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Research Article
Enhancing Shallot (*Allium wakegi* Araki) Shoot Growth Using Seaweed Extract and Benzylaminopurine in Tissue Culture Medium

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Abstract

Background and Objective: Shallot c.v. lembah palu is one of the main commodities of Central Sulawesi and is a raw material for the fried shallot processing industry. The research aimed at finding out the initiation of shallot shoots c.v. lembah palu on various concentrations of seaweed (*Caulerpa* sp.) extract and benzyl amino purine *in vitro.* **Materials and Methods:** The research was designed as a factorial completely randomized design, consisting of two factors. Each factor has three levels. The first factor was the concentration of seaweed extract 0, 20 and 40%. The second factor was the concentration of BAP 0, 2 and 4 ppm. Each treatment combination was repeated three times. The effect of the treatments was analyzed using analysis of variance and the mean difference between the treatments was analyzed with Honestly Significant Difference (HSD) at the level of 5%. **Results:** The results show that there is significant effect interaction between concentration treatment of seaweed extract and BAP on the number and length of the shoots on 6, 12 and 18 days after planting as well as the time of shoots emergence. The interaction between without BAP treatment and 20% of seaweed extract gave the highest number and the longest shoots which is significantly different from other treatments. **Conclusion:** The addition of both seaweed extract and BAP on higher concentration (40% and 4 ppm) raised the shoots faster, yet it was insignificant different with the treatment of 20% of seaweed extract without BAP.

Key words: *Caulerpa* sp., shallot, biomass, nutrient solution, chlorophyll

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Shallot is one of the important commodities of spice vegetables, although not a necessity, this commodity is almost always needed by consumers[1]. Shallot is used as a seasoning dish, raw materials for the food industry and traditional medicines[2].

In the province of Central Sulawesi, particularly in the Palu Valley, there are superior local shallot commodities that have been recognized by the central government as the national superior shallot (under the name of lembahpalu varieties). Shallot c.v. lembahpalu (*Allium wakegi* Araki) is an important commodity that provides business opportunities because it is the main ingredient of fried shallot Palu. Nevertheless, the shallot productivity of the last few years has fallen sharply[3]. Statistics show that in 2011 the productivity reached 7.84 t ha^{-1}, dropped to 4.12 t ha^{-1} in 2012 and more drastically to 3.37 t ha^{-1} in 2013[2,4].

One of the causes of the decline is the limited availability of quality seeds and very limited seed production technology. During this time, conventional seed propagation by using tubers as a source of new crops has not been able to meet the needs of farmers. Thus, efforts should be made to provide seeds quickly through tissue culture techniques[5,6].

Various studies to obtain a better protocol for shallot propagation have been reported[7], such as sterilization techniques, callus induction and plant regeneration through embryogenesis. Propagation of organogenesis buds is still very limited. The addition of growth regulators from the cytokinin group combined with auxin reported having increased interaction of plantlet production and the number of leaves of *Allium ascalonicum*. However, the use of other organic materials that have been proven for a long time to improve conventional plant growth, namely seaweed has never been tested *in vitro*.

Yusuf *et al.*[8], states that nutrient concentrations in seaweed and seaweed processed products are generally very low, so the effect of improving plant growth and production is thought to be derived from the work of the growing growth regulator they contain[9]. Sharma *et al.*[10], reported indoleacetic acid (IAA) content in acid extracts of five seaweed species tested ranging between 2.74 and 46.8 mg g^{-1} of seaweed. Biostimulant activity through bioassay using *Vigno mungo* L. plant with various extract methods from the five types of seaweed proved to increase the dry weight of the results and accelerate the growth of root stem cuttings.

Bioassay using soybeans for various types of seaweed proves that *Caulerpa* sp. commonly found in Banggai Kepulauan District Central Sulawesi with the local name Lato, responded better than other types of seaweed, instead of growth regulators to spur the germination of soybeans[11].

This study aimed to determine the initiation of shallot shoots of lembah palu variety on various concentrations of seaweed extract and BAP *in vitro*.

MATERIALS AND METHOD

Study area: This experiment was conducted from September-December 2018, at the Plant Biotechnology Laboratory of the Faculty of Agriculture, Tadulako University, Indonesia.

Research tools: The tools used in this research are autoclave, electric oven, water distillation, analytical scale, pH meter, Laminar Air Flow Cabinet (LAFC), culture bottle, measuring cup, beaker, Petri dish, tweezers, scalpel, surgical blade, hand sprayer, pipette, stirring rod, culture shelf, Bunsen burner, 60 mesh filter and documentation tool.

