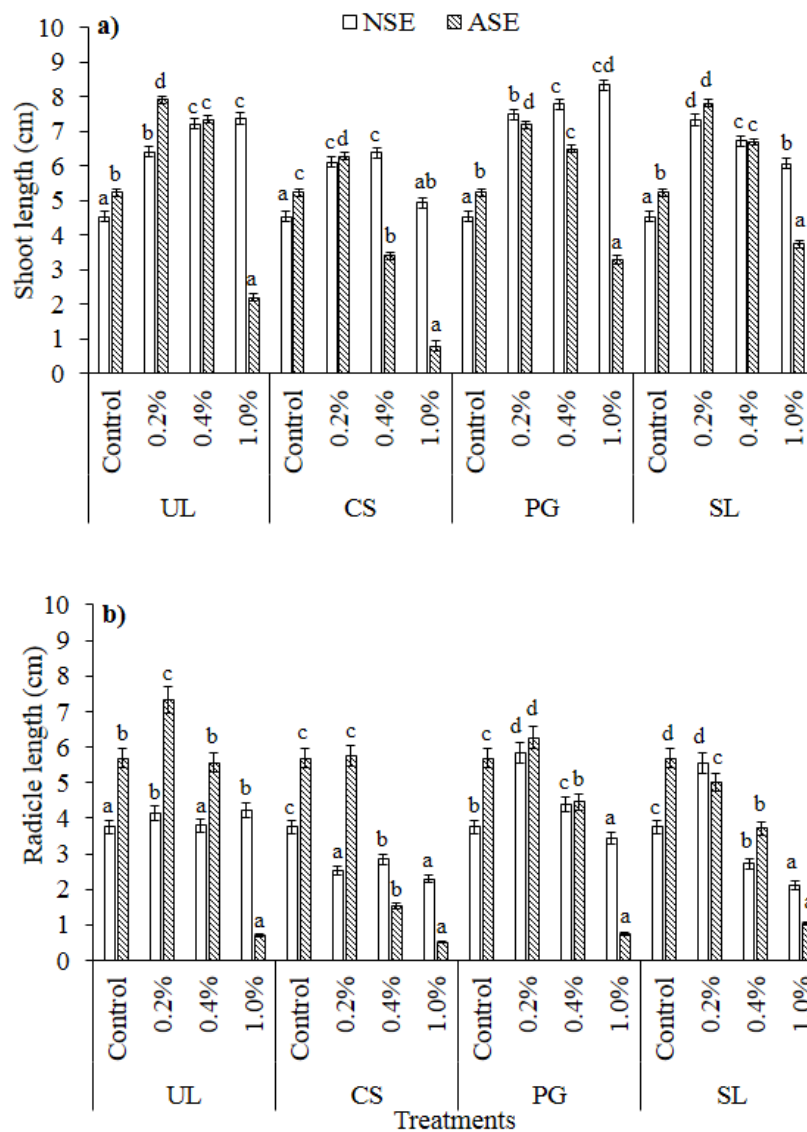


Figure 1. Effect of neutral seaweed extracts (NSE) and alkaline seaweed extracts (ASE) of *Ulva lactuca* (UL), *Caulerpa sertularioides* (CS), *Padina gymnospora* (PG) and *Sargassum liebmannii* (SL) at different concentrations on **a)** shoot length and **b)** radicle length tomato seedlings.

Columns denoted by a different letter are significantly different at $p \leq 0.05$.

Values represent average ($n=100$ seedlings); bars represent standard error.

