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The Presence of IAA in Liquid Extract of *Sargassum polycystum* from Lombok Promotes Germination and Vegetative Growth of Selected Agricultural Plants

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Plant growth hormones play an important role in the uptake of mineral nutrition from soil to improve the growth of plants. Therefore, understanding the source of plant growth hormones is important. Macroalgae are an important source of plant growth hormones from the natural environment. This study investigated the types of plant growth hormones present in the liquid extract of *Sargassum polycystum* collected from Lombok, as well as the application of the seaweed extract in stimulating the germination and growth of selected agricultural plants. Liquid extract of *S. polycystum* collected in Batulayar, the coastal area of West Lombok contained 0.43 mg/mL IAA. The application of 0.2% seaweed extract significantly improved the germination of *Sesamum indicum* seeds by approximately 11% compared to the control. Even though the application of 0.2% seaweed extract did not affect the growth of *Phaseolus vulgaris* seedlings in terms of plant height, root length and number of root branchesthe treatment significantly enhanced the vegetative growth of *Vigna radiata* by increasing the number of leaves and fresh weight. This suggested that IAA present in the liquid extract of *S. polycystum* might have induced an increased germination rate in *Sesamum indicum* and vegetative growth in *Vigna radiata* plants, but the treatment was not effective in improving the growth of seedlings.

Keywords: *Sargassum polycystum*; IAA; germination; seedling; growth; *Sesamum indicum*; *Vigna radiata*; *Phaseolus vulgaris*

I. INTRODUCTION

Biofertilisers are defined as organic compounds from living organisms that promote the growth of seeds, plants or soil bacterial consortia by increasing the availability of essential nutrients such as nitrogen, phosphate, potassium and other minerals (Reddy & Saravanan, 2013). The most exceptional feature of using biofertilisers is the improvement of plant productivity per unit area in a short time, reducing the consumption of energy as well as water and soil contamination while increasing the soil fertility (Carvajal-Muñoz & Carmona-Garcia, 2012). Biofertilisers are also well-known as a renewable and environment-friendly fertiliser of which the usage may lead to sustainable economic development (Srivastava, 2002). Therefore, the search for various sources of biofertilisers becomes

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necessary nowadays. Recent studies indicated that marine macroalgae are one of the potential sources of biofertilisers that provide several types of growth hormones and mineral nutrients which improve seed germination and promote seedling growth for several kinds of horticultural plants (Sunarpi *et al.*, 2019).

Marine macroalgae had been reported to produce various bioactive compounds with a wide range of biological activities that allow them to be used as soil conditioners and fertilisers. Liquid extracts of macroalgae have previously gained importance as foliar sprays for several plants since long ago in several countries such as the United Kingdom, Japan, and New Zealand (Kumar *et al.*, 2012). Algae-based fertilisers also have the ability to fix nitrogen, increase soil fertility and impart beneficial effects of the plant growth promoting rhizobacteria to the crops. Some algal-based fertilisers can also be produced as metabolic byproducts during the wastewater treatment processes, making them a renewable source for sustainable agriculture (Win *et al.*, 2018).

Macroalgae contained significant amount of macro- and microelements which could induce germination and seedling development in plants (Godlewska *et al.*, 2016). *Sargassum weightii*, *Caulerpa racemosa* and *Turbinaria ornata* were reported to contain several kinds of macro- and micro-nutrients such as Na, Mg, K, Fe, Mo and N and several plant growth hormones such as auxins, cytokinins and gibberellins (Kiseleva *et al.*, 2012; Uthirapandi *et al.*, 2018). In addition, macroalgae also contained polysaccharides (galactans, fucoidans, alginates and laminarin), proteins (lectins), polyunsaturated fatty acids, and pigments (chlorophylls, carotenoids and phycobiliproteins).

The effect of marine macroalgal extracts on the germination, growth and yield of several crops had been widely investigated (Manimala & Rengasamy, 1993; Moller & Smith, 1998; Rama Rao, 1990; 1991; Whapam *et al.*, 1993). Seaweed liquid fertiliser increased not only the seed germination and plant growth during the vegetative stage (Rao & Chatterjee, 2014), but also the growth and production of several crops, like sunflower (Chbani *et al.*, 2015), tomato (Polo & Mata, 2018), and soybean plants (Kocira *et al.*, 2018).

