Chapter 10 Growth promotion and productivity of tomato using two plant biostimulants: Arbuscular mycorrhizal fungi and seaweed extract

Capítulo 10 Promoción de crecimiento y productividad de tomate utilizando dos bioestimulantes de plantas: Hongo micorrízico arbuscular y extracto de alga marina

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Abstract

Plant biostimulants include different substances, compounds, and formulations of growth-promoting microorganisms, such as those derived from arbuscular mycorrhizal fungi (AMF) or seaweed extracts (SWE), which are used to regulate or enhance physiological and morphological processes in plants. This study analyzed the morphological implications of the addition of two biostimulants, AMF *Rhizophagus intraradices* and a SWE obtained from *Ulva lactuca* (both alone and in combination), in tomato plants (*Solanum lycopersicum*). The responses evaluated were related to plant growth, flowering, and crop productivity. Likewise, the success of AMF colonization in plants was also assessed. The application of AMF increased the length and root area of the plants. The SWE induced an early flowering and thus a greater number of fruits with greater weights. However, the combination of both biostimulants (AMF + SWE) was less beneficial for the plants, which was reflected in a decrease in both foliar and root growth as well as in the number of flowers and fruits. In addition, it was also observed that the SWE showed a positive effect over mycorrhizal establishment, as evidenced by greater root colonization. In the present study, evidence is presented of the benefits of using SWE to improve plant performance, in addition to the positive effects observed in the establishment of mycorrhizal symbiosis between *R. intraradices* and tomato plant roots. These results constitute an important contribution to the research on biostimulants, their development, and functional design, highlighting those complementary effects and the action mechanisms of each biostimulant should be considered.

Marine Algae, Biostimulant, Mycorrhizal symbiosis, Tomato, *Ulva lactuca*

Resumen

Los bioestimulantes de plantas incluyen diferentes sustancias, compuestos y formulaciones de microorganismos promotores del crecimiento, como los derivados de hongos micorrícicos arbusculares (HMA) o extractos de algas marinas (EA), que se utilizan para regular o potenciar los procesos fisiológicos y morfológicos en las plantas. Este estudio analizó las implicaciones morfológicas de la adición de dos bioestimulantes, el HMA *Rhizophagus intraradices* y/o extracto obtenidos a partir del alga *Ulva lactuca* (tanto solos como en combinación), en plantas de tomate (*Solanum lycopersicum*). Las respuestas evaluadas se relacionaron con el crecimiento de las plantas, la floración y productividad del cultivo. Así mismo, se observó el éxito de la colonización de la raíz en la plantas por el HMA. La aplicación del HMA incrementó la longitud y área radicular de las plantas. El EA indujo una floración temprana y por lo tanto mayor número y peso de frutos. Sin embargo, la combinación de ambos bioestimulantes (HMA + EA) resultó menos benéfico para la planta, reflejándose en una disminución tanto en el crecimiento foliar y radicular como en número de flores y frutos. Además, también se observó que el EA mostró un efecto benéfico sobre el establecimiento del HMA, mostrando una mayor colonización de raíces. En el presente trabajo, se presenta evidencia de los beneficios del uso de los EA para mejorar el rendimiento de la planta. Así como el efecto positivo sobre el establecimiento de la simbiosis micorrícia entre *R. intraradices* y las raíces de las plantas de tomate. Los resultados obtenidos son una contribución importante a la investigación de bioestimulantes, al desarrollo y diseño funcional de los mismos, en donde debe considerarse los efectos complementarios y los mecanismos de acción de cada bioestimulante.

Algas marinas, Bioestimulantes, Simbiosis micorrícica, Tomate, *Ulva lactuca*

Introduction

Recent decades have borne witness to substantial increases in the use of biostimulants for agricultural purposes, and biostimulant research has primarily intensified in the search for new compounds that increase crop yield and quality (Calvo *et al*., 2014). A plant biostimulant can be defined as any substance or microorganism that improves nutritional efficiency, tolerance to abiotic stress, or the quality of particular traits, regardless of its nutrient content. Biostimulants may be obtained from a wide variety of natural sources and are mainly categorized as follows: humic and fulvic acids, protein hydrolysates and other N-containing compounds, botanical and seaweed extracts, inorganic compounds, and beneficial fungi and bacteria (du Jardin, 2015).