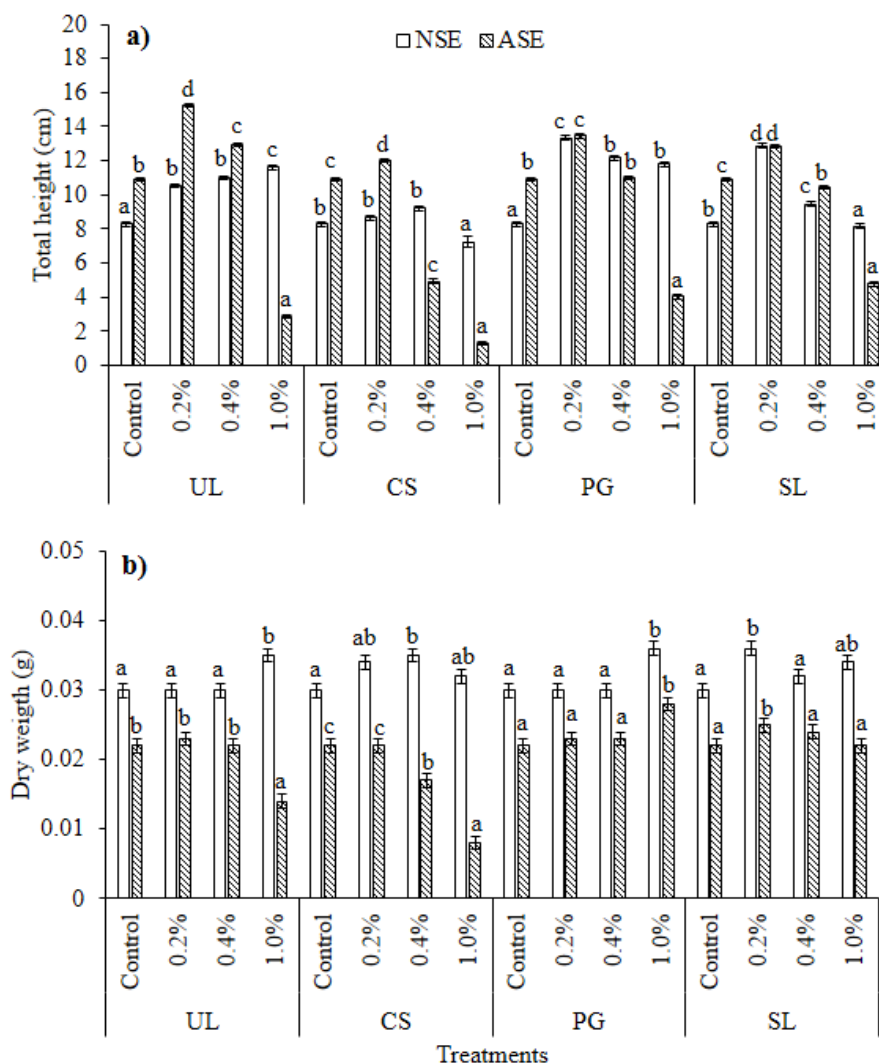


Figure 2. Effect of neutral seaweed extracts (NSE) and alkaline seaweed extracts (ASE) of *Ulva lactuca* (UL), *Caulerpa sertularioides* (CS), *Padina gymnospora* (PG) and *Sargassum liebmannii* (SL) at different concentrations on **a)** total height and **b)** dry weight of tomato seedlings. Columns denoted by a different letter are significantly different at $p \leq 0.05$. Values represent average ($n=100$ seedlings); bars represent standard error.

4. Discussion

The commercial seaweed extract industry employs a wide range of proprietary extraction processes in order to disrupt the cell and release beneficial components in to the extract; some of the processes include, alkali extraction, acid extraction and cell bust technology (Arioli *et al.*, 2015). The

chemical composition of extract largely depends on the method of extraction and on the chemical products used during the production process. Therefore, the biological activity of extracts of the same seaweed raw material obtained by different extraction processes may be considerably different (Khairy & El-Shafay, 2013; Kim 2012).

The results in this research showed that the selected extracts tested can be a source of macro-elements which are beneficial for plant cultivation, especially the extracts from the *U. lactuca* and *P. gymnospora*, that were the most effective treatments. However, not all extracts showed the same type of effect; in some cases, like *S. liebmannii* and *C. sertularioides*, the effect was to delay germination. The presence of various bioactive compounds in seaweed extracts that can stimulate and/or inhibit seed germination may help explain this difference. For example, according to Battacharyya *et al.* (2015) the increased germination percentage at low concentrations may be due to the presence of growth promoting substances such as phytohormones and micronutrients. Previous, papers reported that the four algae used in this research have different chemical compositions and the results revealed that mineral content, and changes in pH, and EC of neutral and alkaline extracts can affect their bioactivity on germination and growth of tomato plants (Hernandez-Herrera *et al.*, 2014; 2016). For example, the increased germination percentage at low concentrations (0.2 %) may be due to the presence of growth promoting substances such as phytohormones and micronutrients. A better response in germination rate is associated with lower mean germination time and consequently higher seedling vigor and higher shoot and radicle length. In the present study, whereby seed germination and seedling vigor showed significantly positive effects after priming of 48 h with both type of seaweed extracts (NSE and ASE). The tomato seed imbibed with lower concentration of the bioprimer treatments of NSE and ASE of *U. lactuca* and *P. gymnospora* at lower concentrations (0.2 %), with low EC (0.77 up to 1.7 dS m⁻¹), exhibited the same result, higher germination rates.

Additionally, it is known that higher concentrations of (1.0 %) the *Ulva lactuca* liquid extracts can inhibit the germination of mung bean (Castellanos-Barriga *et al.*, 2017) and the SLE of *Caulerpa sertularioides* and *Sargassum liebmannii* in tomato seeds (Hernández-Herrera *et al.*, 2014). Similarly, the higher concentrations (1.0 %) of the algal extracts (NSE and ASE) in which the EC was higher (2.98 up to 4.0 dS m⁻¹) inhibited the germination and growth parameters.

According to Reinhardt and Rost (1995) most plants are more sensitive to salinity during germination and seedling growth. This may be due to the absence of salts in the medium, thereby allowing seeds to more efficiently imbibe water. Usually, an increase in salinity causes a decrease in seed germination; this could be attributed to osmotic stress or specific ions toxic as Na⁺ and Cl⁻ that affect the germination by limiting water absorption by the seeds (Khajeh-Hosseini *et al.*, 2003; Dodd & Donovan, 1999), which affect the mobilization of stored reserves (Bouaziz & Hicks, 1990). Also, plants exposed to acid as well as alkaline pHs stress are normally subjected to metal toxicity and hence decrease in the root growth and the total biomass (Pavlovkin *et al.*, 2009; Heredia *et al.*, 2002; Arduini *et al.*, 1998).

