Effect of green bean sprout extract on in vitro shoot multiplication of taro *Colocasia esculenta* L. var. *antiquorum*

A I Latunra¹, S R S Anggraini¹, M Tuwo¹ and Baharuddin²

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin, Makassar, South Sulawesi, 90245, Indonesia.
²Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Hasanuddin, Makassar, South Sulawesi, 90245, Indonesia.

E-mail: baharunhas@yahoo.com

Abstract. Japanese taro *Colocasia esculenta* (L.) Schott var. *antiquorum* is a good export-valued food commodity and is being developed in Indonesia. Lack of availability of taro seedling is one of a limiting factor in development of Japanese taro. In vitro propagation is an alternative to obtain seeds in large quantities and in a relatively short time. In vitro culture is very dependent on composition of media used, especially growth regulators. This study aims to determine effect and to find optimum concentration of green bean sprout extracts *Phaseolus radiatus* in shoot multiplication of Japanese taro through in vitro. Plantlet were derived from cultures with ± 3 weeks of age. MS media was supplemented with different concentrations of green bean sprout extracts for shoot multiplication experiments. The concentration was used is 0; 0.5; 1; 1.5; 2 ppm and MS + NAA 1.5 ppm + 1.5 ppm BAP as a comparison of synthetic hormones. The parameters observation were number of shoots, wet weight and percentage of live explants. The results showed that MS media with supplemented of 1.5 ppm green bean sprout extract was optimum concentration in Taro shoot multiplication. Therefore, green bean sprout extract can be used as natural growth regulator in media of Taro shoot multiplication.

1. Introduction

Japanese taro or Satoimo *Colocasia esculenta* (L.) Schott var. *antiquorum* is a good export-valued food commodity and is being developed in Indonesia. As many as 50% of Japan's population of ± 120 million people consume Japanese taro as a staple food besides rice. So that at this time Japan's needs are reaching ± 360,000 ton per year while production capacity in Japan continues to decline to 250,000 tons per year, due to land tightness and climatic factors which make it impossible to farm. Japanese taro also has other advantages, namely as a source of calcium and high calories, but low carbohydrates making it can be consumed as food and good for diabetics. In addition, Japanese taro contains hyalitrotic acid which is a collagen-forming compound, one type that slows the skin's aging process [1].

Japanese taro cultivation has constraints including limited land, inadequate cultivation systems, lack of soil fertility, higher pest attacks and plant disease problems, as well as limited numbers of available seeds. Japanese taro tubers for seeds have been imported from China, therefore, there are some risks including received tubers that have rotten up to 25%, bringing dangerous pests from China, tubers fail to be sown, and because of imports, the price of tubers is more expensive. To overcome these obstacles, in vitro propagation is an approach that can be done to obtain high-quality Japanese taro plant seeds [2]. The limited
availability of planting material has caused problems to arise. To overcome this problem, in vitro propagation can be an alternative to taro planting material available to farmers. In vitro culture can be produced in large quantities of seeds and in a relatively short time [3]. The success in using the in vitro method depends on the media used. Culture media that meet the requirements are media that contain macro nutrients and micro nutrients, vitamins, growth regulators, and glucose in certain levels and proportions. Of the many types of media, Murashige and Skoog (MS) contain a good amount of nutrients to meet the needs of the large number of plant cells in culture [4].

Research has been carried out for multiplication of shoots and micro tubers in taro plants using natural ingredients namely Phaseolus radiatus green beans and chemicals as a comparison, among others BAP (Benzyl Amine Purin) and NAA (Naphthalene Acetic Acid). Plant bioactive compound extraction can be done on green bean sprouts. Green bean sprouts are a type of vegetable that is commonly consumed, easily obtained, economical, and does not produce compounds that have toxic effects. Green bean sprout extract had a concentration of auxin growth regulator of 1.68 ppm, gibberellins 39.94 ppm, and cytokinin 96.26 ppm [5]. Multiplication of Japanese taro subculture in vitro is expected to be more effective for producing shoots. Therefore, it is necessary to conduct research to determine the ability of natural ingredients from green bean sprouts Phaseolus radiatus to be a reference in the selection of natural ingredients and fermentation results as organic growth regulators to substitute synthetic growth regulators in tissue culture containing organic compounds to outgrowth multiplication of Japanese taro subculture in vitro.