Even in small amounts, biostimulants improve plant growth and performance by modulating metabolic responses like respiration, photosynthesis, protein synthesis, nutrient absorption, and biotic and abiotic stress responses (Posmyk and Szafrańska, 2016). Biostimulants may also improve crop sustainability by preventing the excessive application of fertilizers and consequently their negative impacts that result in environmental pollution (Halpern *et al*., 2015).

Marine algae have been used for thousands of years to improve soil fertility and crop productivity through either direct application or as soil amendments after composting (Craigie, 2011). After the first process to produce liquid seaweed extracts (SWE) was developed in the 1950s, a variety of commercial SWE products are now available worldwide for agricultural and horticultural purposes (Khan *et al*., 2009). In Mexico, marine macroalgae are abundantly available in temperate and tropical waters and thus constitute a low-cost, local resource for coastal agricultural production with great potential for eventual commercial exploitation (Hernández-Herrera *et al*., 2014 a, b). Algal extracts have been found to act as chelators, improving the structure of the soil and the use of mineral nutrients in plants after composting, which favors root growth. As biostimulants, SWE promote seed establishment and germination and increase growth, yields, flower and fruit production, resistance to biotic and abiotic stress, and postharvest shelf life (Khan *et al*., 2011, Craigie, 2011). SWE are comprised of a complex mixture of components that vary depending on the source seaweed, harvest time, and extraction process (Shekhar *et al*., 2012). Moreover, they contain a wide range of organic and mineral components, including unique and complex polysaccharides that are not present in terrestrial plants like laminarins, fucoidans, and alginates (Khan *et al*., 2009).

The biostimulant effects of SWE are often attributed to plant growth hormones and low molecular weight compounds (Tarakhovskaya *et al*., 2007), although larger molecules like polysaccharides and polyphenols have also been implicated (Zhang *et al*., 2003; Battacharyya *et al*., 2015). Most commercial kelp extracts are made from brown kelp, including *Ascophyllum nodosum*, *Fucus*, *Laminaria*, *Sargassum*, and *Turbinaria* spp. (Hong *et al*., 2007). Although commercial manufacturing processes for extracts are generally patented, they include the use of water, acids, or alkalis as extractants (with or without heating) or the physical manipulation of seaweeds using low-temperature or high-pressure grinding. The final products are prepared in either liquid form or as dry formulations and can be combined with plant fertilizers and micronutrients (Craigie, 2011). In Mexico, six seaweed species are used to produce 14 biostimulant products, which are commercialized as biofertilizers or root promoters (Hernández-Herrera *et al*., 2018). According to our research, the production of these commercial products is based on conventional solvent extraction and hydrolysis under hydrothermal treatment using several methods with acid, neutral, and alkaline conditions. SWE can be applied near the root of the plant by mixing extracts with irrigation water, which can then be applied with drip irrigation systems to crops. They can also be used as foliar sprays with a variety of flowers, vegetable crops, and trees (Battacharyya *et al*., 2015). The algal extracts have biostimulant activity at low concentrations (i.e., diluted to 1:1,000 or more), which suggests that the observed effects are different from those associated with a direct nutritional function.

Arbuscular mycorrhizal fungi (AMF) are one of the most important groups of soil fungi, and they form symbiotic associations with the roots of ~ 80% of all plant species (Aguilera *et al*., 2007; Buendia *et al.*, 2016). AMF positively stimulate plant growth, promote abiotic stress tolerance, and improve resistance to both pests and diseases. Likewise, AMF improve the nutritional status of plants, which is reflected in increased yields and fertilization efficiency (Alfonso and Galán, 2006). Symbiosis between AMF and host plants requires a sequence of molecular recognition events leading to the morphological and physiological integration of the two symbionts (Barea *et al*., 2008). Several factors influence the establishment and functioning of mycorrhizal symbiosis. In particular, edaphological characteristics (e.g., soil texture, pH, organic matter content, humidity levels, nutrient levels, and the organisms present), climatic conditions (e.g., light, temperature, and geographic location), and biotic factors (e.g., interactions with other organisms) affect spore germination, root colonization, and AMF efficiency (Khalil *et al.*, 1992).

