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CALLUS FORMATION PROCESS ON RUBBER PLANT SHOOT EXPLANTS (*Hevea brasiliensis* Muell. Arg.) WITH MS MEDIUM

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ABSTRACT

Rubber plant (*Hevea brasiliensis* Muell. Arg.) is one of the plantation commodities that is important due to its role as the income source, boosting the economic growth in around rubber plant area, open job opportunities, foreign exchange source as well as re- lated to environmental conservation of biological resources. Propagation of rubber plant (*Hevea brasiliensis* Muell. Arg.) are currently still using conventional method, namely grafting. This research aims to determine the process of callus formation in a combina- tion of different NAA and BAP concentrations. The research design uses research type observation with cross sectional type (cross sectional design). Media that is used i.e. MS with BAP and NAA combination concentration, that is: P1 : control MS0, BAP 0 mg/l + NAA; P2 : BAP 1.5 mg/L + NAA 0.05 mg/L, P3 : BAP 2 mg/L + NAA 0.1 mg/L, P4 : BAP 2.5 mg/L + NAA 0.2 mg/L. Each treatment was repeated six times. The parameters observed were callus formation process for 3 weeks. Based on the results of data analysis shows that the process of callus formation begins with swelling of explants starting the first week, the second week changes color to greenish yellow and third week callus is formed in BAP treatment P2 : 1.5 mg / L +NAA 0.05 mg / L.

Keywords: *Hevea brasiliensis*, callus, MS, NAA, BAP.

INTRODUCTION

Plant cultivation techniques using conventional methods in the soil or sand medium often face technical, environmental, and time constraints. Tissue culture techniques can be done as an alternative propagation of plants not by using soil media, but in artificial medium in a tube. This can be done because each plant cell has the ability to develop into a complete living body through regeneration.

Plants can be propagated by vegetative using in-vitro culture techniques with callus or cell culture techniques (Montoro *et. al.*, 2010). Callus culture is the maintenance of small parts of plants in sterile artificial environments and con- trolled conditions. Callus is a collection of amorphous (non-shaped or undif- ferentiated) cells that occur from tissue cells that divide continuously *in vitro* or in a tube, and are not organized. Therefore, it gives the appearance of an irregularly

shaped cell mass.

In tissue culture techniques (*in vitro*), callus can be induced by adding suitable growth regulators to the culture media, for example adjusted auxin and cytokines. If the auxin concentration is greater than the cytokines, the callus will form, whereas if the concentration of cytokines is greater than the concentration of auxin, then the callus is not formed, but the shoots. Even though endogenous hormones also affect (Jimenez *et.al.*, 2001). In addition to growth regulators, the addition of vitamins and proteins is also needed for callus growth. Callus induction in plant tissue culture techniques is needed to produce somatic cell diversity on *in vitro* culture and regenerate these cells into somatic embryos (Srichuay *et.al.*, 2014).

The research on callus formation in rubber plants has been carried out a lot (Montoro *et. al.*, 2010; Moradpour *et. al.*, 2016; Nayanakantha and Seneviratne, 2007; Seneviratne, 1996; Seneviratne *et.al.*, 1996; Srichuay *et.al.*, 2014). Callus in rubber plants is formed because of the right concentration of auxin and cytokines (Montoro *et. al.*, 2010). In general, callus of rubber plants is formed at week 3 after the initiation has been done. The process of forming callus of rubber plants for 3 weeks has never been reported.

METHOD

This research was conducted in plant tissue culture Laboratory of PGRI Argopuro University, Jember. The material used is the apical shoots of rubber plant. This research uses Complete Random Designwith 4 treatment factors, that is P1 : control BAP 0 mg/l; P2 : BAP 1.5 mg/L + NAA 0.05 mg/L, P3 : BAP 2 mg/L + NAA 0.1 mg/L, P4 : BAP 2.5 mg/L + NAA 0.2 mg/L.

The tools used in this study is a petridish, stir bar, Erlenmeyer, measuring cups, culture bottles, tweezers, scalpel, scissors, laminar air flow, oven, analytic scale, bunsen lamp, autoclave, culture shelf, alcohol sprayer, tissue, aluminum foil, plastic wrap, filter paper, rubber band, hand sprayer, pH meter, matches, measuring pipette, cotton and stationery. Materials used include rubber, Wood Plant Medium and Murashige & Skoog Medium, BAP 2 mg/L, 70% of alcohol NAA 0.1 mg/L, and 95% of aquades, clorox and rubbing, sugar, agar and alcohol.

Preparation of tols and materials needed for research. Media production was according to media manufacturing standards and given NAA and BAP according o treatment. Media and equipment sterilization of explants were put into culture

bottles that al ready contained media. Explants were observed for 2 months. Inoculated explants were observed based on parameter observations. The parameter observed was the process of callus formation.

RESULT AND DISCUSION

In this research, the treatment that has been succeeded in forming callus is P2 treatment, that is on BAP 1,5 mg/l + NAA 0,05 mg/l concentration (Table 1).

Table 1. Observation Towards Callus Formation

Treatment	Repetition	Callus Formation
P1: control MS0	P1R1	-
	P1R2	-
	P1R3	-
P2 : BAP 1.5 mg/L + NAA 0.05	P2R1	+
	P2R2	+
	P2R3	+
P3 : BAP 2 mg/L + NAA 0.1 mg/L	P3R1	-
	P3R2	-
	P3R3	-
P4 : BAP 2.5 mg/L + NAA 0.2 mg/L	P4R1	-
	P4R2	-
	P4R3	-

Callus formation is marked by the swelling or the appearance of white greenish tissue on explants surface. The explants that show the existence of callus formation is white clots indicated by the arrow of Figure 1. On media treatment which combined with growth substance regulator concentrate, callus is started to be inducted on MS treatment with BAP 1.5 mg/l + NAA 0.05 mg/L concentration (MS1). Process of callus formation begins with swelling of explants starting the first week, the second week changes color to greenish yellow and third week callus is formed in BAP treatment 1.5 mg / L + NAA 0.05 mg / L (P2).

Figure 1. Callus on MS treatment with BAP 1.5 mg/l + NAA 0.05 mg/l. a.) First week; b.) Second week; c.) Third week; d.) Ninth week.

Callus is formed through three phases, which is induction, cell division, and differentiation. Callus formation is determined by explants resources, nutrient composition on the medium and environmental factor (Voronova, 2016; Ward and Jordan, 2001). The explants that come from meristem tissue grow faster than the

tissue from thin-walled cell and consist of lignin. Callus formation is divided into five phases, i.e., lag phase, exponential phase, linier phase, decelera- tion phase, and stationer phase. Callus formation is started by cells that start to divide and then the rate of division comes to its top, and the cell will experience slowness but the expansion rate increase. Then the cell will experience a decrease in the rate of division and elongation of cell, last phase, the cell will experience a fixed amount and size.

In this study, the lag phase began in the first week, which showed swelling of the rubber meristem explants. It is a callus without regenerating a clearorgan usually called loose or compact callus (Ikeuchi *et. Al.,* 2013). The last phase, which is the stationary phase, occurs when the callus is 9 weeks old. Cal- lus that forms organ regeneration is often called muscular callus, bud, or embryonic (Ikeuchi *et. Al.,* 2013). Cell division and growth stop, it is time for the callus to be sub-cultured to grow new shoots or calluses.

CONCLUSION

Process of callus formation begins with swelling of explants starting the first week, the second week changes color to greenish yellow and third week callus is formed in BAP treatment 1.5 mg / L + NAA 0.05 mg / L.

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