Sunarpi *et al.* (2007) identified 88 species of macroalgae in West Nusa Tenggara. Previous study reported that extracts of several macroalgal species including *Sargassum crassifolium, Sargassum cristaefolium*, *Sargassum aquifolium* and *Turbinaria murrayana* could stimulate plant growth (Nikmatullah *et al.*, 2014). However, the potential of other macroalgae including *Sargassum polycystum* from West Nusa Tenggara as plant growth stimulant has not been explored.

This study aimed to identify the plant growth hormones present in the liquid extract of *S. polycystum* from Lombok, as well as to evaluate the application of the seaweed extract in stimulating seed germination and growth of selected agricultural plants.

II. MATERIALS AND METHODS

A. Preparation of Seaweed Liquid Extract

Samples of *S. polycystum* were collected at the Batulayar beach of West Lombok. After thorough washing with seawater, the samples were placed in new polyethylene bags, kept in an ice box containing slush ice and transported to the laboratory. About 1 kg of samples were cut into small pieces and processed in a blender with distilled water at a 1:1 ratio. Liquid extract of the seaweed was prepared according to the procedure outlined in Godlewska *et al.* (2016) by heating the seaweed slurry in a 45°C water bath for 30 min, and the slurry was filtered using Whatman no.1 filter paper. The filtrate obtained was taken as the 100% extract of *S. polycystum*, which was further diluted to 0.2% for use in each experiment.

B. Determination of Plant Growth Hormones in Seaweed Extract

Plant growth hormones present in the liquid extract of *S. polycystum* collected from Lombok were determined using the high-performance liquid chromatography (HPLC) according to the procedure modified from Godlewska *et al.* (2016). Standard solutions at a concentration of 0.1% were prepared for each of the following plant growth hormones: indole acetic acid (IAA), 6-napthalene acetic acid (NAA), gibberellic acid (GA_3), zeatin (ZA), kinetin, abscisic acid (ABA) and 2,4 dichlorophenoxyacetic acid (2,4-D). The HPLC protocol was optimised using standard solutions of each plant growth hormone mentioned above.

HPLC analyses were carried out on a Shim-pack CLC-ODS column (Shimadzu, Japan). Each appropriately diluted sample (liquid extract of seaweed and the standard solutions of each plant growth hormone) was automatically injected into the HPLC column and separated at a column temperature of 30°C, pressure of 50 kg/cm², continuous flow rate of 0.5 mL/min, using methanol/distilled water (7:1 v/v) as the mobile phase. The composition of plant growth hormones in the liquid extract of *S. polycystum* was determined by comparing the peaks generated in the chromatogram of the seaweed extract with that of each plant growth hormone standard.

C. Experimental Design

Three experiments were carried out to evaluate the effect of the liquid extract prepared from *S. polycystum* on the germination of *Sesamum indicum* seeds, the growth of *Phaseolus vulgaris* seedlings and the vegetative growth of *Vigna radiata*. Each experiment which consisted of a control group with non-treated samples (seeds, seedling or plants) and a treatment group where samples were treated with 0.2% liquid extract of *S. polycystum* was carried out in triplicate. The sesame and green bean seeds were used in the germination and seedling growth experiments because of their small size that can maximise the number of samples fitted into the containers for each replicate; the common bean plants were chosen for the vegetative growth experiment due to our familiarity in handling them in the soil medium.

1. Application of Seaweed Extract in Improving Seed Germination

Each replicate consisted of 100 dry S*esamum indicum* seeds that had been washed and placed on several sheets of moistened cotton wools layering a Petri dish. Sesame seeds for the control group were placed on cotton wools moistened with distilled water. Meanwhile, those sesame seeds for the treatment group were placed on cotton wools moistened with 0.2% *S. polycystum* liquid extract. All replicates for this experiment were kept in a dark room and grown under the room temperature for germination. The observation was carried out for one week and the number of germinated seeds were counted.

2. Application of Seaweed Extract in Improving Seedling Growth

Each replicate consisted of five *Phaseolus vulgaris* seeds grown on the MS agar medium prepared according to Salisbury and Ross (1992). The green bean seeds for the control group were grown on the standard MS agar medium while those of the treatment group were grown on the MS agar medium supplemented with 0.2% *S. polycystum* liquid extract. Measurements of the growth parameters (plant height, root length, and number of root branches) were taken 3 weeks after planting.

