Potato tuber (*Solanum tuberosum* L.) formation due to the application of different concentrations of coconut water in *in-vitro*

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**Abstract:** This study apply coconut water to support the process of formation of potato tubers *in vitro*. The study was conducted at the laboratory of tissue culture, Experimental Garden of Vegetable Research Center, Berastagi, North Sumatera. The experiment used Completely Randomized Design with single treatment factor, the coconut water concentration of 0 ; 75 ; 150 and 225 ml L⁻¹. The results show that the application of coconut water provides a significant effect on the number, diameter and fresh weight of potato micro tubers, but the effect is not significant at the time of potato micro tuber formation. The application concentration of coconut water 225 mL L⁻¹ affected the potato micro tuber formation in *in-vitro*.

1. Introduction

As horticultural commodities in food system, the efforts to develop potatoes were carried out through mass intensification and extensification programs. The potato production centers in Indonesia are located in Southern Sulawesi, Northern Sumatra, Western Sumatera, Jambi, Western Java, Eastern Java and Central Java [1]. The productivity of potato in Indonesia from 2015-2019 fluctuated and reached 18.20-19.27 tons ha⁻¹ [2].

The use of continuous potato tubers can lead to lower the quality and productivity of potato. The low quality of potato seeds affects quality and productivity of potatoes due to degenerative diseases virus accumulated during vegetative propagation. The potato seed tuber appearance agronomically highly dependent on seed dormancy and the physiological age of potato seed tubers. The use of seed potatoes repeatedly resulting in loss of vigor and increased disease. Traditional potato storage as storing seed potatoes in the basement and in the darkroom can result in decreased seed weight, overgrowth, as well as pests and diseases [3].

An alternative effort to increase the productivity and quality of potato seeds is from *in vitro* cultures. The utilization of potato *in vitro* provides benefit both in terms of storage, transportation and production of potatoes. *In vitro* potato have morphological and biochemical characteristics that were different compared to tubers produced in the field [4]. Potato tubers derived from *in vitro* culture produce seed potato tubers that are pathogen free. *In vitro* potato tubers were used in producing potato seed genetic conservation, analyzing the metabolism of physiological and biochemical and the expression of genes
associated with the formation and growth of potato tubers as a source for transformation of genetic potato [5].

Factors that influence the success of in vitro potato formation are the intensity and quality of light, hormones, culture media, humidity, temperature, explant orientation and ventilation. Some studies used organic materials such as coconut water to induce in vitro potato tubers. The application of coconut water as the growth regulator in potato tubers formation in in vitro has been widely observed. Using coconut water as a natural plant growth regulators (PGRs) is thought to have activity such as cytokinins that play a role in cell division and encourage the formation of potato tuber of in vitro [6].

The application of coconut water as much as 100-200 ml L\(^{-1}\) in the formation potato tuber of in vitro promote the growth of potato [7]. The utilization of coconut water as much as 150 ml L\(^{-1}\) was able to increase the growth and formation of in vitro potato tubers effectively [8]. The concentration of coconut water 250 ml L\(^{-1}\) support the growth of potato plantlets in vitro [9]. The utilization of coconut water as PGRs to increase the growth and formation of potato tubers in vitro has not been explored at this time. This study was conducted to determine the effect of coconut water in Murashige and Skoog (MS) media on the formation potato tuber of in vitro.

2. Materials and Methods
The materials used in this study were explants of potato cultivar Granola, bacto agar, Murashige and Skoog (MS) media with some modification, alcohol, coconut water, aquadest and sucrose, while the main equipments were laminar air flow cabinet, pH meter, autoclave, magnetic stirrer and hot plate.

2.1. Experimental Design
The study was carried out from March to June 2018 at The Laboratory of Tissue Culture, Experimental Garden of Vegetable Research Center, Berastagi, North Sumatera. The Completely Randomized Design (CRD) has been applied with single treatment factor, the coconut water concentration of 0 (A\(_0\)); 75 (A\(_1\)); 150 (A\(_2\)) and 225 (A\(_3\)) ml L\(^{-1}\).

