Eco. Env. & Cons. 27 (August Suppl. Issue) : 2021; pp. (S350-S354)
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ISSN 0971–765X

The effect combination of growth regulator 2,4D and kinetin on callus induction from *Elephantopus scaber* leaf

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(Received 20 October, 2020; Accepted 21 April, 2021)

ABSTRACT

Elephantopus scaber L. is a plant that has benefit as traditional medicine. The plant leaves can be used as medicine caused by their secondary metabolites such as flavonoid, phenol, saponin, tannin, steroid, terpene, and luteolin-7-glucoside. The production of secondary metabolites can be done by *in vitro* culture using culture media and appropriate growth regulators. This study aims to determine the effect of a combination of 2,4-D growth regulators and kinetin on callus induction time, percentage of explants forming callus, fresh weight, dry weight, and callus morphology. This study was a laboratory experimental study with a completely randomized design method with 8 treatments combined concentrations of growth regulators ($D_{0,0}K_{0,0}$; $D_{0,5}K_{1,5}$; $D_{0,5}K_{2,0}$; $D_{1,0}K_{1,0}$; $D_{1,0}K_{1,5}$; $D_{1,5}K_{0,5}$; $D_{1,5}K_{1,0}$; $D_{2,0}K_{1,0}$) and each treatment consisted of four replications. The data obtained was analyzed qualitatively and quantitatively. Qualitative data were obtained from callus morphological (color and texture) descriptions. Quantitative data obtained from callus induction time, the percentage of explants forming callus, fresh weight and dry weight of callus were statistically analyzed using SPSS 24 with a significance value of 0,05. Data analysis measured the effect of growth regulator 2,4-D concentrations and kinetin using the Kruskal Wallis test. Difference between treatment groups were tested using the *Mann-Whitney* test. The results showed that the $D_{1,0}K_{1,5}$ treatment group increased callus most significantly in comparison than another treatment groups, the average induction time of callus 10.8 days after planting. The $D_{1,0}K_{1,5}$ treatment group produced the highest average fresh weight of 119.5 mg and also the highest average dry weight was 78.9 mg. Callus morphology with compact texture and the yellowish color were produced at $D_{0,5}K_{2,0}$; $D_{1,0}K_{1,5}$ treatment group.

Key words : *Elephantopus scaber, 2,4-D, Kinetin.*

Introduction

Tapakliman (*Elephantopus scaber* L.) is a plant belonging to the Asteraceae family which has benefit as a traditional medicine. The chemical contents of tapakliman include lactone sesquiterpan, scabertopin, isochlorogenic acid A and B, epifriedelinol, lupeol, stigmasterol, tricontan-1-ol, dotria-contan-1-ol, lupeol acetate, deoxyelephantopin (Singh *et al.,* 2005; Rajkapoor *et al.,* 2002). Moreover, tapaklimanhas bioactivities such as anti-inflammatory, analgesic and laxative (Jenny *et al.,* 2012). In addition, the tapakliman also has benefit for treating rheumatism, hepatitis, diabetes, insomnia, bronchitis and arthralgia (Kabiru and Por, 2013). Callus culture method has high potential to obtain secondary metabolites *in vitro* which is used as an alternative to isolating secondary me-

tabolites fromtapakliman. Physical and chemical conditions in callus culture can be controlled and regulated to optimize the secondary metabolite compounds production (Murthy, 2014).

2,4-D is a strong synthetic auxin which has the function of triggering callus formation, cell elongation or growth, root initiation and somatic embryogenesis induction Recently, kinetin plays role as cytokinin type that is often used in tissue culture techniques and it has a function in cell division and differentiation of adventitious shoots from callus (Rahman *et al.*, 2019). This study was conducted to determine the effect of combination from growth regulator 2,4-D and kinetin which plays an important role in callus induction and callus quality of tapakliman leaves (*Elephantopus scaber* L.). Hence, it can be used as a basis for further research development in the isolation of secondary metabolites.

Methods

The research was conducted at the Plant Physiology Laboratory, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya for six months. The plant material used for the explants was the meristem of tapakliman leaves (*Elephantopus scaber* L.). The chemicals used include basic media Murashige and Skoog (MS), growth regulators 2,4-Dichlorophenoxy Acetic Acid (2,4-D) and 6-Furfurylaminopurine (kinetin). The leaves of tapakliman (*Elephantopus scaber* L.) were washed and soaked in detergent for five minutes then rinsed with water. Furthermore, the leaves were sterilized in a 20% chlorox solution for 10 minutes, then rinsed three times using sterile distilled water. The leaves of tapakliman were cut ± 1 cm^2 and then put into a culture bottle, covered with aluminum foil and clingwrap. Each culture bottle contained three pieces of explants. Then, the culture bottles were placed in an incubator at a temperature of 20-25 °C equipped with a 20 W fluorescent lamp for six weeks of the culture period. The callus induction time was observed every day for six weeks of culture. After the sixth week of the culture period, callus growth and development observations were carried out by calculating the percentage of callus forming explants, callus fresh and dry weight, and callus morphology (texture and color). After six weeks, the fresh callus was placed on aluminum foil and dried in an incubator oven at 60-70 °C to obtain dry callus biomass.