3. Application of Seaweed Extract in Improving Vegetative Growth

Each replicate consisted of five 10-day-old *Vigna radiata* sprouts grown in a plastic pot containing 8 kg of soil medium mixed with organic fertiliser (3:1 w/w). Bean plants for the control group were watered every week while those plants for the treatment group were additionally sprayed with 0.2% *S. polycystum* liquid extract once a week for 4 weeks. The measurements of the number of leaves and fresh weight were taken 8 weeks after planting.

D. Statistical Analysis

Data were analysed using analysis of variance (ANOVA) and t-test analysis to determine whether treatment with application of 0.2% seaweed extract is effective in improving the germination and growth of selected agricultural plants compared to the control at 5% significance level. The statistical analyses were performed using the SPSS software. The values presented in the graphs were means of three replicates ± standard error.

III. RESULTS

A. Plant Growth Hormones in Liquid Extract of Sargassum polycystum

IAA was detected at a concentration of 0.43 mg/mL in the liquid extract of *S. polycystum* using HPLC. Injection of the IAA standard into HPLC column produced a peak at the retention time of 2.7 min (Figure 1A). A peak at similar retention time produced after the seaweed extract was injected into HPLC column (Figure 1B) confirmed the presence of IAA in the seaweed extract.

B. Effect of Sargassum polycystum *Extract on Germination of* Sesamum indicum

Treatment with 0.2% liquid extract of *S. polycystum* significantly increased the germination rate of *Sesamum indicum* seeds (Figure 2). The sesame seeds treated with 0.2% seaweed extract germinated faster compared to those in the control group.

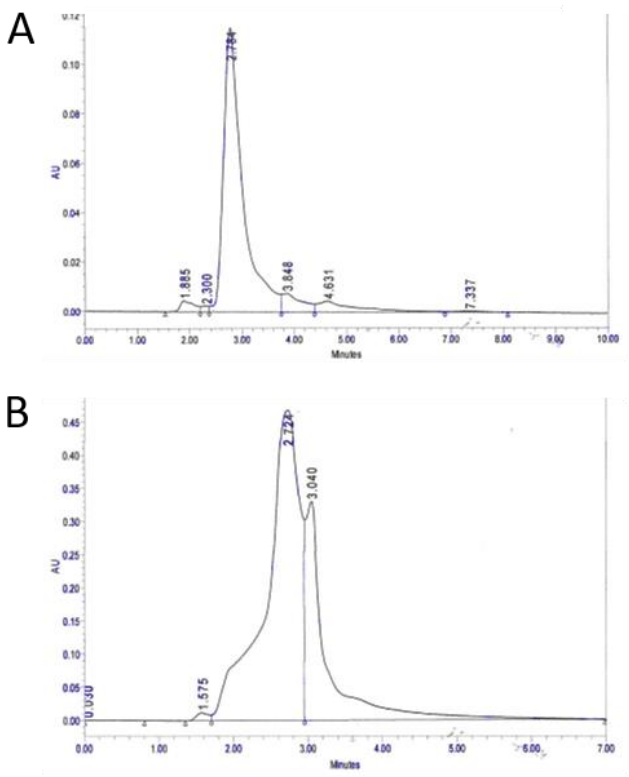

Figure 1. HPLC chromatogram showing the peak of (A) IAA standard and (B) seaweed extract

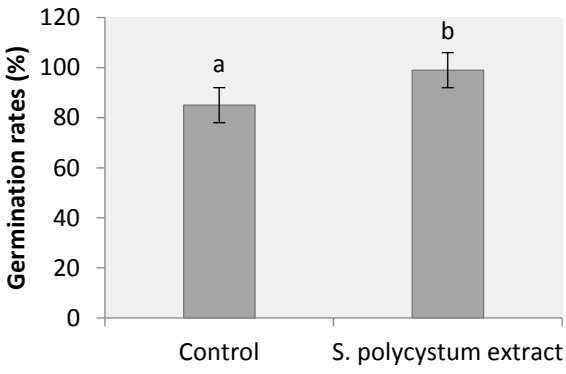

Figure 2. Effect of 0.2% *Sargassum polycystum* extract on *Sesamum indicum* seed germination rates. Bars denoted by different letters were significantly different ($p < 0.05$).