2.2. Shoot and Tuber Induction
MS media was applied along with hydro cloric acid/HCl (0.5 mg L\(^{-1}\)), pyrodoxine (0.1 mg L\(^{-1}\)), thymine (0.1 mg L\(^{-1}\)), glycine (2 mg L\(^{-1}\)), myoinositol (100 mg L\(^{-1}\)), nicotinic acid (0.1 mg L\(^{-1}\)), sugar (30 g L\(^{-1}\)), Ca-P (2 mg L\(^{-1}\)) and coconut water concentration of 0 (A\(_0\)); 5 (A\(_1\)); 150 (A\(_2\)) and 225 (A\(_3\)) ml L\(^{-1}\). The pH media is 5.6-5.8 with HCl or NaOH (0.5 N) before adding agar (8 g L\(^{-1}\)), and be heated until all evenly mixed. After the agar melted, the MS media poured into the culture bottle (25 ml each). The culture bottles containing the MS media were autoclaved for 30 minute at 121°C and 15 Psi. The induction of shoots was done by planting 5 cuttings in the MS media with 2 nodes each, coming from 6-7 week old potato plantlets in aseptic conditions. The shoot and tuber were incubated for 12 weeks in the incubation room with a photoperiod of 16 hours and 17-20±2°C. The culture bottles containing the explants were closed with the black cloth for 8 weeks at 21-2°C. The bottles were observed to check tuber formation and plantlet maintenance every day.

2.3. Measured Variables
The measured variables in this study included time of potato micro tuber formation (days), number of potato micro tubers, diameter of potato micro tubers, fresh weight of potato micro tubers (mg) at 12 weeks.

2.4. The Data Analysis
Data were analyzed with analysis of variance and the mean separation with Duncan’s Multiple Range Test at the 5% level.
3. Results and Discussions

The results of the formation potato micro tubers *in vitro* due to the different concentration of coconut water such as the formation of time tubers, the micro tubers of number, the diameter of tuber and the fresh weight of potato micro tubers shown in Table 1.

Table 1. The formation of time tubers, the micro tubers of number, the diameter of tuber and the fresh weight of potato micro tubers

| Parameter                              | Concentration of coconut water (mL L⁻¹) |
|----------------------------------------|-----------------------------------------|
|                                        | 0 (A₀) | 75 (A₁) | 150 (A₂) | 225 (A₃) |
| The time of potato micro tuber formation (days) | 27,36  | 30,52   | 31,60    | 28       |
| Number of potato micro tubers (pieces)  | 2,2 a* | 2,84 b* | 3,28 bc* | 3,72 c*  |
|                                        | (1,5717) | (1,8008) | (1,9118) | (2,0404) |
| Diameter of potato micro tubers (mm)    | 3,296 a* | 5,624 b* | 6,536 b* | 8,652 c* |
|                                        | (0,9021) | (1,0251) | (1,0653) | (1,1642) |
| Fresh weight of potato micro tubers (mg) | 27,4 a* | 78,6 b* | 106,7 c* | 102 c*   |
|                                        | (0,7260) | (0,7509) | (0,7775) | (0,7729) |

