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The Utilization of Coconut Water Waste As a Growth Media of the In-Vitro Potato Cutting

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abstract

The limited number of potato seed caused the demand to be unfulfilled especially in South Sulawesi. The use of pathogen-free seeds is absolutely necessary that can be obtained through tissue culture. This study aims to determine the effect of adding coconut water on the growth of in vitro potato plant cuttings and the most effective type of treatment. This research used Varying Concentration of the coconut water, then measure its effect on potato growth (a high number of leaves and plantlets). This study consisted of 4 treatments: TQ0 (without coconut milk), TQ1 (100 ml/l), TQ2 (150ml/l), and TQ3 (200 ml/l). The results showed that the addition of coconut water on the growth of micro cuttings of potato (*Solanum tuberosum* L.) gave significant effect on the observed parameters, leaf number and plantlets weight. While TQ2 gave the best effect on the growth of in vitro micro cutting of potato.

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1. Introduction

Potatoes are one of the horticultural commodities which needed every year in the food system in Indonesia (Timmer, 2003; Rahayu et al., 2015). The potato development was implemented through intensification called INMAS (Intensifikasi Massal or mass intensification) and extension programs at production centers (Resosudarmo and Yamazaki, 2011). Indonesia's potato production centers are among others located in West Java, Central Java, East Java, North Sumatra, West Sumatra, Jambi, and South Sulawesi (Fuglie et al., 2006).

Seed potato degeneration, the reduction in yield or quality caused by an accumulation of pathogens and pests in planting material due to successive cycles of vegetative propagation, has been a long tanding production challenge for potato growers around the world (Thomas-Sharma et al., 2015). In most of ountries, a majority of farmers recycle their own seeds or get them from informal sources (Muthoni et al., 2013). This limited number of good quality potato seed caused

The productivity of potato in South Sulawesi from 1998-2002 reached 7.02 tons/ha, and in 2011 reached up to 17 tons/ha, while the yield potential can reach 30 t/ha (Asaad et al., 2005).

The low productivity of potatoes is influenced by many things, the incidence of pests, especially weeds, pathogens and animal pests, reduced water availability, the suitable period becomes shorter, one of the reason the limited of qualified potato seed (Oerke, 2005; Haverkort and Verhagen, 2008). Most farmers use potato seed tubers of the next generation, which deliberately kept aside to be used as seed. This is caused by the high price of potato seed, while the price of potato consumption is relatively low, so farmers are not able to purchase the qualified potato seed. To overcome this atau to resolve this, the use of pathogen-free seeds is absolutely necessary. The seed can be obtained through tissue culture and also accompanied by intensive pathogen testing (Hartus, 2006).

Qualified seeds can be produced by tissue culture (George et al., 2008). It also insures a good regular supply of plants, faster, requires less space and time than that required for conventional methods of producing seedlings (Sidhu, 2010; Aladele and Kuta, 2008). One of the factors which influence the success of tissue culture techniques is the composition of the culture media. There are several natural plant growth regulator of tissue culture media ingredients, one of which is coconut water. It has been long known as a source of growth substance and it is suspected to have an activity of cytokines that play a role in cell division and encourages the formation of organs (Razdan, 2003; Rukmana, 2003; Oka, 2014).

Baque et al. (2011) stated 0 ml/l coconut water effectively enhanced plantlets growth of both *Calanthe* hybrids compared to the relative control (without coconut water). Prihatmanti and Mattjik (2004) added that he use of natural ingredients of coconut water at a concentration of 100 to 200 ml/l for shoot multiplication of *Anthurium andreanum* can improve the growth of the culture in vitro. Furthermore Bey et al. (2005) suggested that a single treatment of coconut water at a concentration of 250 ml/l was able to produce leaves and roots faster on in vitro culture of orchids (*Phalaenopsis amabilis* BL.).

Based on these studies, it is alleged that the use of coconut water as a growing medium can be used to improve the efficiency of the potato plant growth in vitro, which is unexplored precedently. Therefore, this study was conducted to determine the effect of coconut water on Murashige and Skoog media (MS) against the growth of the potato plant (*Solanum tuberosum* L.) in vitro. This study aims to determine the effect of coconut water on the growth of the in vitro potato cuttings and determine the most effective type of treatment for the growth of the in vitro potato cuttings.

2. Method

This study was an experimental study to create variation towards independent variable ie. coconut water concentration, then measure its effect on the dependent variable ie. the growth of potato (number of leaves and plantlets height). This study consisted of 4 treatments (4 levels of coconut water concentration, ie. the TQ0 (without coconut milk), TQ 1 (50 ml / l), TQ 2 (100ml / l), and TQ 3 (150 ml/ l)) and 3 replications with completely randomized design (CRD). This study conducted in Botany Laboratory, Department of Biology Department, Faculty of Science and Technology, UIN Alauddin Makassar. As for the steps, as follows:

2.1 Sterilization. Tools such as culture bottles, petri dish, measuring cups, tweezers, scissors, scalpel handles, empty bottles, pipettes, stir bar, and a flask, were wrapped with thick paper then sterilized in an oven at 180°C for 2 hours, distilled water and the medium sterilized by autoclave for 15 minutes at a temperature of 121°C and the pressure reaches 17.5 psi.

