Formation of potato micro tubers (*Solanum tuberosum* L.) by using BAP and coconut water in the *in vitro* culture

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Abstract: The use of BAP and coconut water can support the process of forming potato micro tubers *in vitro*. The research was conducted at the laboratory of tissue culture, Trial Garden of Vegetable Research Center, Berastagi, North Sumatera. This study used a completely randomized 5×4 factorial design. The first factor was BAP concentration at five levels of concentration, i.e 0; 2.5; 5.0; 7.5 and 10 mg/L and the second factor was coconut water concentration at four levels of concentration, i.e 0; 75; 150 and 225 mL/L. The results showed that the best potato explant growth was found at a combination of 0 mg/L of BAP and 0 mg/L of coconut water. The time of potato micro tubers and the fastest number of potato micro tubers were found in the treatment combination of 5 mg/L BAP concentration and 75 mL/L coconut water of coconut water.

1. Introduction

Potatoes (*Solanum tuberosum* L.) are tubers of five important food commodities in the world, besides wheat, corn, sorghum, and rice. The needs of the market demand for potato in Indonesia has increased every year, but instead of potato production has decreased every year. Potato production in 2016 amounted to 1,213,041 tons while in 2017 it reached 1,164,738 tons [1].

In general, potato tuber propagation is done by asexual way to produce potato tubers that are not free from plant-disturbing organisms (PDO) such as bacteria and viruses that cause a decrease in potato tuber production. One of the causes of the decline in potato production in Indonesia is the low quality of potato tubers caused by bacterial wilt, nematodes, and viruses (PVX, PVY, and PVLR) so that potato tubers attacked by PDO are often used by farmers as seeds to be planted repeatedly [2].

Efforts can be made in obtaining quality potato seedlings to increase potato production in Indonesia by using tissue culture techniques in potato tuber *in vitro* [3]. The use of potato micro tubers derived from propagation *in vitro* has the advantage of producing potato tubers that are free of disease, uniform with its parents, seed supply is not season dependent and can be adapted to the right planting season, can use diverse cultivars and have adapted to the local environment, as well as economic value in storage and transportation [4].

Formation of potato micro tubers is a complex process and can be influenced by genotypes, growth regulators (PGRs), growth nutrients, photoperiods, temperature and increased carbohydrates [5]. PGRs are chemical compounds that regulate various processes in the plant and one of the keys to success in regulating physiological and biochemical processes in potato. PGRs are potentially capable of providing opportunities for growth and development of potatoes. Some studies suggest that PGRs such as cytokines give the best effect to the formation of potato tuber [6].
PGRs used in the formation of potato micro tuber are cytokinins and coconut water. The use of cytokines and coconut water aims to stimulate the formation of shoots, stimulate cell division and enlargement, and tuber formation by stimulating the activity of starch synthesis. The type of cytokinin used in stimulating the growth and development of shoots in vitro culture is 6-benzylaminopurine (BAP) [7]. In addition to BAP, the addition of coconut water into MS media is expected to replace cytokinins and auxins for the formation of potato micro tubers in vitro. Coconut water contains 94% water, amino acids, organic acids, vitamins, sugar, alcohol sugar, lipid and N-diphenyl urea which have activities such as cytokinins and auxins [8].

[4,9] showed that an increase in BAP concentration of 5 mg/L could accelerate the formation of potato micro tubers in vitro. [10] have observed that the use of BAP with a concentration of 5 mg/L can provide the best results for the formation of potato micro tubers in the number of tubers, tuber weights and harvest index of potato micro tubers. [11] have observed that the provision of 150 mL/L coconut water with different levels of maturity of the coconut can encourage vegetative growth in potato plantlets. The types of green early maturing and yellow early maturing coconut also have a similar effect on stimulating plantlet growth. The purpose of this study was to determine the effect of the interaction of BAP concentration and coconut water concentration on the formation of potato micro tubers in vitro.

2. Materials and Methods
2.1 Materials
Murashige and Skoog (MS) media, BAP, coconut water, potato plantlets, HCl, NaOH, 70% and 96% alcohol, tween 20, HgCl, NaOCl, agar, culture bottles, autoclaves and laminar air flow cabinet (LAFC).

2.2 Methods

2.2.1 Experimental Design. The study was conducted at the Tissue Culture Laboratory, Trial Garden of Vegetable Research Center, Berastagi, North Sumatera. The study lasted from February to June 2018. Research using completely randomized design with two treatment factors, namely the BAP concentration (0 (B0); 2.5 (B1); 5.0 (B2); 7.5 (B3) and 10 (B4) mg/L) and coconut water concentration (0 (A0); 75 (A1); 150 (A2) and 225 (A3) mL/L).