Moreover, the seaweed liquid extracts contain vitamins and precursors that have the potential to increase seed germination and growth in vegetables and fruit crops (Lakkakula *et al.*, 2015; Latique *et al.*, 2014; Vinoth *et al.*, 2012; Hong *et al.*, 2007; Stephenson, 1974). It is known that seed priming enhances germination capacity, overcomes dormancy and improves stand establishment, which results in higher yields of agronomic and vegetable crops (Rasheed *et al.*, 2010; Afzal *et al.*, 2009; Farooq *et al.*, 2005, 2007). The possible cause for the early emergence of the seeds treated with seaweed extract was the completion of pre-

germination metabolic activities, causing in the seed trigger for radicle protrusion (Ozbingol *et al.*, 1999). There was a significant decrease in mean germination time, which can be attributed to early reserve breakdown as well as reserve mobilization. It might also be owed to possible early activation or de novo synthesis of cell wall degrading enzymes (Hisashi & Francisco, 2005). In the present research, supplementation with neutral and alkaline seaweed extracts at lower concentrations (0.2 %) improved the shoot and root growth, similar previous reports on vegetables (Satish *et al.*, 2015a, b; Vinoth *et al.*, 2014). The results suggest that the two extraction processes have a differential activity effect on plants growth. The seeds treated imbibed with alkaline seaweed extracts showed higher radicle length in comparison with seeds treated with neutral seaweed extracts, this could be due to the uptake of magnesium, potassium, nitrogen and iron from the seaweed extract present in higher amounts. Vijayakumar *et al.* (2018) also reported a similar finding with *Codium tomentosum* on the growth of *Capsicum annum*.

Also, auxins are known to occur endogenously in brown and green macroalgal species. Alkaline hydrolysis can be used to obtain IAA in its active form (Ueda & Bandurski, 1969 Avery *et al.*, 1942; Avery *et al.*, 1941) that promote rooting (Guiry & Blunden, 1991). The seaweed extracts include cytokinins, auxins, and abscisic (Tarakhovskaya *et al.*, 2007). Also, other organic compounds such as polyamines, brassinosteroids, enzymes, proteins, vitamins, and polyphenols (to improve seedling growth in a similar way to hormones), had a positive effect on the cellular metabolism in treated seeds, leading to enhanced seedling growth (Rengasamy *et al.*, 2015; Zewail, 2014; Stirk & van Staden, 2014; González *et al.*, 2013; Stirk *et al.*,

2004; Rolland *et al.*, 2002; Crouch & van Staden, 1993).

Additionally, recent advances suggest that carbohydrates can also act as plant biostimulant. The common polysaccharides found in seaweed extracts include ulvans, alginates, fucoidans, laminarans, lichenan-like glucans and fucose containing glucans (Arioli *et al.*, 2015; Khan *et al.*, 2009) as well as neutral sugars and sulfate, that showed positive correlation between the sulfate content of polysaccharide enriched extracts and shoot dry weight as well as chlorophyll content in tomato plants (Mzibra *et al.*, 2018). Similarly, Hernández-Herrera *et al.* (2016) reported that the supplementation with neutral and alkaline polysaccharide enriched extracts at lower concentrations improved the shoot and root growth, especially with alkaline polysaccharide enriched extracts. In contrast, dry weight of plants showed higher increase with neutral polysaccharide enriched extracts. It is in concordance with our results, higher total height was obtained in seed primed with alkaline seaweed extracts, and higher dry weight with neutral seaweed extracts. The knowledge of the bio-stimulatory effects of seaweed extracts will have multiple beneficial impacts on agricultural production. However, insufficient information on product composition and the amount of bioactive substances that are delivered to the plants is available. This is critical to establish a functional link between specific molecules and/or specific combinations of molecules that are delivered to the plants. Some initial attempts to link the complex composition of these products to a possible mechanism of action have been reported by Di Stasio *et al.* (2018), have clearly illustrated the need to focus on further detailed studies on their use of seaweed extracts in agriculture and horticulture.

5. Conclusion

The results of this research suggest that bioprimering seeds of tomato with the seaweed extracts obtained in neutral and alkaline condition from *U. lactuca* and *P. gymnospora* at 0.2 % enhance the percentage and seedling rate emergence and faster root and shoot growth. However, the alkaline seaweed extracts (ASE) showed higher results for germination parameters such as lower mean germination time and better seedling vigor index, resulting in an increase in the shoot and root length of plants in response to compounds formed by the alkaline hydrolysis process. The possibility of the existence of minerals and polysaccharides in algal extracts auxin-like activity is high and could be responsible for the effects on growth parameters. Further studies are needed to understand the mechanism involved in such growth stimulation caused by seaweed extracts.

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