2. Materials and Methods

2.1. Preparation of green bean sprout extract solution

Green bean sprouts that will be extracted were previously germinated by soaking the seeds for 24 hours, then drained and spread over a tray covered with a damp towel, preserved with moisture by sprinkling water as needed and placing it in a dark place. Two days later, green bean seeds began to germinate. Green bean seeds that have germinated mixed with distilled water with a ratio of 2:1, little by little while blending 500 grams of mung bean sprouts in 100 ml of water. Blended green bean sprouts was filtered using a filter cloth to obtain the extract. The extract was added to the treatment media with a concentration of 1; 1.5; 2 ppm; 0 ppm as a control and media with growth regulator BAP 1.5 ppm and NAA 1.5 ppm as a comparison. The culture was kept under white light continuously at 25 °C. Observations were carried out every week for 4 weeks.

2.2. Media preparation

Preparation of MS media was done by mixing the composition of MS media, namely stock solution consisting of stock solutions A, B, C, D, E, F and vitamins as needed. The mixture of stock solution was put into an erlenmeyer, then added distilled water up to 1 litre volume. Then the solution was added with 30 g/l of sugar and the acidity of the solution was measured using a pH meter. The pH of the media was set to 5.7. After measuring the pH, the solution was added to agar and boiled. For the treatment used is a mixture of MS media ingredients with the hormone BAP, NAA, and green bean sprout extract. Mixing was done according to the concentration of each needed. Subsequently, the media was sterilized in an autoclave at a pressure of 17.5 Psi, temperature of 121°C for 15 minutes.

2.3. Planting

Planlets planted previously were removed from bottles and then sub cultured using tweezers after which the shoots were separated one by one using a scalpel. Then the shoots that have almost the same shape were planted in the subculture using a sterile scalpel. Explants were implanted into media bottles according to treatment, each culture bottle consisted of 4 explants. Culture bottles were placed on the culture rack in the culture chamber with the temperature of the culture room being used + 20-22 °C and good light intensity.
2.4. Observation
Observed parameters include percentage of live explants (living explants are explants that are able to form new shoots), number of shoots, wet weight of shoots. All parameters were calculated at the end of the study.

2.5. Data analysis
The analysis used were qualitative and quantitative analysis. Qualitative analysis included visual data presented descriptively. Quantitative analysis included data on wet weight of shoots, number of shoots and percentage of live explants. Quantitative data were analyzed using analysis of variance (ANAVA) and followed by DMRT test at 5% level. The design of this study used a single complete randomized design (CRD) with a combination of treatments used on the media, namely:

- A0: 0 ppm (control)
- A1: MS media + 0.5 ppm green bean sprout extract
- A2: MS media + 1 ppm green bean sprout extract
- A3: MS + 1.5 ppm green bean sprout extract
- A4: MS medium + 2 ppm green bean sprout extract
- A5: MS media + 1.5 ppm NAA and 1.5 ppm BAP (comparison)

3. Results

3.1. Growth in number of shoots of Colocasia esculenta (L.) Schoot var. antiquorum
Statistical analysis of the ANAVA test show that the administration of green bean sprout extract with several concentrations had a significant difference in the growth of Japanese taro shoots from 1 week after planting (WAP). The results of the statistical analysis of the Duncan 5% test, the number of shoots at several treatment concentrations are presented in Figure 1.

Figure 1. Number of shoots of Colocasia esculenta (L.) Schoot var. antiquorum

Figure 1 above shows that the number of taro shoots in the treatment without the administration of green bean sprout extract (A0) was significantly different from the treatments of A1, A2, A3, A4, and A5 at 3 WAP observations. The control treatment (A0) and the comparative treatment (A5) had a significant effect with the treatment of green bean extract (A1, A2, A3 and A4). At the end of the observation that was 3 WAP, the treatment which was given mung bean sprout extract concentration of 1.5 ppm (A3) produced the highest number of shoots compared to other green bean sprout extract treatments with an average number of shoots growing at 1.75.
3.2. *Wet weight of shoots of Colocasia esculenta (L.) Schoot var. antiquorum*

Statistical analysis of the ANAVA test show that there were differences in the effect of six treatments carried out on wet weight of shoots of the Japanese taro *Colocasia esculenta* from observation 4 (WAP). The results of the statistical analysis of the Duncan 5% test, measurements of wet weight of shoots of Japanese satoimo *Colocasia esculenta* at several treatment concentrations are presented in Figure 2.