C. Effect of Sargassum polycystum *Extract on Seedling Growth of* Phaseolus vulgaris

The treatment of 0.2% seaweed extract did not affect the seedling growth parameters significantly (Figures 3–5). The plant height of *Phaseolus vulgaris* seedlings was not significantly improved with the application of 0.2% *S. polycystum* liquid extract as foliar spray (Figure 3). Similar root length (Figure 4) and number of root branches (Figure 5) were observed between the control and treatment groups.

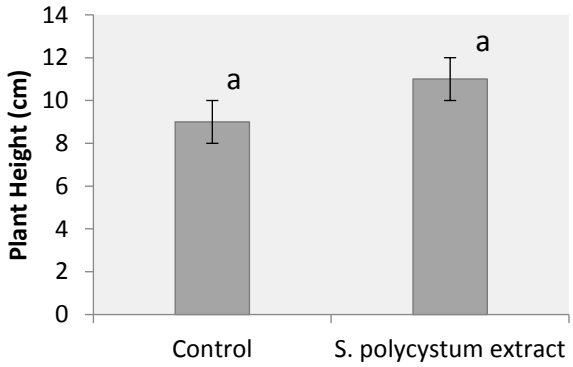

Figure 3. Effect of 0.2% *Sargassum polycystum* extract on the plant height of *Phaseolus vulgaris* seedlings. Bars denoted by the same letter are not significantly different ($p > 0.05$).

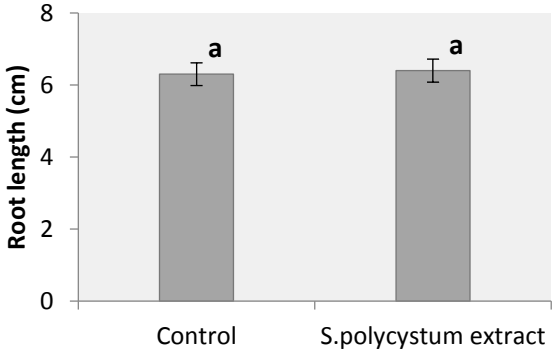

Figure 4. Effect of 0.2% *Sargassum polycystum* extract on the root length of *Phaseolus vulgaris* seedlings. Bars denoted by the same letter are not significantly different ($p > 0.05$).

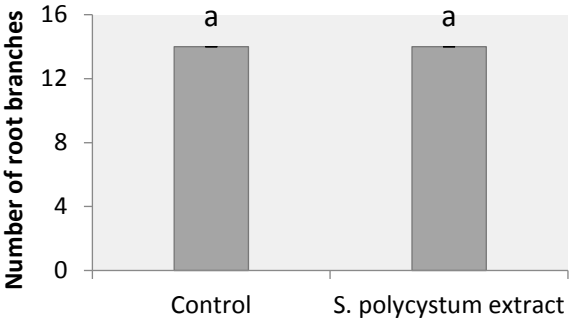

Figure 5. Effect of 0.2% *Sargassum polycystum* extract on the number of root branches of *Phaseolus vulgaris* seedlings. Bars denoted by the same letter are not significantly different ($p > 0.05$).

D. Effect of Sargassum polycystum *Extract on Vegetative Growth of* Vigna radiata

The application of 0.2% *S. polycystum* extract as foliar spray once a week for one month improved the vegetative growth of *Vigna radiata* plants by stimulating the growth of leaves in number (Figure 6) and the increase in biomass (Figure 7). The bean plants sprayed with 0.2% seaweed extract recorded approximately 80% higher leaf number compared with those of the control group. Similar results were also observed for the fresh weight parameter. The treated plants produced higher biomass than those of the control group by approximately 30%.

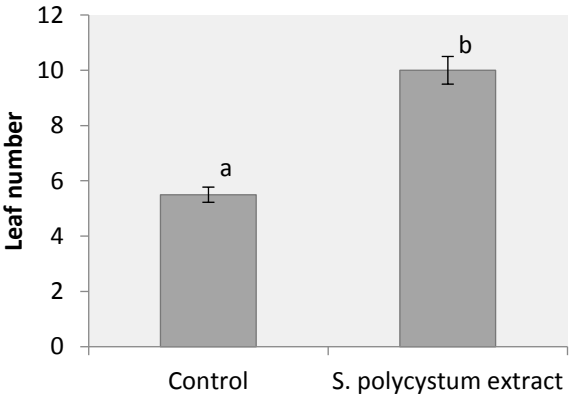

Figure 6. Effect of 0.2% *Sargassum polycystum* extract on the leaf number of *Vigna radiata*. Bars denoted by different letter are significantly different ($p < 0.05$).