2.2.2 Preparation of Plantlets. In vitro potato plantlets were collected from the Tissue Culture Laboratory, Trial Garden of Vegetable Research Center, Berastagi, North Sumatera. Those potato plantlets were germinated, grown and cultured on MS medium. The container was incubated at 25 ± 2øC under 16 hours of light at an intensity of 2.5 × 10² lx. The source of light was fluorescent tubes. Within four weeks, the plantlets were ready for further culture to tuber induction. Plantlets from those plantlets were used in this experiment.

2.2.3 In vitro Culture Condition. After 4 weeks, buds sprouted into plantlets having 5–7 nodes. Single node cuttings (1.5 cm, with leaves and apical meristem) were excised and used as plantlets (Momena et al., 2014). The potato plantlets were placed in a plastic Petri dish both containing Murashige and Skoog (MS) basal medium with BAP concentration and coconut water concentration at pH 5.8, sucrose 30 g and agar 7 g/L for in vitro tuberization.

2.3 Statistics Analysis
Data processing was tested using the F test at a level of 5%, if there were treatments that had a significant effect, further tests would be carried out using the DMRT test at a level of 5%. Data from each treatment were analyzed separately using SPSS version 20.
3. Results and Discussions

3.1 Growth of plantlets

The high plantlet of potatoes at 3 weeks after induction (WAI), the interaction of BAP concentration of 0 mg/L (B₀) with coconut water concentration 225 mL/L (A₁) which increased significantly increased, interaction of BAP concentration of 2.5 mg/L (B₁) with an increased coconut water concentration causing a decrease in the height of potato plantlets, at the interaction of BAP concentration of 5.0 (B₂) and 7.5 (B₃) mg/L with increased coconut water concentration was not significantly different from the height of potato plantlets, where as the interaction of BAP concentration of 10 mg/L (B₄) with increased coconut water concentration of 150 (A₂) and 225 (A₃) mL/L significantly increased in the height of potato plantlets. At 5 WAI, the interaction of BAP concentrations 0 (B₀), 2.5 (B₁), 5.0 (B₂) and 7.5 (B₃) mg/L with increasing coconut water concentrations were not significantly different from high potato plantlets, while the interaction of BAP concentration of 10 mg/L (B₄) with increasing coconut water concentration are150 (A₂) and 225 (A₃) mL/L significantly increased in the high of potato plantlets. At 7 WAI, the interaction of BAP concentration of 0 mg/L (B₀) with a significantly increased concentration of coconut water causes a decrease in the height of potato plantlets, in the interaction of BAP concentration of 7.5 mg/L (B₃) with a concentration of coconut water that is increased, which is 75 mL/L (A₁) significantly increased potato explant height, whereas the interaction of BAP concentrations of 2.5 (B₁), 5.0 (B₂) and 10 (B₄) mg/L with increased coconut water concentration not significantly different from potato explant height. The highest of potato plantlets at 7 WAI was found in the combination treatment of BAP concentration 0 mg/L (B₀) with concentration of coconut water 0 mL/L (A₀) was 14.06 cm (Figure 1).

![Figure 1. Potato plantlets height due to BAP and coconut water](image)

Total leaf plantlets of potato on 3.5 and 7 WAI showed that the interaction of BAP concentration of 0 mg/L (B₀) with increasing concentration of coconut water that causes the number of leaf plantlets of potatoes decreased, the interaction of BAP concentration of 2.5 (B₁), 5 (B₂) and 7.5 (B₃) mg/L with the increasing concentration of coconut water no significant effect on the amount of leaf plantlets of potatoes, while the interaction of BAP concentration of 10 mg/L (B₄) with a concentration of coconut water increases real increased numbers of leaf plantlets of potatoes. The highest number of potato plantlet leaves at 7 WAI was found in the combination treatment of BAP concentration 0 mg/L (B₀) with concentration of coconut water 0 (A₀) mL/L was 20.13 strands (Figure 2).

The highest number of potato explant buds at 3 and 5 WAI was found at a BAP concentration of 10 mg/L (B₄) which was significantly different from BAP concentrations of 2.5 (B₁), 5 (B₂) and 7.5 (B₃) mg/L but not significantly different from the concentration of BAP 0 mg/L (B₀). At 7 WAI, the number of potato explant buds tended to be more likely to be found at a BAP concentration of 10 mg/L (B₄) even though it was not statistically significant different from other BAP concentrations. The highest number of potato explant buds at the age of 7 WAI was found at a BAP concentration of 10 mg/L (B₄), namely: 2.95 units (Table 1).
Figure 2. The number of leaf plantlets of potatoes due to the provision of BAP and coconut water