![Wet weight of shoots](attachment:figure2.png)

**Figure 2.** Wet weight of shoots of *Colocasia esculenta (L.) Schoot var. antiquorum*

Based on figure 2 above, the wet weight of Japanese taro shoots in each treatment of green bean sprout extract with A0 concentration was significantly different from all treatments from 3 WAP observations but A5 with synthetic growth regulators gave a significant effect on the administration of 1 ppm (A1) green bean sprout extract, 0.5 ppm (A2), 1.5 ppm (A3) and 2 ppm (A4). The wet weight of shoots in the treatment of 1.5 ppm (A3) green bean sprouts extract was the heaviest that was 0.54 grams compared with the administration of green bean sprout extract at a concentration of 1 ppm (A1), 0.5 ppm (A2), 1.5 ppm (A3) and 2 ppm (A4). The treatment of synthetic ppm 1.5 ppm NAA, and 1.5 ppm BAP also had the heaviest wet weight compared to all treatments given green bean sprout extract which was 1.34.

3.3. *Percentage of live explant*

Percentage of live explants is presented in Figure 3.

![Percentage of live explants](attachment:figure3.png)

**Figure 3.** Percentage of live explants of *Colocasia esculenta (L.) Schoot var. antiquorum*

Based on the final results of observations of 3 WAP, most of the live shoot explants were 53.33%
and explants of dead or contaminated shoots were 16.66%. In addition to dying from being contaminated by fungus, some Japanese taro shoots die due to 30% browning.

4. Discussion

4.1. Number of shoots
Statistical analysis of ANOVA test showed that the treatment of green bean sprout extract with different concentrations showed a significant effect on the parameters of number of shoots and wet weight. The increase in the number of shoots is one part of the plant tissue culture stage, the multiplication stage. The number of shoots is the most important factor in plant multiplication in tissue culture. Calculation of the number of shoots performed on all shoots that appear in explants both shoots originating from the elongation of shoots and adventitious shoots [6].

Based on the results of statistical analysis Duncan test at a level of 5% conducted, it showed that the administration of green bean sprout extract significantly affected the growth of the number of Japanese taro shoots. In observation of 3 WAP, green bean sprout extract at a concentration of 1.5 ppm (A3) had the highest number of shoots from the extract concentration of other green bean sprouts and the prospective shoots from the taro that grew since observation of 2 WAP were seen. This is because green bean sprout extract has a high cytokinin content. Cytokines are growth regulators that can stimulate bud growth. Based on the results of the study [7] that green bean sprout extract was also very influential on the growth of the orchid with the highest concentration of 150 g/L. The treatment of green bean sprout extract with other concentrations (A1 and A4) was also able to increase the number of taro sprouts even though the results were not optimal, as well as the treatment that there was no addition of green bean sprout extract (A0) was able to spur growth in the number of shoots well. Vitamins are generally needed for plant growth, especially for plant tissues that are actively growing. Vitamins in plants are needed as a catalyst in various metabolic processes. Therefore, it can be suspected that the vitamin in MS media without the administration of green bean sprout extract is able to work optimally so that it can help in stimulating the growth process of Japanese satoimo taro shoots even without the addition of synthetic and natural hormones.

The treatment of green bean sprout extract administration at a concentration of 0.5 ppm (A2) did not have good shoots or died because at 3 WAP the shoots died due to browning. However, at 1 WAP and 2 WAP shoot growth at 0.5 ppm (A2) still survived and the growth of these shoots was well seen from the color of the buds which appeared fresh green. This is because there is a tendency for the cytokinin hormone found in green bean sprouts which can increase the green color of the shoots. Based on figure 2 above it is known that the treatment given synthetic growth regulator 1.5 ppm NAA and 1.5 ppm BAP has the most growth in the number of shoots and more visible candidates for shoots that grow taller than the treatment of green bean sprout extract. Cytokines added with auxin together cause cells to divide rapidly. Cytokinins growth regulators play a role in cell division and differentiation of certain tissues in shoot bud formation and root growth.