The importance of AMF to plant nutrition has provided new insights into the contributions of these symbionts to nutrient assimilation. According to Barea (1991), after AMF establish themselves and develop their hyphae, plants increase their radical exploration area and the absorption of nutrients like N, K, Ca, Mg, B, Fe, and especially phosphate. In tomato plants, various AMF species have been found to positively affect growth (Rodríguez-Yon *et al*., 2004).

Similarly, tomato plants under nutrient- or water-limiting conditions in the presence of mycorrhizal symbiosis can produce the same quality and quantities of fruits as those of plants grown under non stressed conditions. The use of AMF seems a promising option to stabilize tomato production incresing farmers earnings by reducing costs of water irrigation and nutrient supply (Zouari *et al.,* 2014; Fracasso *et al*., 2020).

Nonetheless, few studies have evaluated how plant development is affected by the application of seaweed extracts in conjunction with AMF. Seaweed extracts have been found to positively modulate mycorrhizal interactions by promoting the development of AMF. Mannitol and carrageenan obtained from *Laminaria japonica* were found to induce the growth of AMF *Gigaspora margarita* and *Glomus caledonium* hyphae and root colonization in the trifoliate orange (*Poncirus trifoliata) in vitro* (Kuwada *et al*., 2005 and 2006b). Similarly, favorable results have been observed in the development and growth of papaya, passion fruit, and cucumber when these two biostimulants were applied (Kuwada *et al.,* 2006a; Suhail, 2013a, b). The simultaneous application of two biostimulants can enhance the benefits that are obtained from an individual application, and these may be either additive or synergistic. González-González *et al*. (2020) reported that the joint application of a seaweed extract from *Padina gymnospora* and the AMF *Rhizophagus intraradices* resulted in enhanced root development and increased carbohydrate and protein content in the leaf tissues of tomato plants. Moreover, the individual application of each biostimulant produced an observable synergistic effect on the appearance and number of flowers.

The present study aimed to analyze tomato plant (*Solanum lycopersicum* L. var. Rio Fuego) development under the combined application of extracts from the green algae (*Ulva lactuca* L.) and the AMF *R. intraradices* to identify possible functional links reflected in either positive (i.e., additive or synergistic) or negative effects on plant growth, productivity, and mycorrhizal interactions. The results of this study will be relevant for assessing the importance of the joint use of these two biostimulants for tomato production and the development of environmentally friendly agricultural management strategies with lower costs.

Materials and Methods

Biological material

The experiments were performed with tomato plants (*S. lycopersicum*) using certified seeds (Kristen Seed®, Guadalajara, Mexico). Inoculations of the AMF *R. intraradices* were conducted using the commercial product INIFAP mycorrhizal BIOfertilizer® (Celaya, Guanajuato, Mexico), which consists of spores, hyphae, and root fragments colonized by AMF (64 spores/g of inoculum). The seaweed *U. lactuca* was collected in February 2018 from an intertidal zone of the Port of Topolobampo, Ahome, Sinaloa (25º 36' 00" N, 109º 04' 00" W). The collected algae were washed with tap water, dried in the sun, and pulverized. The extracts were prepared at 0.2% following the methodology of Hernández-Herrera *et al*. (2014a) starting with 2 g of seaweed powder. The SWE were sterilized, filtered using Whatman No. 40 paper, and refrigerated at 4 °C until use.