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

Table 1 shows that the application of coconut water (CW) significantly affect the number of micro tubers, the diameter of the micro tubers and the fresh weight of the micro tubers but did not affect the formation time of potato micro tubers. The time for the formation of potato micro tubers was faster at 28 days with the application of CW as much as 225 ml L⁻¹ (A₃), while the longer formation of potato micro tubers occurred in 31.60 days with the application of CW as much as 150 ml L⁻¹ (A₂). The highest number of potato micro tuber produced (3.72 pieces) was found at CW concentration 225 ml L⁻¹ (A₃) while the least number of potato micro tuber (2.2 pieces) was found in CW concentrations of 0 ml L⁻¹ (A₀). The highest potato micro tuber diameter (8.652 mm) was found in CW concentrations of 0 ml L⁻¹ (A₀) while the smallest (3.296 mm) was resulted by the CW concentration of 0 ml L⁻¹ (A₀). Tuber diameter reflected the accumulation of starch in the tuber, which was very much affected by the amount of sugar absorb by the roots. The higher fresh weight of potato micro tubers was found in CW concentrations of 150 ml L⁻¹ (A₂) while the lower fresh weight of potato micro tubers was found in the CW concentration of 0 ml L⁻¹ (A₀).

Culture media without the application of coconut water produce potato micro tubers which are affected by the absolute absence of light during the formation of potato micro tubers. Without light intensity during the formation of potato micro tubers, it encourages the formation of potato micro tubers by increasing the synthesis of endogenous cytokinins in potato plantlets [10]. Coconut water (CW) was found to be essential to trigger the transition and to enhance initiation of the process of cell division [11] [12]. The combination of CW with a plant growth regulator (PGRs) as cytokinins is the most effective way to improve rates of multiplication. CW is a complex additive which contains many nutritional and hormonal compounds. Therefore, along with plant hormones, CW was very effective in plant regeneration [13] [14]. The concentration of PGRs present in CW affected the leaf number and fresh weight of the potato microtubers [6] [15].

The application of CW in the media is very effective for the growth and formation of potato micro tubers, but the level of hormone content is very small, so it requires exogenous hormone assistance in supporting the growth and formation of potato micro tubers such as cytokinins and gibberellins [16]. CW functioned as a PGRs because it contains cytokinins and other phytohormones that have different functions in plants, such as cell division, tissue differentiation and stimulating shoot growth. Apart from containing PGRs, there are also macro elements (P, K, Ca, Mg, and S) and micro elements (Na, Cl, Mn, Al, Zn, Fe and Cu) which stimulate shoot growth in CW.
When CW was incorporated into the culture medium, potato plantlets showed an increase in shoot size, fresh weight and dry weight of potato micro tubers [8] [17]. CW treatment in potato plantlets had an effect in reducing the fresh weight of potato micro tubers. This happens because the accumulation of sugar in the potato plantlets can be taken from the treatment medium. The glucose content in the culture media continues to accumulate in potato plantlet tissue which results in an increase in simple sugar content, but cannot promote growth in potato plantlet tissue [18]. During the formation, potato micro tubers expand from the sink tissue by taking sucrose from the culture medium to convert into starch. The storage of starch in potato plantlets axillary bud initiation and induction is an indicator of micro-potato micro tubers containing sucrose of media treatment [19] [21].

Increasing the concentration of CW accelerate the formation of potato micro tubers. The application of CW encourages tuber formation. One of the hormones that plays an important role in CW in the formation of potato micro tubers is cytokinins. Cytokinins have an important role in the formation of potato micro tubers and increase the number of potato micro tubers formed. The application use of cytokinins in CW alone is not sufficient to increase potato micro tuber formation, because cytokinins cannot inhibit GA activity or GA synthesis in potato plantlets [22] [23] [24]. The high concentration of coconut water given to potato plantlets affect the fresh weight of the potato micro tubers. The endogenous cytokinins in potato plantlets and exogenous cytokinins in coconut water can control the activity of gibberellic acid in cells, which affects cell division and enlargement in potato micro tubers. The cell division and enlargement of potato plantlets, which are affected by cytokinins cause the formation of potato micro tubers to be more optimal and have an effect on the size and weight of potato micro tubers [10] [25] [26].

4. Conclusion
The application of coconut water has a significant effect on the time of potato micro tuber formation (days), number of potato micro tubers and fresh weight of potato micro tubers (mg). The application of concentration of coconut water 225 ml L⁻¹ affects the in vitro potato micro tuber formation.

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