2.2 Preparation of Stock Solutions. Stock solution required as a raw material in the manufacture of MS medium, such as a stock solution A, B, C, D, E, and F. Preparation of Media: Murashige and Skoog (MS) is added with coconut water with different concentrations, ie, 50 mL/L, 100 mL/ L, and 150 mL/L. The manufacture of medium each stock solution pipetted based on the concentration required and added into a flask and then add 25 g / L of sugar which dissolved in a beaker with a capacity of 1000 ml. The media is divided into 4 levels of treatment,

2.3 Planting. Planting was performed in a prepared room with Laminar Air Flow Cabinet. First, sterilize the room, workbench, tools and materials in order to prevent contamination of the media and the plants. The surface part of Laminar Air Flow Cabinet sprayed with a solution of 70% alcohol and then clean it with sterile tissue. Laminar Air Flow Cabinet can also be sterilized with ultraviolet light which turned on for 60 minutes to kill the existing contamination in the work area. Beside that, planting tools such as tweezers, scalpel, must also be sterilized by soaking it in 90% alcohol. Using tweezers, the source of explants derived from 4-week-old potato were inoculated into the prepared media. After the inoculation of the culture bottle mouth should be covered with plastic paper and labeled according to the concentration of the medium, and placed in tissue culture bottle rack with a culture room temperature of 21°C.

2.4 The Parameters. Parameters measured in this research is a number of leaves formed; plantlets are removed from the bottle then calculated at the end of the observation during

the growth period of 8 weeks. The second is plantlets height (cm); using yarns ranging from the base of the stem which borders the base of the root to the tip of the rod. After the rod measurement using yarns completed, then continued with measuring the yarns using a ruler.

2.5 Data Analysis. The data which was obtained from this study then analyzed with Inferential Statistic method, ie. Anova-Test, if it shows significant results then it will be continued using another advanced test to determine which treatment gives the best results.

3. Result and Discussion

3.1 Leaves Formation

The result of variance analysis shows that coconut water addition has a very significant effect on the formation of potato leaf plantlets”

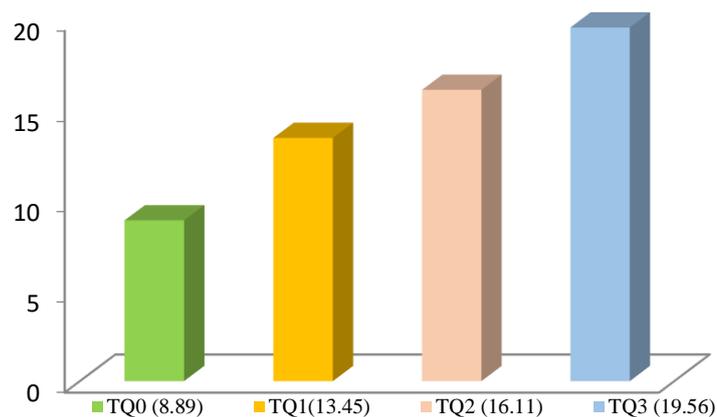


Figure 1. The diagram of coconut water addition traetment towards the average of potato leaf plantlets at 4 weeks after planting.

Figure 1 shows that the highest average of leaves formation increase was found on the TQ3 treatment which is 19.56 while the lowest average was on the TQ 0 treatment (control) in which the treatment produces 8.89 average number of leaves. The results of variance analysis show that coconut water addition on the potato is also very influential on the leaves formation which means that there are one treatment concentrations that were very prominent compared to other treatments. Since the reliability degree was 2.89%, in order to determine which one is the most

influential treatment, it must be followed by the HSD test which showed TQ 3 treatment is the optimum range for leaf parameters.

Leaves are the main photosynthetic organs of vascular plants and show considerable diversity in their geometries (Scarpella et al., 2009). For many species of dicotyledonous plants, the leaf consists of a blade and a petiole that attach to the stem (Kozuka et al., 2005; Perttunen et al., 2001). Leaves will be formed as a leaf (primordium) in an iterative pattern by the shoot of apical meristem which triggered by the plant auxin hormone (Fleming, 2005; Reinhardt et al., 2003). Apical meristem will grow bigger during the cell division and differentiation. Leaves will grow with various sizes due to meristematic activity (Ha et al., 2003; Fiorani et al., 2000). Thiamine needs to be added in tissue culture that caused maximum callus growth, affect root and shoot growth simultaneously, also serve as cofactors in enzymatic reactions (Al-Khayri, 2001; Abrahamian and Kantharajah, 2011), especially in the condition of low cytokinin in the medium. The lack of cytokine will inhibit cell division and prevent shoot formation and axillary shoot proliferation (Hussain et al., 2012).