Greenhouse plant growth conditions and experimental design

Tomato seeds that had been previously germinated in Petri dishes (Copetta *et al*., 2011) were sown in 1-L plastic pots that had been filled with a sterile substrate mixture (sand/vermiculite 1:1 v/v). Two groups of plants were established. The first group consisted of non-inoculated plants, whereas 50 g of the AMF product was incorporated into the individuals of the second group. Inoculation was performed according to the methodology of Menge and Timer (1982), which consists of placing the inoculum in bands measuring 3–5 cm under the surface of the substrate. The plants were kept under greenhouse conditions for 90 d (March–May 2019) at an average temperature of 30 ± 2 °C and an average photosynthetically active radiation (PAR) of 3100 µmol m^{-2} s^{-1}. The pots were watered daily with 100 mL deionized water to maintain humidity, and 100 mL of algae extract at 0.2 % was applied twice a week with or without Rorison nutritive solution: ($MgSO_4$•$7H_2O$, 0.248 g·L^{-1}; $Ca(NO_3)2$•$4H_2O$, 0.476 g·L^{-1}; K_2HPO_4•$3H_2O$, 0.05 g·L^{-1}; FeNaEDTA, 0.025 g·L^{-1}; $MnSO_4$•$4H_2O$, 2.24 mg·L^{-1}; H_3BO_3, 2.88 mg·L^{-1}; $(Na_4)_6Mo_7O_{24}$•$4H_2O$, 0.2 mg·L^{-1}; $ZnSO_4$•$7H_2O$, 0.44 mg·L^{-1}; $CuSO_4$•$5H_2O$, and 0.4 mg·L^{-1}) adjusted with K_2HPO_4 to favor mycorrhizal colonization (Hajiboland *et al*., 2010; Cervantes-Gámez *et al*., 2016). A random block experiment was established with 4 treatments with 14 repetitions each (n = 56 plants).

The treatments were: 1) plants watered with nutritive solution (Control), 2) plants inoculated with AMF (RI), 3) plants treated with the seaweed extract (SWE), and 4) plants inoculated with AMF and treated with the algal extract (RI + SWE).

Mycorrhizal colonization

Ninety days after sowing, six plants were randomly chosen from the treatments inoculated with AMF (RI and RI + SWE), and their roots were carefully removed. The root samples were washed with tap water and stored in ethanol (50%) until analysis. Mycorrhizal colonization was evaluated according to the staining technique of Kormanik and McGraw (1982). The roots were cut into ~ 1-cm fragments and clarified for 5 min at 90°C in KOH (10%). Then, the roots were acidified by immersion in HCL (0.1 M) for 24 h and stained with trypan blue (0.05%) at 90 °C for 40 min. After which, the roots were placed in lactoglycerol (lactic acid: glycerin: deionized water, 14:1:1 v/v/v) for 15 d. The magnified intersection method (McGonigle and Miller, 1990) was used to quantify the percentage of AMF colonization in the roots. The roots were horizontally mounted on slides, and each root was methodically scanned using the 40x objective of an Axiostar plus microscope (Carl Zeiss, Jena, Germany) aligned to the axis of a square lattice. The presence or absence of mycorrhizal structures (i.e., arbuscules, vesicles, or hyphae) that touched a grid axis and crossed the root was recorded, and at least 100 fields per sample were counted. The counts are presented as the percentage of the length of the colonized root (% LCR):

$$\% \, LCR = 100 \times \frac{Number \, of \, intersections \, with \, HMA \, structures}{Total \, number \, of \, counted \, intersections} \quad (1)$$

Growth and yield parameters in tomato plants

Multiple morphological parameters of growth and yield were evaluated in the 14 plants of each treatment 90 d after sowing. Whole plants were carefully removed from their pots, and their roots were washed with tap water. Then, the plants were scanned, and the root area (cm^2), leaf area (cm^2), root length, stem length (cm), number of leaves, and fresh weight (g^{-1}) were measured with the help of ImageJ v. 1.52a software (https://imagej.nih.gov/ij/download.html). The following performance parameters were also evaluated: the number of flowers, number of fruits, and fruit weight (g^{-1}) regardless of maturity.

Statistical analysis

Normality and homoscedasticity of the data were evaluated using the Shapiro-Wilk and Bartlett tests, respectively, and the data were analyzed by one-way analysis of variance (ANOVA) and a post-hoc Tukey HSD mean comparison test to compare the means of the different treatments and conditions. The analyses were conducted with R-Commander (R Foundation for Statistical Computing, Version 3.5.1).