Table 1. Average number of potato plantlets buds at 3, 5 and 7 WAI to the giving of BAP

| WAI | BAP Concentration (mg L⁻¹) | 0 (B₀) | 2.5 (B₁) | 5 (B₂) | 7.5 (B₃) | 10 (B₄) |
|-----|----------------------------|--------|----------|--------|----------|---------|
| 3   | ab                         | 0.70 cd | 0.43 ab  | 0.52 bc | 0.23 a   | 0.82 d  |
| 5   | a*b                       | 1.42 (1.36) | 1.27 (1.30) a* | 1.35 (1.32) a* | 0.97 (1.19) a* | 1.82 (1.50) b* |
| 7   | (1.27) a**                 | 2.33   | 2.37     | 2.68    | 2.22     | 2.95    |

Note: Numbers followed by the same letters on the same line are not significantly different at the 5% probability level (DMRT₀.₀₅), *: transformation using √ (x + 0.5)

Potato explant growth at 3, 5 and 7 WAI has increased specifically. The growth of potato plantlets for height and the best number of potato explants leaves were found in the interaction of BAP 0 mg/L (B₀) with coconut water 0 mL/L (A₀) at 7 WAI but the best number of potato explant buds were found in concentration of BAP 10 mg/L (B₄) at 7 WAI. It is suspected that the growth of plantlets of potatoes without giving BAP concentration and coconut water can support the growth and development of plantlets of potatoes as plantlets potato has a concentration of endogenous optimal for the process of cell division (cell differentiation), whereas the administration of the concentration of coconut water that rose to support the establishment buds and the occurrence of elongation in potato plantlets. If the addition of exogenous PGRs such as BAP and coconut water with a particular concentration in media MS can lead to increased concentrations of endogenous PGRs on potato plantlets that potato explant growth can take place more effectively and more optimal cell differentiation takes place against the increased growth of potato plantlets. The best BAP concentration can optimally support the growth of potato buds for the formation of potato micro tubers.

The combination of cytokinin and auxin contained in BAP and coconut water causes the triggering phase of morphogenesis and proliferation in the formation of shoots affected by the PGRs. The balance between cytokinin and auxin in the BAP with coconut water given in the media MS can regulate and control the growth of plantlets of potato plants, especially the formation of leaves, roots, shoots, callus and elongation at explant potato in vitro [12]. BAP is a PGRs cytokinin synthesis widely used in tissue culture because it has a role in stimulating the cell division, the formation of buds, the tip meristem proliferation and inhibit the formation of roots. Giving higher concentrations of BAP causes the number of buds that are formed more and more, but the growth of buds formed under go inhibition, because BAP act as cytokinesis and can not promote the growth of other organs [13].

The leaves have a close relationship with the process of photosynthesis, plant metabolism and nutrient absorption because the leaf is the main organ in the process of photosynthesis. The process of growth and development requires a PGRs such as auxin leaves contained in coconut water and BAP as cytokinin and other nutrients contained in MS media. Giving auxin derived from coconut water can affect leaf growth, especially the length of the network of vessels. The wider the leaf, the stomata will
increase. Stomata play an important role in the absorption of nutrients and substances needed in plant metabolic processes to produce assimilates. The use of cytokinins can directly affect photosynthesis in potato plants in vitro such as synthesis and degradation of chlorophyll, chloroplast arrangement, electron transfer and enzyme activity [14].

The effect of cytokinins on tissue culture of potato plants can increase the polyperation of axillary buds and can inhibit apical dominance which causes the emergence of axillary buds. The number of shoots affects the number of nodes and micro potato tubers are formed. It becomes a positive indication in the formation of potato microtuber. Each explant potatoes used in the process of tuber formation in vitro consists of a number of buds. In there each shoots are axillary buds that can be pushed to form buds, bulbs in vitro depending on the composition of the growth media and environmental conditions [15].

3.2 For In Vitro Tuberrization

The interaction of BAP 0 mg/L (B0) concentration with coconut water concentrations that increased 75 (A1) to 225 (A3) mL/L was significantly different and accelerated the formation of potato micro tubers. In the interaction of BAP concentrations of 2.5 (B1), 5.0 (B2) and 10 (B3) mg/L with increased coconut water concentration was not significantly different to the time of the formation of potato micro tubers, while the BAP concentration of 7.5 mg/L (B3) by giving coconut water concentrations that increase 0 (A0) and 75 (A1) mL/L are significantly different from the time of formation of potato micro tubers. Coconut water concentrations 0 (A0), 75 (A1) and 225 (A3) mL/L by giving BAP concentrations that increase significantly cause the time of formation of long-formed potato microtubers, while the concentration of coconut water 75 mL/L (A1) with increased BAP concentration, which is 5 mg/L (B2), the formation of potato microtubers takes place more quickly. The fastest time to form potato microtubers was found in the combination treatment of BAP concentration of 5 (B2) mg/L with 75 (A1) mL/L coconut water, which was 19 days (Table 2).