4.2. Wet weight of shoots
Growth of the number of shoots also affects the increase in the wet weight of Japanese taro shoots. Based on the results of the statistical analysis of the Duncan test at a level of 5% showed that the administration of green bean sprout extract significantly affected the wet weight of Japanese taro Colocasia esculenta (L.) Schoot. In observation of 3 WAP administration of green bean sprout extract at a concentration of 1.5 ppm (A3) had the highest wet weight of 0.54 grams compared to the sprouts extract treatment of a concentration of 0.5 ppm, 1 ppm and 2 ppm. This is because the administration of green bean sprout extract can also stimulate the growth and enlargement of explants. The treatments that were given synthetic growth regulator of 1.5 ppm NAA and 1.5 ppm BAP had the highest wet weight compared to the treatment of green bean sprout extract. Auxin also plays a role in the
absorption of water which will encourage cell lengthening and cell enlargement which can increase the wet weight of plants.

4.3. Percentage of live explants

Based on the final results of observations of 3 WAP conducted most of the live shoot explants were 53.33% (16 bottles) and explants of dead or contaminated shoots were 5 bottles of 16.66%. Besides dying from being contaminated by fungi, a subculture of Japanese satoimo taro buds also died because the buds experienced 9 bottles of browning (30%). Contamination began on day 3 until death. It is known that the contaminated bud subculture is characterized by the growth of fungi in the culture. This contamination occurs only at the growth stage, where at the beginning of planting there is no contamination, even contaminated cultures only form buds and spread throughout the surface of the media. Contamination in this study was characterized by the presence of white fungal hyphae found on the surface of the media and spreading on the culture. Apart from dying due to contamination by fungus, some cultures also die from browning. Browning began to occur on the 17th day and death in explant cultures was less able to absorb food, so the culture withered and turned brown.

In the treatment of green bean sprout extract concentration of 0.5 ppm there were no live explants. The shoot died from browning and was contaminated in 3 WAP. Taro plants are thought to have a high secondary metabolite content. Secondary metabolites produced from this phenolic group can stimulate browning in explants. This phenol compound oxidizes due to injury to the explant. Phenol compounds that are oxidized to the media result in the explants being unable to take nutrients from the media so that the explant growth is inhibited and eventually the explants will die. Kavitha [8] stated that phenol compounds which appear in explants will be toxic to cells if in excessive concentrations, which will inhibit their growth. Production of phenol compounds which are limited to explants or callus can still be tolerated by explants, so that culture can still grow. However, if the phenol compound has caused browning on the planting medium, this can inhibit the growth of explants that result in culture death.

Browning in tissue culture is due to increased production of phenolic compounds which are followed by oxidation by the activity of the enzyme oxidase and its polymerization. Phenylalanine ammonia liase is one of the enzymes in phenylpropanoid that is very influential in the browning. One of the main causes of browning in in vitro culture is injury due to tissue cutting. These injuries stimulate stress and cause an increase in activity followed by phenylpropanoid production and cause browning [9]. The budding color variable indicates the formed chlorophyll content. The greener the shoots and leaves the higher the chlorophyll content. Chlorophyll functions in the process of photosynthesis. Observation of color of shoots was based on scoring 0-4. On observations 1-3 WAP the color of the buds formed was light green. The green color was found in the treatment of green bean sprout extract at a concentration of 0.5 ppm (A1), 1.5 ppm (A3), 2 ppm (A4). This is because there is a tendency for the cytokinin hormone found in green bean sprouts to increase the green color. According to Wahidah and Hasrul [10], cytokines can encourage the formation of chlorophyll. But in the control treatment (A0) and growth regulator hormone (A5), the bud color is green. This is due to the work of BAP in encouraging the formation of chlorophyll. Cytokines can support the formation of chlorophyll while auxin works to inhibit it.

5. Conclusions

Green bean sprout extract can be used as a substitute for natural growth regulators which have a significant influence on the growth of shoots and wet weight of shoots Japanese taro Colocasia esculenta (L.) Schoot var. antiquorum. Green bean sprout extract with a concentration of 1.5 ppm is the most optimal concentration for growth and propagation of Japanese taro Satoimo Colocasia esculenta (L.) Schoot var. antiquorum in vitro.
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