The Effect of Explant Types and Plant Growth Regulators On Callus Induction of Geranium (Pelargonium graveolens L’Her) In Vitro

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Abstract. The purpose of this study was to determine the effect of explant types and plant growth regulators on callus induction of geranium (Pelargonium graveolens L’Her). Callus induction was carried out by culturing leaf and petiole explants from ex vitro geraniums on MS + 0.5 mg/l 2.4-D + 0.1 and 0.3 mg/l Benzyladenine (BA) or Kinetin. Each culture was carried out in 5 replications, cultures were incubated in rooms 25°C and 600 lux. The parameters days for callus induction and percentage of callus formation were observed. Geranium callus formation was influenced by types of explants and plant growth regulators. Petiole explants was able to induce callus better than leaf explants. Days for callus induction at second week and percentage of callus formation of petiole explants were 58 and 58% compared to leaf explants were only 53 and 54%. Plant Growth Regulators BA combined with 2.4-D was able to induce callus better than kinetin. The best callus formation was produced from petiole explants cultured on MS + 0.5 mg/l 2.4-D + 0.3 mg/l BA by 84%. Keywords: Pelargonium graveolens L’Her, Callus, Leaves, Petiole, BA, Kinetin.

1. Introduction
Geranium plant (Pelargonium graveolens L’Her) is one of the plants producing geranium oil that was widely used as an ingredient in the cosmetics, perfume industry and can overcome several health problems.¹² The use of geranium oil was increasingly in demand along with the increasing role of natural products for treatment in various disease conditions.³ The high demand for geranium oil had not been well met by total production. World geranium oil production was estimated at only 250-300 ton, while demand for geranium oil was more than 800 tons annually.⁴ The main constraints to increasing geranium oil production were limited land and superior types of geraniums.⁵ Of the 25 Pelargonium species, only 4 species are important in the production of geranium oil, namely P. graveolens, P. odoratissium, P. capitatum and P. radens.⁶ Based on this there is an opportunity to increase geranium oil production through in vitro culture techniques.

In vitro culture techniques in addition to plant propagation were often used for the production of secondary metabolites.⁷ The callus culture technique is one of the in vitro culture techniques used for the production of secondary metabolites. The application of callus culture had several advantages such as production of secondary metabolites, does not require large areas, the number of explants used is
small, free from pests and diseases, independent of season, can be produced in large quantities, saves time and energy.\textsuperscript{8,10}

The division and multiplication of callus was influenced by several factors including plant growth regulators added to the medium and the type of explants used. 2,4-Dichlorophenoxyacetic acid (2,4-D) is a synthetic auxin which is effective for callus induction and stable because it was not readily decomposed by enzymes released by plants. Benzyladenin (BA) was a synthetic cytokinin that was easily obtained, stable and effective to accelerate the development and growth of callus.\textsuperscript{11} Whereas Kinetin is a cytokinin group that functions for cell division and morphogenesis.\textsuperscript{12} The combination of appropriate plant growth regulators was a major factor in the success of callus culture. Giving auxin and cytokines will stimulate cell division and division and increase protein synthesis, consequently cell metabolism will be affected which will later affect callus growth and secondary metabolite production.\textsuperscript{13} Previous studies explained that the growth response of \textit{Pelargonium graveolens} L'Her callus induced in a combination of plant growth regulators such as 2,4-D, NAA + Kinetin or Zeatin, IAA + BA or Kinetin, IAB + Kinetin and IBP + BA showed different results.\textsuperscript{14}

The use of different types of explants can respond to callus growth and the production of different secondary metabolites.\textsuperscript{15} Leaf explants in \textit{Ocimum sanctum} L. cultured on 2,4-D and Kinetin MS medium showed better callus growth compared to stem explants.\textsuperscript{16} While the growth of callus \textit{Labisia pumila} var. alata induced from stem explants showed better callus growth compared to leaf explants.\textsuperscript{17} The use of appropriate explants in each species is a major factor in the success of callus culture. Therefore, this study aims to determine the effect of explant types and plant growth regulators BA or Kinetin combined with 2,4-D on the formation of geranium callus plants (\textit{Pelargonium graveolens} L'Her).

2. \textbf{Material and Methods}

In this study, leaf and petiole of geranium plants (\textit{Pelargonium graveolens} L'Her) ex vitro culture were used as explants. The explant surface was sterilized with a whitening solution (5.25% NaClO) 20% for 5 minutes and followed by 2 rinsing in sterile distilled water for 5 minutes each. Sterile explants were cultured on MS medium with the addition of plant growth regulators, namely 2,4-D (0.5 mg/l) combined with BA or Kinetin (0.1 mg/l and 0.3 mg/l). The culture was incubated at room temperature (25 ± 1) °C and 600 Lux light. All experiments were carried out 5 times.

