

Effect of Benzyl Adenine Concentration on Callus Induction of Geranium Plants (*Pelargonium graveolens* L'Her) from Petiole and Leaf Explants

Moch. Faizul Huda, Serafinah Indriyani, Wahyu Widoretno*

Department of Biology, Faculty of Mathematics and Natural Sciences, University of Brawijaya, Malang, Indonesia

Abstract

Geranium plant (*Pelargonium graveolens* L'Her) is one of the geranium oil-producing plants that has many benefits. Callus culture is a technique that can be used to plant multiplication and increase production of secondary metabolites. This study aims to determine the effect of the concentration of Benzyl Adenine on the formation of geranium callus from petiole and leaf explants. Callus induction was carried out by culturing petiole and leaf explants on MS medium + 0.1 mg.L⁻¹ NAA + Benzyl Adenine (0; 0.5; 1; 1.5 and 2 mg.L⁻¹). Callus morphological parameters, percentage of callus formation, and time of first callus formation were observed. The formation of geranium callus influenced by the explant type and the concentration of Benzyl Adenine. In the 2nd week, the geranium callus was initiated, light green colored with a compact callus texture. At 4th week, the percentage of callus formation containing NAA 0.1 mg.L⁻¹ of petiole and leaf explants was 20% and 8%, whereas the percentage of callus formation on medium containing 0.1 mg.L⁻¹ NAA combined with 0.5-2 mg.L⁻¹ Benzyl Adenine of petiole and leaf explants was 52-80% and 24-52%. The best percentage of callus formation was found on the culture medium containing 1 mg.L⁻¹ Benzyl Adenine, equaled 80% of petiole explants, and 52% of leaf explants.

Keywords: BA, Callus, Leaf, Petiole, *Pelargonium graveolens* L'Her.

INTRODUCTION

Pelargonium graveolens L'Her or known by the name Geranium plant is one of the geranium essential oil-producing plants that have many benefits such as cosmetics, perfume and can overcome several health problems [1-2]. The high demand for geranium oil has not been well fulfilled by total production. Global geranium oil production is estimated at only 250-300 tons per year, while demand for geranium oil is more than 800 tons per year [3]. Based on this there is an opportunity to increase geranium oil production through callus culture techniques.

Callus culture techniques in addition to plant propagation techniques are also one of the techniques for the production of secondary metabolites [4]. Callus culture techniques has several advantages such as controlled environmental factors so that it is not influenced by climate, season, pests and plant diseases and can produce secondary metabolites that are more consistent in a shorter period of time [5].

Growth and formation of callus in culture was influenced by plant growth regulators (PGR) auxin and cytokinin. The use of PGR alone or in combination with the right concentration can induce and increase callus growth so that better

results are obtained. The use of a combination of PGR auxin and cytokinin greatly influences the determination of the type of morphogenesis. The balance of hormones in cells against auxiliary PGR auxin and cytokinin determines the differentiation process [6-7]. The combination of auxins and cytokinins properly stimulates cell division so that it can induce callus formation [8].

Callus induction is strongly influenced by the type and concentration of plant growth regulators. NAA and BA are types of auxins and cytokinins commonly used for callus induction. In *Astragalus nezakate* plants, the response of callus formation and bud regeneration on MS medium with the addition of NAA and BA was more effective than the addition of other plant growth regulators [9]. The use of 0.05 mg.L⁻¹ NAA and 5 mg.L⁻¹ BA could increased the frequency of callus formation in *Catalpa bungei* [10]. *Artemisia absinthium* callus induction on MS medium with the addition of BA combined with NAA, 2,4-D or IBA is also able to form callus up to 100% [11].

The use of different types of explants can also provide a different callus growth response [12]. The use of appropriate explants in each species is a major factor in the success of callus culture. Callus formation from *Eurycoma longifolia* Jack explants was faster than leaf explants [13]. While the use of *Catalpa bungei* stem explants is less responsive in forming callus compared to leaf explants [10].

*Correspondence address:

Wahyu Widoretno

Email : wahyu_widoretno@yahoo.com

Address : Dept. Biology, University of Brawijaya, Veteran Street, Malang, Indonesia. 65145

The results of these studies, it appears that the response of callus formation depends on plant genotype, type of explant and formulation of the medium used. This study aims to determine the effect of Benzyl Adenine concentration on callus formation of geranium plants (*Pelargonium graveolens* L'Her.) from petiole and leaf explants.

