The Effect of Seaweed Extract on The Growth of Shoot of Shallot (Allium wakegi Araki) Lembah Palu Variety on in vitro

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Abstract. Plant cultivation using sand media and conventional soil methods often have time and environmental constraint. One method that is expected to support the supply of quality shallots seeds is to propagate the seeds through tissue culture techniques. This study aims to know the effect of seaweed concentration on the growth of local palu shallot shoot in vitro. This research was carried out at the Biotechnology Laboratory, Faculty of Agriculture, Tadulako University, Palu. This study conducted using a completely randomized design (CRD) of one factor consisting of five levels, namely, without seaweed extract, seaweed extract the concentration of 5%, seaweed extract concentration of 10%, seaweed extract. The concentration of 15%, and seaweed extract concentration of 20%. Every treatment was quadruplicate; therefore, there were 20 experimental plots. Every unit of the experiment was planted with two explants; thus, the total sample amounted to 40. The results showed, adding 20% seaweed extract, in general, gave better growth in all parameters of the number of leaves and leaf length indicated by the number of leaves (7.50) and leaves size (10.80 cm).

1. Introduction
Palu’s local shallot (Allium wakegi Araki) is a marketable vegetable spice that should be used as traditional medicine and flavoring for cooking, such as a heat-lowering compress, reducing sugar levels and blood cholesterol, preventing thickening and hardening of the arteries. (2003). The shallots’ production in Central Sulawesi declined from 2012 to 2013 by 2,872 tons with a percentage reduction of 39.49% from 7,272 tons to 4,400 tons. From 2014 to 2016, shallot commodities production increased significantly, namely by 23, 82% from 6,923.30 tons to 9,088.30 tons [1]. Plant cultivation using sand media and conventional soil methods often have time and environmental constraints; for instance, the multiplication of plants using seeds needs relatively more hours, and the results are sometimes not identifiable by the parent plants. The need for many quality plant seeds, pest, and disease-free must be available in less time often can not be met if using conventional methods, both vegetatively and generatively [2].

The potential of local Palu’s shallot production can reach 12 tons/ha, while farmers' yields are on average only 4.3 tons/ha. The low productivity of Palu local shallots is caused by the low quality of seedlings, cultivation techniques that are generally carried out conventionally, and high pest and disease attacks [3]. To overcome this problem, one method that is expected to support the supply of quality Palu local shallots seeds is to propagate the seeds through tissue culture techniques. The tissue culture technique is an efficient technique for plant clonal cultivation [4].

Factors that also determine the success of tissue culture implementation are the composition of the media used [5]. Interaction between plant growth regulator (PGR) used in culture media will determine the direction of development of the cultured explant[6][7]. To
produce organs such as callus and shoots, it is necessary to provide PGR, such as auxin and cytokinin, with seaweed extract to increase growth-promoting hormone in plants [8].

Seaweeds contain many essential minerals from the sea required by plants [9]. Seaweed similarly consists of growth-enhancing PGR, which can increase plant growth and yield [10]. Seaweed extracts contain amino acids and macro elements such as Cu, Fe, Zn, and Mg, which are less present in terrestrial plants. Seaweed contains alginic polysaccharides, alginic acids, unsaturated fatty acids [11]. The content has good biological activity, facilitates and stimulates active factors that are not specific, and regulates endogenous hormone balance [12].

This study aims to know the effect of seaweed concentration on the growth of local palu shallot shoot in vitro.

2. Material and methods

2.1 Study location

This trial was performed at the Laboratory of Plant Biotechnology, Faculty of Agriculture, University of Tadulako, Palu. From May to July 2019, The explant used are shallot cv. Lembah palu. Medium for tissue culture was MS primary media. Seaweed of Caulerpa sp.

2.2 Research method

This research uses a completely randomized design (CRD) of one factor consisting of five levels, namely, without seaweed extract (R0), seaweed extract concentration of 5% (R1), seaweed extract concentration of 10% (R2), seaweed extract concentration 15 % (R3), and concentration of seaweed extract 20% (R4). All treatments were repeated four times; therefore, there were 20 trial units. Each units of the experiment were planted with two explants; consequently, the total sample was 40.

2.3 Seaweed preparation

Seaweed of Caulerpa sp collected from Salakan District Luwuk province Sulawesi Tengah. The dried seaweed is blended until the powder passes the 60 mesh filter. After that finely ground powder then incubated at a temperature of -20 °C for 20 min. Every 100 g of seaweed and 2 L of aquades was added and then heated while stirring at a temperature of about 75 °C for 2-3 h. The extract was filtered off with a fine sieve; the result obtained was considered 100% seaweed extract. Seaweed extract is then sterilized by autoclaving at 121 °C, 15 psi pressure for 15 min.

2.4 Medium Preparation and explant planting

The media was prepared by inserting macro and micro nutrient stocks into a 1000 ml beaker, then weighing 0.1 g of myoniositol, 1 g of vitamin, 30 g of sucrose, put in sterile distilled water to 1000 ml, divided into 5 parts and poured each. 200 ml each into a 250 ml beaker.

Each solution was mixed with each treatment, namely without 0% (R0) seaweed extract, 5% (R1), 10% (R2), 15% (R3), 20% (R4) seaweed extract. using a stirring rod, the media is heated using a hot plate, to a temperature of 80°C, after which each medium is poured into a sterile bottle with a volume of 25 ml, then covered with plastic tightened with a rubber band, after which label paper is attached to each media. Media was sterilized by using autoclave at a temperature of 121 0C pressure of 17.5 psi for 15 minutes.

