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Laila, Fitriya Nur. 09620024. 2013. **The Effect of ZPT 2,4-D and PEG (Polyethylene Glykol) 6000 wiht The Addition MS (Murashige & Skoog) for Production Secondary Metabolite wiht Callus Stevia (*Stevia rebaudiana* Bertoni M.) Culture.** Thesis. Department of Biology, Faculty of Science and Technology. Thesis Supervisor: Dr. Evika Password Savitri, M.P. Supervisor Religion: Ach. Nashichuddin, M.A.

Kata Kunci: *Calli, Stevia (Stevia rebaudiana Bert. M.), PEG 6000, 2,4-D, Stevioside.*

Stevia rebaudiana Bert. Is a member from Asteraceae family, it produce steviol glycoside (stevioside, rebaudioside A, B, C, D, E and dulcoside A) that can be used as the addotion food material such as food flavoring or the sweetener material in nutrient supplement. The purpose of the research is to know combination concentrate 2,4-D and PEG 6000 that efective in increasing the production of secondary metabolite in callus stevia (*Stevia rebaudiana* Bert. M) wiht *In Vitro*. PEG 6000 which is one of to manipulated growing media wiht *In Vitro* for increasing the secondary metabolite. PEG is a compound that can lower the osmotic potential of the solution, so that the water can not be absorbed, resulting in plants that causes osmotic stress induces proteins, genes coding for enzymes involved in forming the biosynthesis of secondary metabolites.

This research use Completely Randomized Design (CRD) factorial method wiht 12 combinations wiht 3 repetitiol. The first factor is the distribution of 2,4-D (1 mg/L, 2 mg/L, dan 3 mg/L) and the second factor is the distribution PEG 6000 (0 mg/L, 5 mg/L, 15 mg/L, dan 25 mg/L). The parameter that analyse is callus appear day (day), presentage (%) exsplant callus (gr), callus weight, callus morphology (callus pattern adn color), and secondary metabolite stevioside. The data that the researcher get is analyse by ANOVA two way and to know the differentiation is analyse by *Duncan Multiple Range Test* (DMRT) wiht significant standartd 5 %. To secondary metabolite stevioside is test using *High Performance Liquid Cromatography* (HPLC).

The result of the research shows that 2,4-D influence to the appearing callus day whit growt anerag 15 day that is in 2 mg/L concentrate. PEG 6000 is influence to the callus weight, the treatment 5 mg/L PEG 6000 can be able to maintain callus weight than the treatment others PEG, that is 0,2511 g. The combination 2,4-D and PEG 6000 in flurence to the synthesis secondary metabolite, suntetic combination 1 mg/L 2,4-D and 25 mg/L PEG 6000 is the efective combination to get the secondary metabolite stevioside that is 4,792 mg/g. The callus morphology research is (callus pattern adn color) is getting the callus thet has complite pattern and the color is brown because of the osmosis stress PEG 6000, that callus moorphology is has high secondary metabolite stevioside content in this research.