MATERIALS AND METHODS

Petiole and leaf explants of geranium plant (*Pelargonium graveolens* L'Her) were used for callus induction. The explants surface was sterilized using a 20% commercial whitening solution (containing 5.25% NaClO) for 5 minutes and rinsed by using sterile distilled water twice each for 5 minutes. Sterile explants were cultured on MS medium with the addition of plant growth regulators, namely NAA (0.1 mg.L⁻¹) combined with several concentrations of plant growth regulators BA (0; 0.5; 1; 1.5 and 2 mg.L⁻¹). Each treatment was repeated 5 times (bottle), each bottle was cultured with 5 explants. The culture was incubated at room temperature (25 ± 1)°C and 600 Lux light.

The effect of each combination treatment of plant growth regulators on petiole and leaf explant was determined by observing callus morphology and percentage of callus formation, including time of first callus formation. Quantitative data were analyzed using ANOVA, and the averages compared using the Duncan multiple range tests (DMRT) at a significance level of 5% (P <0.05).

RESULTS AND DISCUSSION

Geranium callus can be induced from petiole and leaf explants cultured on MS medium with the addition of plant growth regulator Benzyl Adenine (BA) combined with NAA. Formation of the geranium callus was initiated in the 2nd week

after induction. The formed geranium callus was light green with a compact callus texture first visible on the edge of the explant cut, followed by the entire explant surface (Fig. 1).

Induction of geranium callus influenced by the type of explant and BA concentration, both factors have a significant effect on callus formation and growth. At 4th week after induction, the percentage of callus formation from petiole explants showed better results compared to leaf explants, which was 54.4% compared to 32.8% (Fig. 2). The percentage of callus formation was different. It indicates that the ability of petiole and leaf explant of geranium was different.

After it was induced in MS medium by the addition of several concentrations of Benzyl Adenine, the percentage of geranium callus formation increased significantly. The addition of PGR BA on MS medium combined with NAA was able to produce a better callus formation percentage of petiole and leaf explant compared to the percentage of callus formation on MS media with NAA addition (control). The percentage of callus formation on the medium containing 0.1 mg.L⁻¹ NAA was 20% of petiole explant and 8% of leaf explant, whereas the percentage of callus formation on the medium with the addition of 0.5-2 mg.L⁻¹ BA combined with 0.1 mg.L⁻¹ NAA was 52-80% of petiole explant and 24-52% of leaf explant.

The effect of different concentrations of PGR BA could affect the percentage callus formation of geranium. The percentage of callus formation on the medium with the addition of 1 mg.L⁻¹ BA with a combination of 0.1 mg.L⁻¹ NAA indicated the best results and increased significantly (± 4 times higher than the control) equaled 80% of petiole explants and 52% of leaf explants (Fig. 3).

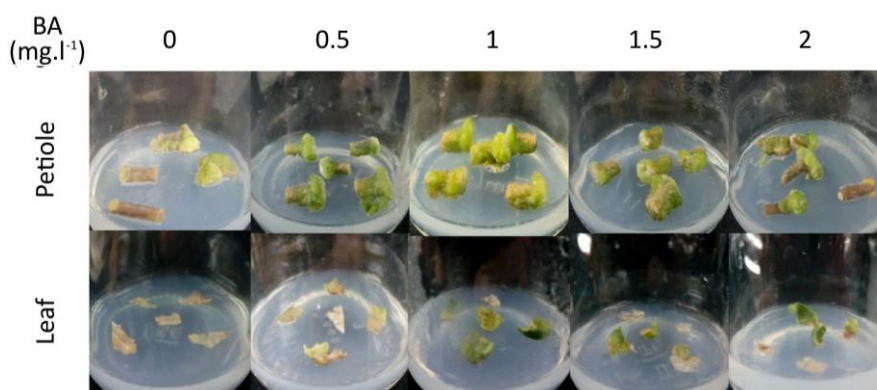


Figure 1. Response of callus growth from petiole and leaf explants on the medium with the addition of plant growth regulator 0.1 mg.L⁻¹ NAA combined with several concentrations of BA