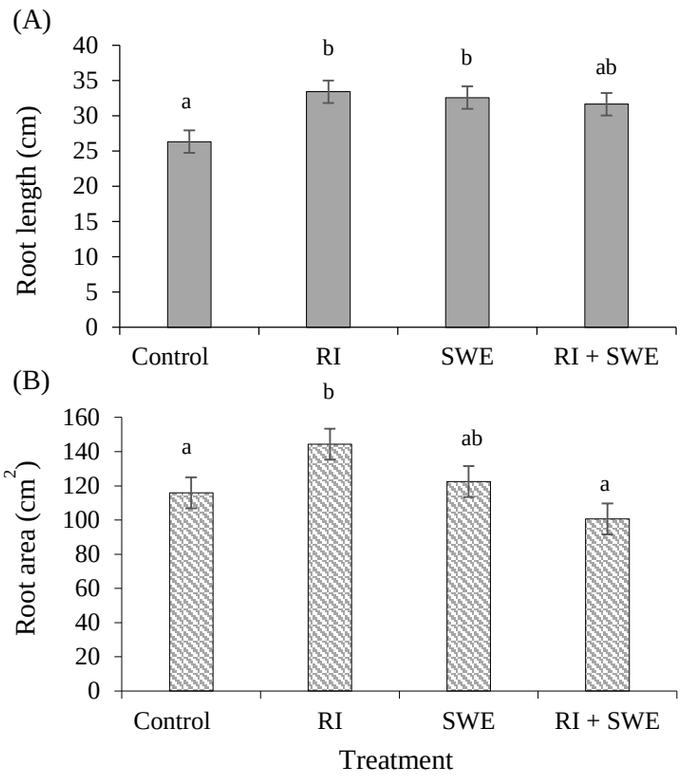
Results and Discussion

Growth promotion in tomato plants

Algal extract application and AMF inoculation significantly increased root length. However, no significant differences were present among plants that were treated with both biostimulants (RI + SWE), the control plants, or plants in either the SWE or RI treatments (Graphic 1A). When analyzing the root area, a positive effect was only observed in the plants in the RI treatment (144.3 cm^2) when compared to those of the control and RI + SWE treatments (Graphic 10.1B). Root growth promotion has been widely documented in response to AMF inoculations. In particular, Copetta *et al*. (2011) found that inoculation with *Glomus mosseae*, *G. caledonium*, *G. viscosum*, *G. intraradices*, and *G. coronatum* promoted root development in tomato plants. In addition to increasing the root area, symbiosis also optimizes nutrient transport, particularly that of phosphate (Gamalero *et al*., 2004). However, AMF can stimulate root formation due to the physiological activity of these endophytes that promotes the synthesis of growth regulators like auxins and cytokinins (Gianinazzi-Pearson *et al*., 1991). Root proliferation in response to algal extract treatment is believed to be related to the presence of bioactive compounds (e.g., thiamine, riboflavin, vitamin K, auxins, cytokinins, gibberellins, polysaccharides, and minerals) in the extracts that play important roles in regulating cell division (Gollan and Wright, 2006; Zodape *et al*., 2008).

The results obtained in this study differ from the additive effects reported from the joint application of AMF *R. intraradices* and a *Padina gymnospora* extract (González-González *et al*., 2020), which may be due to differences in the composition and origin of these extracts.

Graphic 10.1 Root growth in tomato plants inoculated with the arbuscular mycorrhizal fungi (AMF) *Rhizophagus intraradices* (RI), a seaweed extract (SWE) from *Ulva lactuca*, or treated with both the AMF and extract (RI + SWE). The values correspond to the mean ± standard deviation (n = 14). Different letters denote significant differences (P ≤ 0.05) based on Tukey tests



None of the treatments resulted in positive growth promotion in aerial tissues. The foliar area, leaf number, stem length, and shoot fresh weight were lower in the treated plants when compared to those of the control plants (Table 10.1). These results do not correspond to what was reported for both biostimulants when applied individually. It is possible that the growing conditions (particularly the substrate) utilized to evaluate mycorrhization limited plant development.