This study used a completely randomized design (CRD) consisting of eight treatments with four repetitions. The combination of concentrations tested, namely:

1. 2,4-D 0,0 mg/l + Kinetin 0,0 mg/l (control)
2. 2,4-D 0,5 mg/l + Kinetin 1,5 mg/l
3. 2,4-D 0,5 mg/l + Kinetin 2,0 mg/l
4. 2,4-D 1,0 mg/l + Kinetin 1,0 mg/l
5. 2,4-D 1,0 mg/l + Kinetin 1,5 mg/l
6. 2,4-D 1,5 mg/l + Kinetin 0,5 mg/l
7. 2,4-D 1,5 mg/l + Kinetin 1,0 mg/l
8. 2,4-D 2,0 mg/l + Kinetin 1,0 mg/l

This study producedtwo data types, namely qualitative and quantitative data. Qualitative data were analyzed descriptively whereas quantitative data were statistically analyzed using SPSS 24, normality test was performed using Kolmogorov-Smirnov (>0.05), homogenity test using Levene's test (>0.05), using Kruskal-Wallis test to determine significant differences between the two or more groups of independent variables on the dependent variable (<0.05). Then, Mann-Whitney test to determine the differences between treatment groups (<0.05).

Results and Discussion

Callus induction time and the percentage of explants producing tapakliman (*Elephantopus scaber* L.) leaf callus on MS medium with a combination of growth regulator 2,4-D and kinetin concentrations

Lestari (2011) revealed that growth regulators had the function of spurring to the phytohormones formation that already exist in plants or replacing the hormonesrole while plants were not able to produce hormones properly. Tapakliman (*E. scaber* L.) leaf

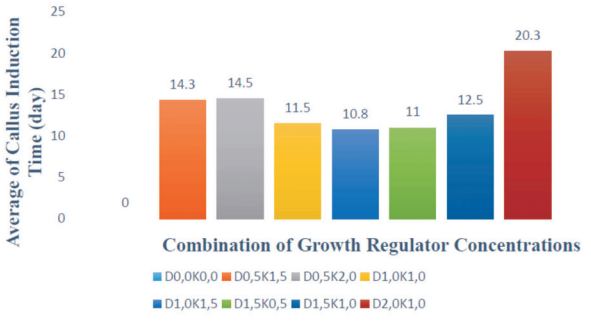


Fig. 1. The corellation between the tapakliman callus induction time with combination of growth regulators 2,4-D and kinetin concentrations

explants which were competent showed a response when the growth regulator 2,4-D and kinetin were added to MS media which was characterized by explant expansion and an irregular cell mass or called callus is formed.

The callus formation as a result of cell division occured due to injury response and supply of natural and artificial hormones from outside into the explants (George and Sherrington, 2007). The auxin growth regulator 2,4-D had a significant role in the callus formation process. Cytokinin kinetin plays a role in triggering cell division and elongation and it can accelerate callus growth and development (Rahman *et al.*, 2019). Naturally, the auxin hormones play a role in stem and internodes elongation, abscission, apical dominance and root parenthood. Meanwhile, cytokinin hormones being part in shoot differentiation, cell division and apical dominance. The combination of plant growth regulators concentration to certain plants type has an effect on the callus induction duration.

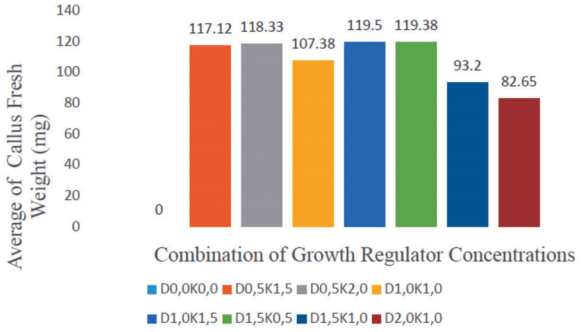


Fig. 2. The relationship between tapakliman callus fresh weight with combinationof growth regulator 2,4-D and kinetin concentrations

Callus formation percentage from explants was determined by counting the number of explants that formed callus divided by the total number of explants planted in each combination then multiplied by 100%. In all treatment combinations, the concentration of growth regulators produced callus with a 100% in percentage, except for control group.

