Phytohormone content in brown macroalgae Sargassum from Lombok coast, Indonesia

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Phytohormone content in brown macroalgae *Sargassum* from Lombok coast, Indonesia

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Abstract. Exessive use of inorganic fertilizers in rice production systems, causes various negative effects in the environemnt, such as reducing soil fertility and increases pollution, which disturbs agricultural sustainability. Understanding raw materials for developing organic fertilizers which are cheap and adaptive to our environment, is an important study recently. Many researchers reported that brown algae could increase growth and yield of several species of plants. This due to brown algae extract contain plant growth hormone and essential elements to stimulate growth and production of plants. This article report growth and yield of rice plants suplied with mixtured extract of brown algae. Several species of brown algae were collected in coastal beach of Lombok. The seaweeds were cutted into small peaces, extracted with boiled water for 30 minute and filtered using filter whatman no.1 to get solid and liquid extract. Mixtured solid extracts were added in soil media. On the other hand, the mixtured liquid extracts were sprayed to rice plants during vegetative growth. The results shown that mixtured solid extracts increased growth and yield of rice plants. However, mixtured liquid extract did not stimulate growth and yield of rice plants. This suggests that brown algae is a potential raw material for development of organic fertilizers, which are adaptive to environment to support sustainable agriculture.

1. Introduction

Rice production system is very dependent to the application of inorganic fertilizers. The dose of application is increased from year to year, and it has reached to the exessive level of 300 kg ure, 100 kg TSP and 100 kg KCl per hectare. Since the maximum capacity of rice plants to absorb mineral nutritions, like nitrogen, phosphore and kalium, is 46%. This means that more than 50% ions released by inorganic fertilizers into environment, leaching into ecosystem, irigated water and vegetation, polluting our environment. This condition is dangerous not only for environment, but also for human health.

Exessive application of inorganic fertilizers also decreases soil fertility [1,2]. They kill soil microorganisms around rhizosfer whic plays an important role in nutritional availability which are ready to be absorbed by root system. In addition, the application of inorganic fertilizers in high dose to soil, induces mineral mining in every one cycle of rice cultivation. During fertilization, the soil is supplied with three elements: N, P and K. However, rice plants absorbs not only three elements, but also they absorb nearly all of macro and micro essential elements. Therefore, mineral nutrition in soil is decreased in type and quantity in every harvesting time. This cultivation practice decreases soil fertility continuesly from time to time. Therefore, it need to find out another type of fertilizers, which ara adaptive to environment to stop environmental destructive.

Previous researchers reported that liquid extract macroalgae contained plant hormones and solid extract macroalgae contained esential elements, which are stimulating growth and increasing yield of several species of

plants [3,4]. Moreover, since liquid extract contains plant growth hormones, like IAA, NAA and gibberellin, therefore liquid extract influence germination of several seeds. This is due to theoritical documented in some literatures that growth plant hormones, plays an important role as signal transductions for catalytic enzymes activities, like protease, amylase and lipase, in the process of germination [5-8].

In addition, except it effect ongermination, it has been also reported that liquid extracts also stimulated growth of tomato seedlings , and vegetables [9,10]. This indicates that plant growth hormones in liquid extracts, stimulates enzymes involve in remobilization of macromolecules to become small molecules to support growth as occurred in soybean cotyledones , *Vigna mungo* L. , and tomato plants [11,12].

Liquid extracts of macroalgaes has also been reported to influence vegetative growth of several plant species. When vegetative growth begin, root system of plants begin to absorb essential elements from environment and transported to young leafs. On the other hand, phtosyntates are translocated into young leaf and root system. All of that mechanism need hormone to induces activities of the enzymes involve of that mechanism. That is the theoritical reason liquid extract induces vegetative growth of bean plant (*Phaesolus vulgaris* variety Paulista), grapevine (*Vitis vinifera* L.) , tomato (*Lycopersicum esculentum* Mill.) [13-15].

Like liquid extract, solid extract has also been reported to affect growth and production of plants, due to essential elements content of solid extract [4]. Biofertilizer developed based on solid extract of red algae, could increase the growth of green bean , maize plant [16,17]. In addition, biofertilizers developed from solid extract of green algae, influenced chlorophyl and proline content, and growth of sunflower plants, vegetable , soybean , rice , cucumber, and tomato plants [18-20]. However, there remains limited information regarding the beneficial phytohormone and micronutrient content in brown macroalgae *Sargassum* in Lombok coastal areas. This study aims to elaborate these potentials in brown macroalgae *Sargassum* for further understanding regarding its potential as biofertilizer raw material.

2. Materials and Methods

2.1. Sample colection and extraction

Brown algae (*Sargassum crassifolium*, *Sargassum cristafolium* and *Sargassum polycystum*), was collected in Batulayar coastal beach West Lombok. Then, the samples were rinsed and dried in shadow place without direct contact with sun. After that, dried samples were extracted using distillated water according to the modified procedure developed by Godlewska et al. [4]. The samples were cutted into small peaces using scissors. Moreover, the cutted samples were mixed with distillated water with the comparison 1 kg sample plus 3 L water. The mixed samples were boiled at 95^{o} C water bath for 30 minute. Then, the mixed samples were filtered using filter cloth. Finally, solution and pelled obtained were called as liquid and solid extracts of brown algae mixture extract respectively.