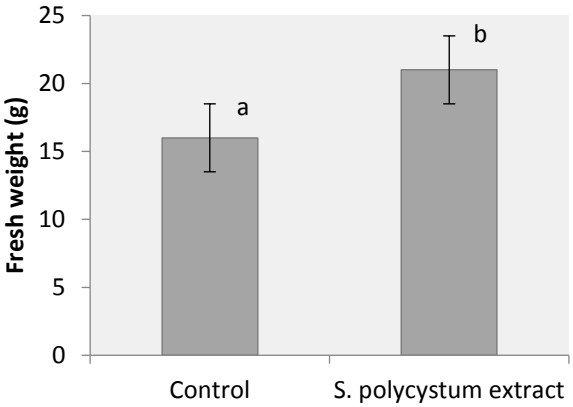

Figure 7. Effect of 0.2% *Sargassum polycystum* extract on the fresh weight of *Vigna radiata*. Bars denoted by different letter are significantly different ($p < 0.05$).

IV. DISCUSSION

It is widely known that plant growth hormones affect the physiological efficiency of plants including growth, photosynthesis and accumulation of assimilates. The liquid extract of *S. polycystum* prepared in this study contained the plant growth hormone IAA at a concentration of 0.43 mg/mL seaweed extract. This amount is considerably enough to induce plant growth (Salisbury & Ross, 1992; Taiz & Zeiger, 1998).

The application of IAA was reported to increase the germination rate, plant height, number of branches and leaves, total chlorophyll content and dry weight in *Lens culinaris* (Naeem *et al.*, 2004). In this study, the application of 0.2% *S. polycystum* liquid extract did not influence the development of *Phaseolus vulgaris* seedlings, but the treatment increased the germination rate of *Sesamum indicum* seeds and improved the vegetative growth of common bean plants.

The results showed that the germination rate of *Sesamum indicum* seed increased by about 11% with the application of 0.2% *S. polycystum* extract. This might be due to the role of IAA in inducing the activity of catalytic enzymes, such as amylase, protease and nuclease. These enzymes would break down polymers like carbohydrates, lipids, proteins and nucleic acids in endosperm of seeds into small molecules, including glucose, fatty acids, amino acids and nucleotides, which were transported to growing points to support germination (Buchanan *et al.*, 2000). The application of 0.2% *S. polycystum* extract as a foliar

spray once a week for one month increased the leaf number and biomass of bean plants compared to the control. These data clearly indicated that IAA contained in the seaweed extract stimulated the vegetative growth of bean plants, which were in line with the findings in Abou El-yazied *et al.* (2012). IAA application induced the up-regulated expression of gibberellin biosynthesis genes and produced new cell wall polysaccharides so that growth may continue for longer periods. Auxin, which is a class of hormone including IAA, was reported to initiate a signal transduction pathway to result in the production of secondary messengers that directly activate pre-existing H^+-ATPases and stimulate the expression of several genes related to plant growth and development (Takahashi *et al.*, 2012).

The application 0.2% *S. polycystum* liquid extract did not significantly affect the root growth of *Phaseolus vulgaris* seedlings, as the root length and number of root branches were the same in both control and treatment groups. There was an increase in the height of seedlings treated with seaweed liquid extract, but the effect was not significant. This might be due to the concentration of auxin (IAA) in the seaweed extract applied was too low to induce growth in those areas. Khandaker *et al.* (2018) reported that flower injection of 90 mg/L IAA significantly improved all physiological and reproductive parameters of the okra plants. Therefore, a higher amount of *S. polycystum* extract could be considered for application in further research. However, it is also likely that the growth of seedlings appeared unaffected by the addition of seaweed extract in the MS medium because the growth of seedlings was dependent on the availability of resources in cotyledon.

V. CONCLUSION

The liquid extract of *S. polycystum* contained IAA that enhanced the germination rate and vegetative growth, but application of this seaweed extract as foliar spray did not affect the seedling growth of selected agricultural plants. The results suggested that liquid extract of *S. polycystum* could be developed into a bio-stimulant that could be used to increase the germination, growth, and yield of agricultural plants.

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