Fresh weight and dry weight of tapakliman leaf callus (*Elephantopus scaber* L.) on MS media with a combination of growth regulator 2,4-D and kinetin concentrations

In tissue culture, the resulting biomass depends on the cells dividing rate, multiplying, and followed by cell enlargement (Abdelmaksood *et al*., 2017).

Fresh weight measurement is an easy and fast method of measuring callus growth. Physiologically, fresh weight consists of two materials, namely water and carbohydrates. However, the fresh weight measurement is less accurate because there is water content and this condition requires to measure the dry weight Dry weight is the weight that contains only the metabolic products of plants whose water content has been removed by the drying process. Plant production is more accurately expressed by dry weight, because it is not affected by water content (Al Ajlouni *et al.*, 2012; Al Taha, 2013).

The addition of auxin and cytokinin growth regulators in the media and endogenous growth regulators interaction could affect callus growth. Growth is related to increase in cells number and volume, new protoplasm formation, and also increase in fresh weight and dry weight. The process of callus formation is inseparable from cell division, enlargement and extension (Rahman *et al.*, 2019).

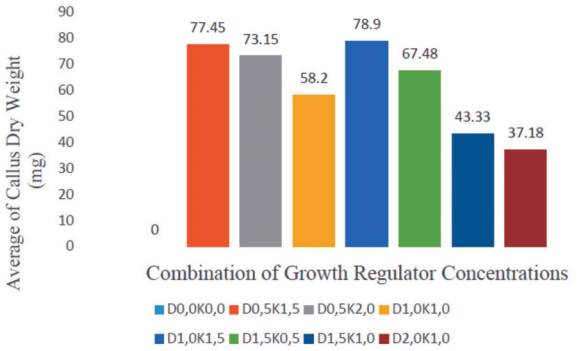


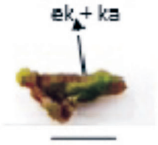
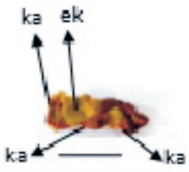
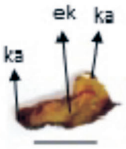
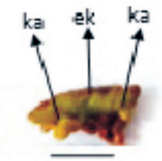
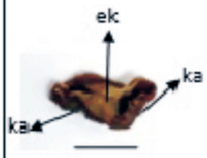
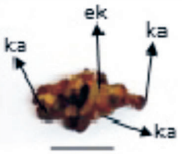
Fig. 3. The relationship between tapakliman callus dry weight with combination of growth regulator 2,4-D and kinetin concentrations

Morphology of tapakliman (*Elephantopus scaber* L.) leaf callus on MS media with a combination of growth regulators 2,4-D and kinetin concentrations

Observation of callus morphology from tapakliman leaf explants was done descriptively including color and texture, which was carried out at week 6 after the culture period. The callus color and texture describe the quality and growth from callus. Embryogenic callus has a friable to compact texture, nodular and is white to yellowish in color (Sharma, 2017). Callus that has a compact texture is considered as a good condition caused it can accumulate more secondary metabolites.

The texture of crumb callus has rapid cell division compared to the compact callus texture. The color change in callus indicates that there is a growth phase and regeneration power in callus cells.The white color in callus indicates the plastid content with contains starch, then grows into a clear membrane system until chloroplast grains are formed. This condition is exposed to light exposure which causes the callus to turn green. The brownish color of callus is due to the phenol compounds and mechanical treatment. Furthermore, the greenish-green color of callus is a good callus because of its high cell division activity (Ali *et al.*, 2007).

Table 1. Morphology of tapakliman leaf callus explants at week 6 from the culture period.

No.	Treatment	Morphology	Figure
1	2,4-D 0,0 mg/L + K 0,0 mg/L	Brown explant and no callus grew	
2	2,4-D 0,5 mg/L + K 1,5 mg/L	Callus was brownish green with a compact texture. Callus grew in all over the explants surface	
3	2,4-D 0,5 mg/L + K 2,0 mg/L	Yellowish callus and green explants had compact texture. Callus grew in the wound area	
4	2,4-D 1,0 mg/L + K 1,0 mg/L	Brownish callus and green explants had compact texture. Callus grew in the wound area	
5	2,4-D 1,0 mg/L + K 1,5 mg/L	Yellowish callus and green explants had compact texture. Callus grew in the wound area	
6	2,4-D 1,5 mg/L + K 0,5 mg/L	Blackish callus and green explants had a compact texture. Callus grew in the wound area	
7	2,4-D 1,5 mg/L + K 1,0 mg/L	Blackish callus and green explants had a compact texture. Callus grew in the wound area	
8	2,4-D 2,0 mg/L + K 1,0 mg/L	Callus was brownish in color with a compact texture. Callus grew in all over the explants surface	