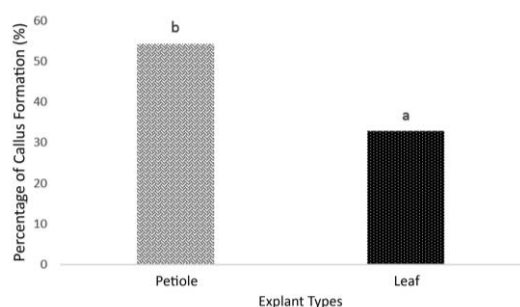


Figure 2. Effect of explant types on percentage of formation of geranium callus at week 4 of culture. **Note:** The same lowercase notation shows no significant effect (DMRT $P < 0.05$)

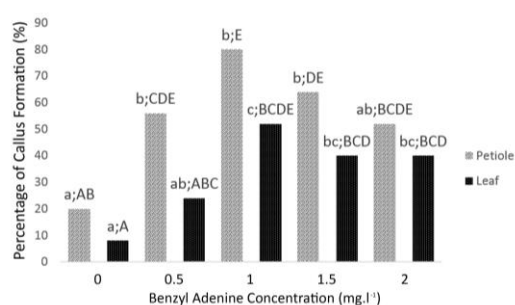


Figure 3. Effect of explant type and various concentrations of PGR BA combined with 0.1 mg.l⁻¹ NAA on the percentage of geranium callus formation. **Note:** The same lowercase notation indicate no significant effect between different PGR BA concentrations, while the same uppercase notation indicates no significant effect between different types of explants (DMRT $P < 0.05$)

The different responses of the formation and growth of different callus of petiole and leaf explants could be influenced by the physiological conditions of each explant. Different explants indicated different cleavage responses [14]. Several factors, such as plant genotypes, explant sources, mediums, and plant growth regulators on the medium, influenced formation and growth of callus [15]. Younger tissue and actively splitting explants provided faster and more responsive callus formation [16,17].

Besides being influenced by the type of explants, the formation and growth of callus were also influenced by the balance of plant growth regulators used. Benzyl adenine was a stable and effective synthetic cytokinin to accelerate callus development and growth [18]. The combination of suitable plant growth regulators was a major factor in the success of callus culture [10]. The difference in frequency of callus formation could be influenced by the

balance of concentration of plant growth regulators used [17].

The addition of exogenous plant growth regulators into the medium increased the concentration of endogenous hormones in the cell. It can trigger the process of growth and tissue development [19]. The right combination of plant growth regulators (auxin and cytokinin) will stimulate cell division [8]. Plant growth regulators exclusively regulate the division and growth of plant cells. It served to trigger multiplication callus cells that were important for the production of secondary metabolites [20].

CONCLUSION

Geranium callus formation could be induced from petiole and leaf explants. In the 2nd week, the geranium callus was initiated. The percentage of callus formation of petiole explants (54.4%) was better than that of leaf explants (32.8%). The addition of PGR BA combined with NAA could increase the percentage of callus formation from petiole and leaf explants. Benzyl Adenine, with a concentration of 1 mg.l⁻¹, was able to form callus with the highest percentage of explants petiole (80%) and leaf explants (52%).

REFERENCES

- [1] Saxena, G., C.V. Praveen, R. Laiqur, B. Suchitra, R.S. Shukla, K. Sushil. 2008. Selection of leaf blight-resistant *Pelargonium graveolens* plants regenerated from callus resistant to a culture filtrate of *Alternaria alternata*. *Crop Prot.* 27. 558-565.
- [2] Benslima, A., B.A. Manel, B. Mohamed, T. Al-Ibrahim, B. Tahia, A. Nouredine, G. Radhouane. 2013. Antioxidant properties of *Pelargonium graveolens* L'Her essential oil on the reproductive damage induced by deltamethrin in mice as compared to alpha-tocopherol. *Lipid Health Dis.* 12(30). 1-9.
- [3] Rabuma, T. 2015. In vitro propagation of geranium (*Pelargonium graveolens* L.) from nodal culture. *J. Agr. Sci. Tech.* 4(2). 23-34.
- [4] Palupi, A.D., Sholichatun, D.M. Soerya. 2004. The effect of 2,4-D and BA on essential oil production from neem (*Pogostemon cablin* Benth.) callus culture. *BioSMART.* 6(2). 99-103.
- [5] Purnamaningsih, R., M. Ashrina. 2011. The effect of BAP and NAA on callus induction and artemisinin content of *Artemisia annua* L. *Berita Biologi.* 10(4). 481-489.
- [6] Decruse, S.W., A. Gangaprasad, S. Seenii, M.V. Sarajini. 2003. Micropropagation and ecorestoration of *Vanda spathulata*, an