Further research conducted by Kristina and Shahid (2012) proved that although the cytokinin content decreased by 10-fold, the content of this cytokinin can still be used in tissue culture. A number of cytokinin content contained in coconut water is suspected to match against leaf formation. The results of the study are obtained by observing the leaf formation parameters against the use of coconut water as a growth regulator on the in vitro ginger (*Curcuma xanthorrhiza* Roxb.) Shoot multiplication has also been carried out by Seswita (2010). She suggests that the coconut water administration by 15% resulted in the highest number of shoots and leaves.

3.2 The Increase of Plantlets Weight

The result of variance analysis at the age of 4 weeks after planting showed that the addition of coconut water was very significant in terms of potato plantlets weight increase. The observation result from the average of plantlets weight gain with various concentrations can be seen in Figure 2.

The highest average of potato plantlets weight increase is 0.4 grams, it was found on TQ3 treatment while the lowest average was on TQ0 treatment where such treatment resulted in average weight of 0.16 g (Figure 2). The result of variance analysis about the effect of coconut water addition showed a very significant effect on potato plantlets weight addition. It can be

concluded that the administration of coconut water on the potato plants are very influential on increasing of potato weight, which means that is also a very prominent treatment concentration compared with the other treatment. Because in this experiment, the coefficient of variability is 1.68%, therefore the test continued with HSD test.

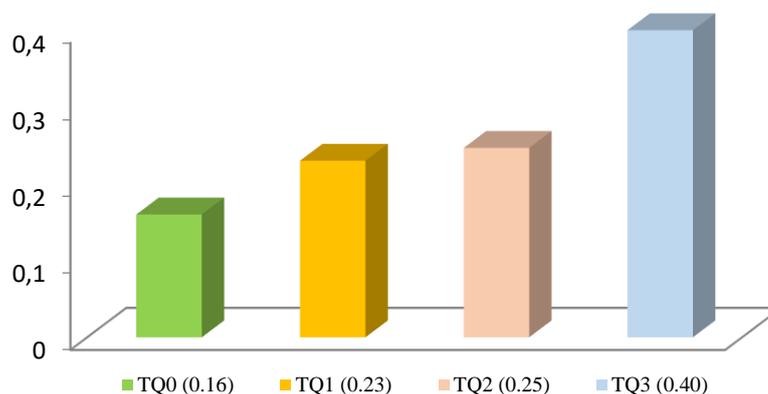


Figure 2. The diagram of coconut water addition treatment towards the average of potato weight gain at 4 weeks after planting.

HSD test results showed that the difference between treatment of TQ1 and TQ2 is not significant, but significantly different on TQ3. The optimum range is at a concentration of 150 ml/l (treatment P3) for the plant weight increase parameter.

Wet weight of potato plantlets also depends on the formed organs grown in vitro. In addition to coconut water with a concentration of 150 ml/l showed a significant effect on the formation of roots, leaves, and plantlets height. The plantlets weight increase was influenced by those three parameters.

The wet weight increase of plantlets determined by solid components such as cell walls, proteins, carbohydrates, the number and size of cells also the amount of water. A cell wall is formed by the presence of lignin, pectin and hemicellulose. Proteins are formed by the reaction of amino acids forming in the cytoplasm. Carbohydrates are formed from photosynthesis result i.e sucrose that can be respired and other solid materials such as proteins or accumulated in other carbohydrates in the cell wall, amyloplast and others (Kačuráková et al., 2000; Wu, 2009; Hammarström and Hammes-Schiffer, 2009). Boron can affect the metabolism of carbohydrates transport in the phloem which can add the wet weight of the plantlets. The phosphorus plays a role in the formation of carbohydrate and energy metabolism to form ATP, ADP, AMP, and

PPi. While potassium is very important in carbohydrates formation in young plants. Deficiency of potassium, phosphorus and boron causes the accumulation of carbohydrates and stunted growth (Marschner, 2002).

4. Conclusion

The addition of coconut water on the growth of micro potato cutting (*Solanum tuberosum* L.) showed a significant effect on the observed parameters i.e the formation of roots, number of leaves, plantlets height and weight. Treatment with the addition of coconut water volume of 150 ml/l on MS medium gave the best effect on the growth of in vitro potato micro cutting. The future studies should use a higher concentration of the coconut water as an additional composition of Murashige and Skoog media (MS) to the in vitro potato micro cutting grown.

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