The effect of each combination of plant growth regulators and difference in explants used was determined by observing the day of callus appearance, callus growth percentage and callus morphology. Quantitative data were analyzed using ANOVA and the averages were compared using the Duncan Multiple Range Test (DMRT) at a significance level of 5% (P <0.05).

3. \textbf{Results and Discussion}

The results of this study indicated that the formation of geranium callus began to be seen in the second week after initiation. Geranium callus were light green and whitish green, however, each callus had a varied texture. Callus formed on the medium with the addition of BA had a compact texture while callus formed on the medium with the addition of Kinetin had a friable texture (Figure 1). Compact callus can be caused by decreased proliferation in cells that are dividing, this activity was influenced by the auxin contained in the original explant. Giving high concentrations of auxin affects the endogenous auxin content in explants. In addition, the administration of low cytokinin concentrations can also affect the formation of compact textures.\textsuperscript{18} The formation of friable textured callus was affected by the presence of endogenous auxin hormones in the already formed callus. Friable callus requires a balanced combination of auxin and cytokinin.\textsuperscript{19}
Control 0,1 mg/l BA 0,3 mg/l BA 0,1 mg/l Kinetin 0,3 mg/l Kinetin

Figure 1. Response of callus growth from leaf and petiole explants cultured on MS medium with the addition of 0,5 mg/l 2,4-D and combined with plant growth regulators BA or Kinetin at several concentrations.

Most explants were able to form callus with a percentage of callus formation ranging from 20-76% (2nd week) and 16-84% (4th week). Callus growth response was influenced by the type of explants used and plant growth regulators added to the medium. The addition of plant growth regulators BA or Kinetin combined with 2,4-D can increase the percentage of callus formation. The percentage of callus formation in MS medium with the addition of 2,4-D (control) was 20% (2nd week) and 16% (4th week), while the percentage of callus formation in MS medium with the addition of BA ranged from 68-76% (2nd week) and 64-84% (4th week) and Kinetin by 40-64% (2nd week) and 52-60% (4th week) (Figure 2).

There are interactions between the types of explants, the types of plant growth regulators and the concentrations of plant growth regulators to the percentage of callus formation. The best treatment was showed by explants of petiole cultured on media containing 0,3 mg/l BA, then sequentially followed by leaf explants (0,3 mg/l BA), petiole explants (0,1 mg/l BA), leaf explants (0,1 mg/l BA), petiole explants (0,3 mg/l Kinetin), leaf explants (0,3 mg/l Kinetin), petiole explants (0,1 mg/l Kinetin) and leaf explants (0,1 mg/l Kinetin). The treatment of leaf and petiole explants on media containing plant growth regulators BA or kinetin was able to form a better callus compared to control treatments (0,5 mg/l 2,4-D).

The success of callus formation depends on the types of explant, the types of plant growth regulators and the concentrations of plant growth regulators. The appropriate combination of all these components is a major factor in the success of callus formation. The response of callus formation was often influenced by the physiological differences of each explant. The combination of auxin and cytokinin is also an important thing that must be considered. Auxin and cytokinin are plant growth regulators that can enhance cell development and affect cell physiological development.20

In this study, it was known that the different types of explants showed an influence on the formation of callus, although not significant, while the differences in the type of plant growth regulator showed a significant effect on callus formation. The effect of the concentration of plant growth regulators gives different results for each explant. Callus formation was strongly influenced by the interaction and balance between plant growth regulators added to the medium and plant growth regulators contained in cultured cells.21 Several explants experienced growth retardation and caused tissue death. One of the influential factors is browning the tissue. Tissue browning as a result of the activity of copper-containing oxidase enzymes such as polyphenol oxidase or tyrosinase that were released or synthesized and available under oxidative conditions when tissue was injured.22
Figure 2. Effect of explant types and plant growth regulators on the percentage of formation of geranium callus at week 2 and week 4 of the culture period. (A. 2nd week, B. 4th week). Note: Different letters in the notation indicate a significant influence between the types of explants, the types of plant growth regulators and the concentrations of plant growth regulators.
4. Conclusion
Geranium callus formation can be induced from leaf and petiole explants. The average callus formation was initiated at 2nd week after culture. The percentage of callus formation from petiole explants tended to be better compared to leaf explants even though it was not significant. While BA plant growth regulators were be able to form a better callus percentage compared to Kinetin significantly. Concentrations of 0.3 mg/l in medium added with BA and Kinetin were be able to form a better callus compared to concentrations of 0.1 mg/l in each plant growth regulator although not significant.

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