- exquisite orchid. *Plant Cell Tiss. Org. Cult.* 72. 199-202.
- [7] Miyashita, T., O. Takafumi, S. Fukashi, A. Hajime, H. Yoichiro. 2009. Plant regeneration with maintenance of the endosperm ploidy level by endosperm culture in *Lonicera caerulea* var. *emphylocalyx*. *Plant Cell Tiss. Org. Cult.* 98(3). 291-301.
- [8] Widiastoety, D. 2014. Effect of auxin and cytokinin on the growth of *Mokara* orchid plantlets. *J. Hort.* 24(3). 230-238.
- [9] Erisen, S., M. Yorgancilar, E. Atalay, M. Babaoglu, A. Duran. 2010. Callus induction and plant regeneration of the endemic *Astragalus nezaketiae* in Turkey. *Electron. J. Biotechn.* 13(6). 1-7.
- [10] Juan, L., W. Lihua, L. Jing, W. Junhuy. 2010. Effect of different plant growth regulators on callus induction in *Catalpa bungei*. *Afr. J. Agric. Res.* 5(19). 2699-2704.
- [11] Zia, M., R. Rehman, M.F. Chaudhary. 2007. Hormonal regulation for callogenesis and organogenesis of *Artemisia absinthium* L. *Afr. J. Biotechnol.* 6(16). 1874-1878.
- [12] Tarrahi, R., F. Rezanejad. 2013. Callogenesis and production of anthocyanin and chlorophyll in callus cultures of vegetative and floral explants in *Rosa gallica* and *Rosa hybrida* (Rosaceae). *Turk. J. Bot.* 37. 1145-1154.
- [13] Rosmaina, Zulfahmi, P. Sutejo, Ulfiatun, Maisupratina. 2015. Callus Induction of *Eurycoma longifolia* Jack by leaf and petiole explant. *Jurnal Agroteknologi.* 6(1). 33-40.
- [14] Savita, V., G.S. Virk, A. Nagpal. 2010. Effect of explant type and different plant growth regulators on callus induction and planlet regeneration in *Citrus jambhiri* Lush. *Environ. We. Int. J. Sci. Tech.* 5. 97-106.
- [15] Kumari, S., R.K. Pandey, K. Uttam. 2015. In vitro callus induction from two different explants stem and leaf in *Charthamus tinctorius* Linn. *Eur. J. Exp. Biol.* 5(2). 1-4.
- [16] Zulkarnain. 2011. Kultur jaringan tanaman. Bumi Aksara. Jakarta.
- [17] Durrani, N.S., D. Ahmad, A. Jalal, H. Rajab, M.S. Khan. 2017. The effect of explant sources and growth regulators on callus induction and regeneration in different tomato cultivars. *J. Anim. Plant Sci.* 27(2). 481-489.
- [18] Indah, P.N., D. Ermavitalani. 2013. Induksi kalus daun nyamplung (*Calophyllum inophyllum* Linn.) pada beberapa kombinasi konsentrasi 6-Benzylaminopurine (BAP) dan 2,4-Dichlorophenoxyacetic Acid (2,4-D). *Jurnal Sains dan Seni Pomits.* 2(1). E-1 – E-24.
- [19] Lestari, E.G. 2010. Peranan zat pengatur tumbuh dalam perbanyakan tanaman melalui kultur jaringan. *Jurnal Agrobiogen.* 7(1). 63-68.
- [20] Adil, M., X. Ren, D.I. Kang, L.T. Thi, B.R. Jeong. 2018. Effect of explant type and plant growth regulators on callus induction, growth and secondary metabolites production in *Cnidium officinale* Makino. *Mol. Biol. Rep.* 45. 1919-1927.