Table 10.1 Effect of the arbuscular mycorrhizal fungi (AMF) *Rhizophagus intraradices* (RI), seaweed extract (SWE) from *Ulva lactuca*, and both the AMF and algal extract (RI + SWE) on the growth of aerial tissue in tomato plants. The values correspond to the mean ± standard deviation (n = 14). The different letters within columns denote significant differences (P ≤ 0.05) between treatments using a Tukey test.

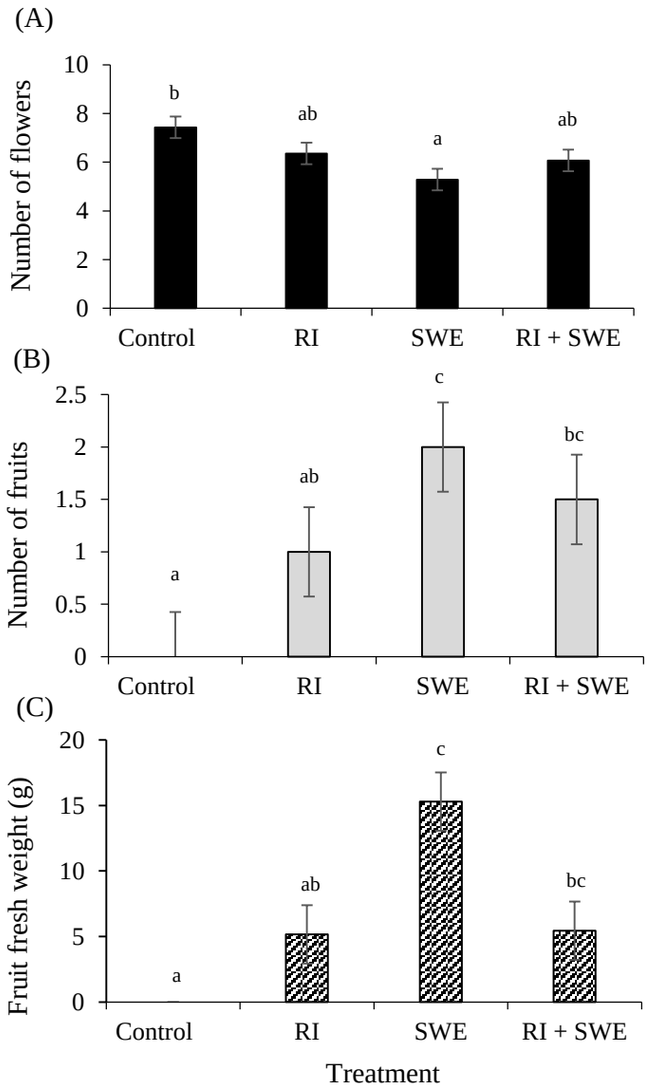
	Foliar area (cm^2)	Number of leaves	Stem lenght (cm)	Sprout fresh weight (g)
Control	185.3 ± 11.6[a]	70.9±8.7[a]	54.6±7.9[a]	16.2±1.6[a]
RI	179.8 ± 10.5[a]	73.3±5.4[a]	56.7±7.7[a]	15.5±1.6[a]
SWE	170.5 ± 12.3[a]	62.3±10.3[a]	47.2±5.0[a]	15.4±1.5[a]
RI + SWE	161±15.6[a]	63.7±13.8[a]	48.3±5.4[a]	14.4±2.1[a]

Tomato plant productivity

Parameters related to productivity showed significant differences between treatments. The number of flowers per plant was significantly lower in the SWE treatment compared to that of the control but similar to the number of flowers registered in the RI and RI + SWE treatments (Graphic 10.2A).The number of fruits per plant was significantly higher in all biostimulant treatments, with the highest number of fruits being found in the SWE treatment (2), followed by the RI + SWE (1.5) and RI (1) treatments. The control plants did not show fruit development on the day of the evaluation (Graphic 10.2B). The analysis of fruit fresh weight indicated a similar trend to that of the number of fruits per plant.