Planting materials used are tubers of shallot varieties lembah palu. Other materials used include MS medium, seaweed extract (*Caulerpa* sp.), BAP, sterile distilled water, sucrose, phytagel, 70% alcohol, detergent, bactericide, fungicide and Bayclin (bleach contains 5.25 % NaOCl).

Research protocol: This study was prepared based on a Complete Randomized Design (CRD) with two factors. The first factor was the concentration of seaweed extract consisting of three levels ie 0 (R_0), 20 (R_1) and 40% (R_2). The second factor is the concentration of BAP, which consists of three levels i.e. without BAP (B_0), 2 ppm BAP (B_1) and 4 ppm BAP (B_2). Thus there are nine combinations of treatments. Each treatment combination was repeated three times, so there were 27 experimental units. Each experimental unit was planted with two explants, resulting in a total sample of 54. The observation parameters included the average on the time of the appearance of buds, shoot numbers and shoot lengths were measured at 6, 12 and 18 Days After Planting (DAP). 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.

Seaweed extraction: The dried seaweed is blended until the powder passes the 60 mesh filter. The finely ground powder is then incubated at a temperature of -20°C for 20 min. Furthermore, for 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 hrs. 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[11].

Sterilization explants: The shallot bulbs are burned (passed on a Bunsen flame), then the outer shell is opened and the next layer of skin. The tuber is washed with running water until clean, then shaken in a detergent solution at 90 rpm for 15 min. Then the tubers are rinsed with running water until free of foam, then rinsed with sterile aquades several times. After that, the tubers were shaken in a solution of fungicide and bactericide each 2 g L^{-1} for 24 hrs. Tuber then rinsed sterile aquades 3 times. Sterilization was continued on the laminar airflow using Bayclin 20% for 20 min, then rinsed with sterile aquades 3 times[7,13].

Planting: Planting conducted in a Laminar Air Flow Cabinet (LAFC). Before planting, about a quarter of the bulbs (tops) are separated. The base is then planted in the media as many as two tubers per bottle. Cultures maintained on the shelves with the source of lighting coming from TL lamps (40 watts), storage space temperature ranges from ±25°C[2].

RESULTS

The results showed that the interaction between seaweed extract and BAP treatment significantly affected the time to shoot appear the number of shoots (Table 1) and the length of the shoot (Table 2).

The parameters at the time of shoots appear (Table 1), treatment without adding any seaweed extract and without adding BAP was the slowest shoot appear treatment (4.17 days), while the fastest one (2.33 days) was treatment with without adding seaweed extract and adding 4 ppm of BAP, a similar result to treatment with adding 40% seaweed extract and without adding any BAP. This indicates that by adding seaweed extract at concentration 40% on to medium no need to add any more BAP, similarly adding 4 ppm BAP on to medium no need to any more seaweed extract to stimulate the shallot shoot appear faster.

Shallot treated with 20% seaweed and without any BAP on medium produced the highest number of shoots (2.67) at 6, 12 and 18 DAP (Table 1). However, this result was not significantly different from shallot treated with 20% seaweed and 2 ppm BAP (2.17)and shallot treated with 40% seaweed extract and 4 ppm BAP (2.17) at 6 DAP. This result also similar to shallot growing at 12 and 18 DAP.

Mean shoot lengths 6, 12 and 18 DAP (Table 2) showed that shallot treated with 20% seaweed extract and without BAP produced the highest shoot length 4.48, 8.97 and 10.13 cm, respectively. While the lowest shoot length at 6 and 12 DAP were 0.65 and 1.73 cm respectively occurred in the shallot treated with 40% seaweed and without BAP and at 18 DAP 2.65 cm occurred in shallot treated with without seaweed and 4 ppm BAP (Table 2).

Table 2: Average length shoot at 6, 12 and 18 DAP

	Length Shoots (cm)		
Treatments	6 DAP	12 DAP	18 DAP
R_0B_0	1.30^{ab}	4.91^{b}	7.46^{bc}
R_0B_1	3.38^{c}	6.65^{c}	7.89^{bc}
R_0B_2	1.06^{ab}	2.33^{a}	2.65^{a}
R_1B_0	4.48^{d}	8.97^{d}	10.13^{c}
R_1B_1	3.78^{cd}	7.45^{cd}	8.85^{bc}
R_1B_2	1.95^{b}	4.57^{b}	7.58^{bc}
R_2B_0	0.65^{a}	1.73^{a}	2.90^{a}
R_2B_1	2.45^{b}	5.92^{bc}	7.61^{bc}
R_2B_2	1.12^{ab}	3.32^{a}	6.01^{b}