Conclusion

1. The combination of growth regulator 2,4-D and kinetin concentrations had a significant effect on the duration of callus induction, the percentage of explants forming callus, fresh weight and dry weight of callus. The addition of growth regulators combination was affected various callus induction times. The percentage of explants forming callus is 100%, and the resulting callus biomass is influenced by the dividing cells rate, multiplying followed by cell enlargement.
2. The combination of growth regulator 2,4-D and kinetin concentrations were affected the callus morphology. The callus was produced with a compact texture and changing colors indicating cell differentiation. In addition, the callus color describes a visual appearance that can be seen that the cells are still actively dividing or dying.
3. The combination of growth regulator 2,4-D and kinetin which is suitable for the callus induction process was found at a concentration of 2,4-D 1.0 mg/l + K 1.5 mg/l and the result showed average of the fastest induction time with 10.8 ± 0.957 days after planting. The highest average fresh weight and dry weight were 119.50 ± 10.568 mg and 78.90 ± 13.766 mg, respectively. The explants could produce callus with a compact texture and yellowish color.

Suggestion

Further research is recommended to use a combination of other growth regulators with varying concentration ranges.

References

Abdelmaksood, A.W.M., Zaydetovna, K.L. and Arnoldovna, T.O. 2017. Effect of different plant growth regulators on the in vitro induction and maintenance of callus from different explants of *Hyoscyamus muticus* L. *J. Appl Environ Biol Sci*. 7 : 27-35.

Al-Ajlouni, Z., Ajlouni, M., Shatnawi, M., Shibli, R., Makhdme, I., Abu Rohman, S. and Al-Ghazawi, A.L. 2012. Callus induction, plant regeneration, and growth on barley (*Hordeum vulgare* L.). *South Western Journal of Horticulture, Biology, and Environment.* 3(1) : 25-39.

Ali G., Hadi, F., Ali, Z., Thariq, M. and Khan, M. A. 2007. Callus induction and in vitro complete plant regeneration of different hormonal concentration. *Biotechnology*. 6(4) : 561-566.

Al-Taha, H.A.K. 2013. Effect of shock and gradual drought by PEG on callus growth and proline accumulation in sour orange (*Citrusaur antium*). *Adv. Agric. Bot. Int J Bio Soc*. 5 (2) : 77-83.

George, F. P and Sherrington, P. D. 2007. *Plant Propagation by Tissue Culture*. Eversly. Handi Book and Dictionary of Commercial Laboratories Exigetic Limited.

Jenny, A., Sasha, D., Paul, S., Dutta, M., Uddin, Z. and Nath, A.K. 2012. Antibacterial activity of aerial part of extract of *Elephantopusscaber* Linn. *Bull. Pharm. Res*. 2(1) : 38-41.

Kabiru, A. and Por, L.Y. 2013. *Elephantopusscaber*: Traditional uses, pharmacological actions and chemical composition. *Advances in Life Science and Technology*. 15 : 1-13.

Murthy, H.N., Lee, E.J. and Paek, K.Y. 2014. Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. *Plant Cell Tissue Organ Cult*. 118 : 1-16.

Rahman, N.N.A., Rosli, R., Kadzimin, S. and Hakiman, M. 2019. Effect of auxin and cytokinin on callus induction *Catharanthus roseus* (L.). G.Don. *Fundamental and Applied Agriculture.* 4(3) : 928-932.

Rajkapoor, B., Jayakar, B. and Anandan, R. 2002. Antitumour activity of Elephantopusscaber Linn. against Dalton's ascetic lymphoma. *Indian Journal of Pharmaceutical Sciences*. 64(1) : 71-73.

Singh, S. D., Krishna, V., Mankani, K. L., Manjunatha, B. K., Vidya, S.M. and Manohara, Y.N. 2006. Wound healing activity of the leaf extract and deoxyelephantopin isolated from *Elephantopus scaber* Linn. *Indian Journal of Pharmacology*. 37(4) : 238-242.

Sharma, A., Lal, D. and Sutradhar, M. 2017. Effect of growth regulators on callus morphology of Rice anther culture. *Journal of Applied Biology and Biotechnology*. 5 (3): 068-071.