The fruits of the SWE treatment were significantly heavier compared to those of the plants of the RI or RI + SWE treatments (Graphic 10.2C). It is important to highlight that all productivity parameters were evaluated 90 days after sowing, and thus the lowest number of flowers observed in the SWE treatment corresponds to the highest number of fruits in the same treatment.

Graphic 10.2 Productivity of tomato plants inoculated with the arbuscular mycorrhizal fungi (AMF) *Rhizophagus intraradices* (RI), an algal extract from *Ulva lactuca* (SWE), or treated with both the AMF and the extract (RI + SWE). The values correspond to the mean ± standard deviation (n = 14). The different letters denote significant differences ($P \leq 0.05$) between treatments using a Tukey test.



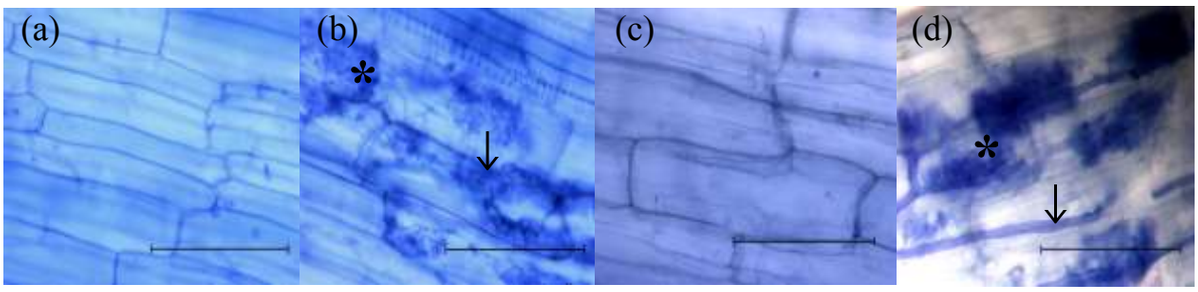
Our results agree with those previously reported for *Ecklonia maxima* seaweed extracts in which accelerated flowering and/or fruiting in tomato plants was observed due to the presence of macro- and micronutrients and growth regulators (Hamed *et al.*, 2018). Furthermore, AMF inoculation has been found to increase tomato yields. For example, William (2007) reported a 40% increase in tomato yield in the nursery after inoculation with *Glomus mosseae* and *Scutellospora calospora*. In other studies, inoculation with autochthonous AMF was found to increase yield by 26% (Regvar *et al*., 2003) while inoculation with *G. fasiculatum* resulted in an increase of 13% (Mohandas, 1987). Similar to what was observed with regard to the promotion of root growth, no synergistic or additive effects were found in the AMF + SWE treatment. This contrasts with previous studies that have found positive effects on crop productivity when two biostimulants were applied. For example, cucumbers inoculated with the AMF *G. intraradices* and *Acoulospora laevis* in combination with the foliar application of a commercial seaweed-based product were found to significantly increase the number of fruits per plant (52.28%), yield per plant (82.42%), and total yield (82.46%) with respect to those of the control plants (Suhail, 2013b). In tomato plants, the joint application of *R. intraradices* and a *P. gymnospora* seaweed extract resulted in a greater number of flowers when compared to those of plants treated with only one of the two biostimulants (González-González *et al*., 2020).

The results of this study differ from those reported for cucumber and tomato plants due to the differences in experimental conditions, particularly those related to the algal species employed.

Colonization of AMF Rhizophagus intraradices

In the roots, where the RI and RI + SWE treatments were applied, the presence of fungal structures characteristic of symbiotic arbuscular-type associations were observed, highlighting intra-radical and arbuscular hyphae (Figure 10.1b and 10.1d). The presence of vesicles was not observed in any field analyzed. As expected, the roots of the non-inoculated plants (control and SWE treatment) did not show the presence of AMF. The results indicated that no significant differences were present in the number of intra-radical hyphae or arbuscules between the plants of the RI and RI + SWE treatments. However, when analyzing the number of arbuscles, significant changes were found between the treatments, with the RI + SWE treatment showing 8-fold the number of arbuscles than those of the RI treatment. Similar results were obtained when analyzing the colonized root length percentage (CRL%) with significantly higher values observed in the (RI + SWE) treatment (Table 2). These results indicate that the *U. lactuca* extract had a positive effect on the establishment of mycorrhizal symbiosis between *R. intraradices* and the plant roots.