2.2. Phytohormones analysis in liquid extract using HPLC

Plant growth hormones in liquid extract of brown algae, like *Sargassum crassifolium*, *Sargassum cristafolium* and *Sargassum polycystum* were meausred using HPLC according to modified procedure developed by Godlewska et al. [4]. Firstly, standard solution (0.1%) of phytohormones, such as kinetin, GA3, NAA and IAA, was prepared. Then, Liquid extract of brown algae was filtered using Allpure filter 0.45 µm before it was injected into HPLC column (the shimpact CLC-ODS column", Shmadzu, Japan). After that, the sample was injected into column HPLC using automatic sampler injection, which was automatically diluted in the column. Moreover, the sample was separated at temperature 30^{o}C, pressure 50 kg/cm^{2}, retention speed 0.5 mL per minute using methanol/water (7:1, v/v) as moving phase. Finally, phytohormone content was analized by comparing between chromatogram peak of sample and standard. The data were expressed as mean value ±SD.

2.3. Essential elements analysis in solid extracts and plant tissue using AAS

Essential elements, such as nirogen, phosphore, potassium, calcium, manganese and ferrum in solid extract of brown algae and plant tissue, were measured using AAS (Atomic Absorbance Spectroscophy) according to modified procedure developed by Godlewska et al. [4]. Firstly, standard solution (0.1%) of essential elements, like nitrogen, phosphore, potassium, calcium, manganese and ferrum, were prepared. After that, 10 gr of the solid extract and plant tissue, was destructed using 100 mL HCl 90% at the temperature of 400^{o} C. Then, standard and sample solutions were injected into AAS column as recommended procedure. Finally, the essential elements content in samples were determined by comparing between chromatogram peak between standards and samples. The data were expressed as mean value of three replicates ± SD.

3. Results and Discussion

3.1. Morphology and ecology of brown algae

Figure 1. Morphology of *Sargassum crassifolium, Sargassum cristaefolium* and *Sargassum polycystum*.

S. crassifolium has oval phyloid with serrated edges, rounded or tapered ends and duplicates. Phyloid is the petiole in seaweed. Cryptostomata are not clearly visible. The cauloid is cylindrical with irregular branching. Midrib is clearly visible from the base to the tip of phyloid. They usually grows in the intertidal zone on rock substrats [21].

The algae was grouped into the algae which has compressed primary branches. Some dominant morphological characters in this species are shape of receptacles, leaves, vesicles and stems. This species has flattened triangular receptacles. Moreover, this species has duplicated leaves with fine teeth. The thalli of this algae is up to 9 cm high with discoid holdfast. Main and primary branches of this algae have terete and smooth characteristics, where cryptostomata are present. Cryptostomata are randomly distributed in the main and primary branch of algae. In addition, it also has been reported that the habitat of this algae is coral flats and subtidal zone. They usually grows in the lower portions of the intertidal zone on rock substrats or shallow subtidal zones.

S. polycystum has dark brown thallus, small spike, slightly flattened appearance and branching like a tree. The leaves have an oval shape. This type group has discoid holdfast. The holdfast functions to be firmly attached to rocky habitats and to be able defends itself in current water. Moreover, *S. polycystum* has air bladder which functions to float if it is submerged in water when the water is in intertidal tide. Habitat of *S. polycystum* is quite dominant in the beaches with dominant coral reef substrates and strong tides [22].

3.2. Plant growth hormone and essential element content of brown algae

Table 1. Plant growth hormone and essntial element content of brown algae extract

Brown Algae	Hormone Content (mgml^{-1})				Element Content(% d.w.)					
	IAA	NAA	GAs	Kinetin	N	P	K	Ca	Fe	Mg
Sargassum crassifolium	0.232	0.009	0.002	0.007	0.51	0.02	3.14	0.86	57	8.7
Sargassum cristaefolium	0.591	0.011	0.005	0.008	0.52	0.08	6.11	0.85	55	8.5
Sargassum polycystum	0.441	0.007	0.008	0.010	0.50	0.07	6.0	0.86	50	8.6

The content of plant growth hormone and essential elements in liquid and solid extract of brown algae respectively is shown in Table 1. The data in Table 1 shows that liquid extract of brown algae species contained different type of phytohormones. For example, all *Sargassum* species contained similar phytohormone, IAA, with the highest concentration in *S.cristaefolium*. However, these *Sargassum species* vary in its phytohormones contents, such as kinetin, GA3 and NAA. Based on this data, it can be assumed that these brown algae may could stimulate growth and production of plants [23,24].

Except phytohormones, solid extract of several brown alga species used in this experiments contained several essential elements, such as nitrogen, phosphore, potassium, calcium, ferrum and manganese as shown in Table 1. The content of those elements in solid extracts of brown algae was relatively similar with those requested by plant tissue [6-8]. Moreover, the concentration of K, Ca, Mn and Fe relatively higher in solid extract of brown algae compared with those of teresterial plants, approximately 0.52, 0.08, 6.11, 0.85, 55, and 8.5% dry weight. This composition implies that solid extract provide essential elements in soil media, which are available to be absorbed by root sysem to support growth and production of plants.

4. Conclusion

In conclusion, brown macroalgae *Sargassum* species contain different amount of phytohormones and also micronutriets which are essential for plant growth. IAA is the most common growth hormone found in all *Sargassum* species. The highest content was found in *S.crassifolium*. Furthermore, the essential micronutrient contents in *S.cristaefolium* and *S.polycystum* were higher amount compared to *S.crassifolium*. Further studies regarding effects of environmental factors and seasonal changes on these contents should be considered.

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