Different letters indicate significant differences between treatments, DAP: Day after planting, R_0B_0: No seaweed and no BAP, R_0B_1: No seaweed and 2 ppm BAP, R_0B_2: No seaweed and 4 ppm BAP, R_1B_0: 20% seaweed and no BAP, R_1B_1: 20% seaweed and 2 ppm BAP, R_1B_2: 20% seaweed and 4 ppm BAP, R_2B_0: 40% seaweed and no BAP, R_2B_1: 40% seaweed and 2 ppm BAP and R_2B_2: 40% seaweed and 4 ppm BAP

Table 1: Average number of shoots and time of shoots appear

		Number of Shoots		
Treatments	Time of shoot appear(days)	6 DAP	12 DAP	18 DAP
R_0B_0	4.17^{b}	1.50^{a}	1.50^{a}	1.50^{a}
R_0B_1	2.83^{ab}	1.67^{a}	1.67^{a}	1.67^{a}
R_0B_2	2.33^{a}	1.33^{a}	1.33^{a}	1.33^{a}
R_1B_0	3.00^{ab}	2.67^{b}	2.67^{b}	2.67^{b}
R_1B_1	3.00^{ab}	2.17^{ab}	2.17^{ab}	2.17^{ab}
R_1B_2	2.67^{a}	1.33^{a}	1.50^{a}	1.50^{a}
R_2B_0	2.33^{a}	1.50^{a}	1.67^{a}	1.67^{a}
R_2B_1	2.83^{ab}	2.17^{ab}	2.17^{ab}	2.17^{ab}
R_2B_2	2.83^{ab}	1.33^{a}	1.83^{ab}	1.83^{ab}

Different letters indicate significant differences between treatments, DAP: Day after planting, R_0B_0: No seaweed and no BAP, R_0B_1: No seaweed and 2 ppm BAP, R_0B_2: No seaweed and 4 ppm BAP, R_1B_0: 20% seaweed and no BAP, R_1B_1: 20% seaweed and 2 ppm BAP, R_1B_2: 20% seaweed and 4 ppm BAP, R_2B_0: 40% seaweed and no BAP, R_2B_1: 40% seaweed and 2 ppm BAP and R_2B_2: 40% seaweed and 4 ppm BAP

DISCUSSION

Results showed that the addition of 20% seaweed extract on MS base medium, without BAP, generally gave better bud initiation with a higher average (2.67) and shoot length (10.13 cm). The response is better than the growth figures produced by using benzyl amino purine growth regulators. The use of seaweed extract as a source of organic and nutrient material has been done for a long time[14,15], known as a soil conditioner[16]. Many seaweed-based products have been used as additional nutrients[17] and as biostimulants[18] or organic fertilizers (biofertilizer)[19] to increase plant growth and yield[20-22]. From these results, it is indicated that seaweed (*Caulerpa* sp.) also contains substances that can improve the mechanism of plant growth[23]. Although the working mechanisms can not be fully understood, a strong suspicion of good substances at low concentrations can be demonstrated in this study, where lower concentrations (20%) give better growth than higher concentrations (40%). This result is reinforced by the statement of Crouch and van Staden[24], that seaweed extract is bioactive at low concentrations, diluted by a ratio of 1: 1000 or more. Some previous researchers generally state as biostimulants[25], components contained in seaweeds such as macro and micronutrients[18,26], amino acids, vitamins, cytokines, auxins and abscisic acids affect plant cell metabolism by improving growth and yield[27-30].

The presence of a debilitating effect of a combination of treatments containing BAP and seaweed extracts may be an indicator that high cytokinin content such as BAP at *Caulerpa* sp. Negative effects of concentration accumulation can be suspected as the cause as it is generally known that at high concentrations, growth regulators are inhibiting growth[12]. Concentrations that exceed the optimum limit are characteristic of slowing growth, at higher concentrations, growth can experience obstacles beyond control. However, it cannot be concluded whether the tested *Caulerpa* sp contains growth regulators from the cytokinin or auxin group as no analysis of these components, was carried out yet. Some types of seaweed contain auxin, such as Indoleacetic Acid (IAA)[10] and several other types contain cytokinins[31].

CONCLUSION

Seaweed extract of *Caulerpa* sp. 20% better initiate shallot shoots than other treatments and BAP concentration of 2 ppm can stimulate the initiation of buds but result in lower number and length of shoots compared seaweed extract at 20% concentration.

SIGNIFICANCE STATEMENT

This study discovered the potential effect of seaweeds on the growth of shallot *in vitro* that can be beneficial for researchers and growers to produce shallot seedling. This study will help the researchers to uncover the critical areas of horticulture that many researchers were not able to explore. Thus a new theory on using natural seaweed to replace the use of synthetic plant growth regulator to produce *in vitro* shallot seedling may be arrived at.

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