Figure 10.1 *Rhizophagus intraradices* fungal structures in tomato plant roots: (a) control, (b) inoculated with the arbuscular mycorrhizal fungi (AMF) *Rhizophagus intraradices* (RI), (c) treated with a *Ulva lactuca* algal extract (SWE), and (d) inoculated with the AMF and treated with the extract (RI + SWE).



Symbols:*, arbuscles; ↓, intraradical hypha; scale bars represent 100mm 40X

Table 10.2 Mycorrhizal colonization of tomato roots. Registration of arbuscles, hyphae, and the colonized root length percentage (CRL%). The values correspond to the mean (n = 6). The different letters denote significant differences ($P \leq 0.05$) using a student t-test.

	Hypha	Arbuscles	%CRL
RI	49.5[a]	8.8[a]	49.2[a]
RI + SWE	52.3[a]	65.5[b]	82.6[b]

The information available regarding the influence of algal extracts on the growth, colonization, and establishment of AMF includes the involvement of low molecular weight compounds like 5-deoxy-5-methylamino-adenosine, alginic acid, mannitol, and a variety of polysaccharides present in marine algae that facilitate phosphorus acquisition (Kuwada *et al*., 2005; Kuwada *et al*., 2006b; Khan *et al*., 2009; Paszt *et al*., 2015). *Ulva lactuca* extracts contain amino acids, fatty acids, vitamins (Ortiz *et al*., 2006; Frikha *et al*., 2011), polysaccharides, polyphenols, organic acids (Violle *et al*., 2018; Dominguez and Loret, 2019), mucilage (Abirami and Kowsalya, 2011), flavonoids, and terpenoids (Sava and Sîrbu, 2010; Elmegeed *et al*., 2014; Alagan *et al*., 2017). It is probable that some of these compounds directly intervene in the establishment of mycorrhizal symbiosis, favoring spore germination and the growth and branching of germinative hyphae. Furthermore, vitamin E, which is present in *U. lactuca* (Khan *et al*., 2009; Ortiz *et al*. 2006) and *Chlorella pyrenoidosa* extracts, has been found to stimulate the growth of *G. margarita* and *G. caledonium* (Kuwada *et al*., 2006a). In addition, the enhanced fungal growth and root colonization may have been due to the polysaccharides or oligosaccharides contained in the SWE. Another possible explanation for the improvement in lateral root growth by the SWE may have been a change in the hormonal balance as a consequence of the auxins contained in the SWE, which are root hair growth regulators that promote elongation through the up-regulation of associated root epidermis genes, and thus mycorrhization may have consequently been improved as a result of greater root development (Gonzalez-Gonzalez *et al*., 2020).

Conclusions

When AMF and SWE were used individually, each was found to positively stimulate plant growth and performance in different but complementary ways. AMF promoted growth and root development, whereas SWE promoted flowering and tomato fruit formation. In addition, the *U. lactuca* extract stimulated the development of fungal structures and *R. intraradices* colonization (%) in tomato plant roots. However, no advantageous effects were observed from the joint application of the two biostimulants. In agriculture, the application of biostimulants like AMF and SWEs could substantially improve sustainability efforts. However, in Mexico, the production and use of plant biostimulants is still limited, and they have rarely been incorporated into established cultivation practices, partly due to a lack of understanding regarding their usefulness and application. Therefore, accurate information is needed for biostimulants to replace organic and agrochemical fertilizers. Consequently, greater collaboration between farmers, the industrial sectors, researchers, and government entities is required to improve production systems and the quality of biostimulants to develop and implement improved, environmentally friendly agricultural practices.

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