| Table 2. Time of formation of potato microtubers (days) to the giving of BAP and coconut water |
|---------------------------------------------------------------|
| Time of formation of potato microtubers (days)              |
|                                                              |
| A0 (0 mL/L) | A1 (75 mL/L) | A2 (150 mL/L) | A3 (225 mL/L) |
| B0 (0 mg L⁻¹) |            |              |              |
| B1 (2.5 mg L⁻¹) | 38.8 Ac | 32.8 Ab | 40.0 Ab | 35.6 Ab |
| B2 (5.0 mg L⁻¹) | 23.6 Ab | 19.0 Aa | 23.2 Aa | 23.2 Aab |
| B3 (7.5 mg L⁻¹) | 34.2 Bbc | 37.2 Bb | 32.6 Abab | 20.4 Aa |
| B4 (10 mg L⁻¹) | 4.2 Ac | 31.2 Aab | 31.4 Aab | 31.0 Aab |

Note: Numbers followed by different letters (uppercase seen according to row and lowercase seen according to column) have a significant effect on the 5% test level (DMRT), ~: potato tubers do not form until 12 weeks of observation.

The interaction of BAP concentration of 0 mg/L (B0) with coconut water concentration of 150 (A2) and 225 (A3) mL/L was significantly different from the number of micro potato tubers and increasing the number of potato micro tubers formed, at BAP concentration are 2.5 (B1), 5 (B2) and 7.5 (B3) mg/L by giving increased concentrations of coconut water not significantly different to the number of potato tubers formed, while the concentration of BAP 10 (B4) mg/L by giving 225 mL/L (A3) coconut water concentration significantly increased the number of micro potato tubers formed. The number of potato micro tubers was more often found in the interaction of BAP concentration of 0 mg/L (B0) with coconut water concentrations of 150 mL/L (A2) and 225 mL/L (A3) which were 4.6 and 4.2 pieces (Figure 3).
Calculation of the time for the formation of potato micro tubers is needed to determine the appropriate treatment in accelerating the process of the formation of potato microtubers. The results of the analysis of BAP and coconut water interaction data show that there is a significant influence on the time of formation of potato microtubers. The fastest time to form potato microtubers was found in the combination of BAP 5 (B$_2$) mg/L with coconut water 75 (A$_1$) mL/L, while the higher number of tubers was produced by the combination of BAP 0 (B$_0$) mg/L with coconut water 150 (A$_2$) mL/L. It is suspected that the growth of potato plantlets without the addition of BAP and coconut water concentrations can support the growth and development of potato plantlets, because potato plantlets have optimal endogenous concentrations for cell division (cell differentiation). While the addition of BAP and coconut water exogenous with certain concentration can increase the concentration of endogenous on potato plantlets that potato explant growth can take place more effectively and lead to more optimal cell differentiation takes place against the increased growth of root plantlets of potatoes.

PGRs not only support the growth of potato plantlets but also can support the process of micro-potato tuber formation in vitro. Cytokines found in MS media such as BAP and coconut water can encourage the formation of potato micro tubers in buds and shoots from potato plantlets in vitro. Cytokines are contained in coconut water and BAP supposed to influence the metabolism of carbohydrates, causing the occurrence of micro-induction bulb. Cytokines play a role in stimulating cell division and organ formation. The cytokines present in BAP and coconut water have been shown to have considerable regulatory effects on the source-zinc relationship during the formation of potato micro tubers in vitro [16]. During the growth stage of the tuber, starch is synthesized and actively stored in the form of amyloplast in the tuber cell. The synthesis of high assimilation and entry into tubers has an important role in tuber growth. Significantly BAP and coconut water can change the total balance of plant biomass to potato micro tubers through shoot reduction [17].

The function of cytokines in the formation of tubers in vitro is to regulate the activity of cell division and form a new container as a result of assimilation in addition to regulating the rate of cell division and cell elongation. In the early stages of curing there is swelling and enlargement of the tubers which is a result of enlargement of cells that function as new storage cells. In the process of carbohydrate metabolism, BAP and coconut water play a role in the process of regulating the activity of enzymes that synthesize starch, especially enzymes of phosphorylase and flour synthetase [18].

In the induction of potato micro tubers in vitro, the production of potato micro tubers both in quality and quantity is influenced by temperature, composition of growth media and the quality of plantlet growth for induction of potato micro tubers. Potato plantlets obtain energy through the addition of sucrose and coconut water contained in MS media. The excess sucrose in MS media for potato plantlets is not converted into energy but will be stored into starch at the end of the stolon. Then Stolon will swell and form potato micro tubers [19].
4. Conclusion
Giving BAP treatment showed a significant influence on the micro-potato tuber induction. Coconut water is given very significant effect on potato micro tubers time that happened, but the effect is not significant on the number of micro-potato tubers are formed. Giving BAP 5 mg/L with 75 mL/L coconut water can accelerate the induction of potato micro tubers in vitro.

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