2.5 Parameters

The observation parameters included the average on the first time of the appearance of shoot. Numbers of leaves were measured at 6, 12, and 18 days after planting (DAP), and leaves length were measured at the end of the experiment. The data obtained were analyzed using an analysis of variance [12] and (F test 5%) was carried out to determine the effect of treatments on the observed parameters. If it was significant, then a 5% Honestly Significance Difference (HSD) test was used to separate the significantly different mean.
3. Results and discussion

3.1 Shoots appear

The results of the analysis of variance showed that the treatment did not affect the emergence of shallot shoots. The average shoot buds are presented in Figure 1.

![Figure 1. Average of time to Appear of shallot shoot in Various Caulerpa sp. Seaweed Extracts concentration.](image)

Figure 1 shows that the average shoots appeared by additional seaweed extract at concentrations 20% shoots growing faster than other seaweed extract concentrations.

3.2 Number of Leaves

The analysis of variance showed that the concentration of the extract significantly affected the number of leaves. The average number of leaves is presented in Table 1.

| Concentration | Number of leaves |
|---------------|-----------------|
|               | 7 DAP           | 14 DAP         | 21 DAP         |
| 0%            | 2.75<sup>a</sup> | 4.75<sup>a</sup> | 5.88<sup>a</sup> |
| 5%            | 3.13<sup>ab</sup> | 4.88<sup>a</sup> | 6.00<sup>ab</sup> |
| 10%           | 3.38<sup>ab</sup> | 5.00<sup>ab</sup> | 6.75<sup>ab</sup> |
| 15%           | 3.63<sup>ab</sup> | 5.75<sup>ab</sup> | 7.25<sup>ab</sup> |
| 20%           | 4.38<sup>b</sup> | 6.13<sup>b</sup> | 7.50<sup>b</sup> |
| HSD 5%        | 1.44            | 1.18           | 1.51           |

Means followed by the same letter in the same column are not significantly different at the 0.05 HSD level.

Results of the HSD test (Table 1) show that the number of leaves in 7, 14, and 21 days after planting (DAP), the concentration of seaweed extract 20% produces more leaves which different from without seaweed extract, but not different from other treatment with seaweed extract (Table 1), except in 14 DAP where concentration of seaweed extract 20% difference from 5% of seaweed extract treatment.

3.3 Leaf Length

The results of the diversity analysis showed that the concentration of seaweed extract significantly affected leaf length. The average leaf length is presented in Table 2.
Table 2. Average Leaf Length (cm) of Shallot at Various Concentrations of Caulerpa Seaweed Extract

| Concentration | Leaves length |
|---------------|---------------|
| 0%            | 9.48<sup>a</sup> |
| 5%            | 9.53<sup>b</sup> |
| 10%           | 9.68<sup>b</sup> |
| 15%           | 10.00<sup>b</sup> |
| 20%           | 10.80<sup>b</sup> |

Means followed by the same letter in the same column are not significantly different at the 0.05 HSD level

The HSD test (Table 2) shows that extract from seaweed at concentrations of 20% produces longer leaves, which is significantly different than without seaweed extract; however, it is no different from other treatments within additional seaweed.

The growth of explants in tissue culture is affected by factors such as the type of media, the choice of explants, sterilization, and the composition of growth regulators used. Media variation can be in the form of modification of essential components in the media, namely by adding other substances to the media that might increase explant growth, such as vitamins, coconut water, amino acids, and fruit juices [7].

The treatment of seaweed extract had no significant effect on the parameters of shoots emerge because shoot growth was not uniform in all treatments; this could be due to each tuber quality index or explants used had different growth rates. Besides, the addition of growth regulators at low and high concentrations will not stimulate plant cell growth because it is in a condition that is less or excessive so that the development of plant cells is inhibited. (Tiwari et al. 2007 and Bulo et al. 2018) The interaction of application between PGR given to the media and those produced by cells endogenously determines the direction of development of a culture [24]. [7] states that the genotype and composition of the media used are factors that determine tissue culture techniques' success.

Treatment of 20% seaweed extract, in general, gives better growth in the parameters of the number of leaves and leaf length; these results indicated that seaweed also contains substances that can improve the mechanism of plant growth (Arioli et al. 2015; Michalak et al. 2016; and Ali et al. 2016). Although the mechanism at work is not yet fully understood, a strong suspicion is that a substance has a good effect on the concentration of 20% seaweed extract [17]. [18] stated that seaweed extracts contain bioactive at low concentrations diluted with 1: 1000 or more. The seaweed primary metabolite content is economically valuable; the secondary metabolite content of seaweed has the potential to be a producer, various bioactive metabolites with comprehensive activities as antibacterial, antiviral, antifungal, and antioxidant [19] [20] [21].

From these results, it has been proven that the role of seaweed extract can support the development of shallot shoot, especially the number of leaves and leaf length at a concentration of 20% seaweed extract Caulerpa sp. This finding may mean that Caulerpa sp has prospects to use in tissue culture media because it contains growth regulators. For example, PGR in seaweed includes auxin, cytokinins, and gibberellins[12] [22] [23].

4. Conclusions
The higher the seaweed extract concentration used, the better Lembah Palu shallot shoot growth marked by the number of leaves and longer shoot.

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