Biosaintifika 14 (2) (2022): 219-225 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Callus Development from Potato (*Solanum tuberosum*) Stem at Various Concentrations of Benzylaminopurine

Megananda Aprilia, Nintya Setiari*, and Yulita Nurchayati

Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Jalan Prof Soedarto, Tembalang, Semarang, Central Java, Indonesia, 50275

*Corresponding Author: nintyasetiari@yahoo.co.uk

Submitted: 2022-04-20. Revised: 2022-06-26. Accepted: 2022-08-31

Abstract. Potato has the potential for food diversification.The propagation method in a short period is needed. One of the methods used is plant tissue culture. Benzyl Amino Purine (BAP), determine the success of propagation by tissue culture method. This research aimed to study the use of different BAP concentrations for callus development from stem explants. The explants put on culture medium added by 0, 1, 2, 3 ppm BAP. The development of the stem explant observed every week for a month. The results showed that callus formed in all media. Without BAP treatment, callus were formed after 2 weeks and got browning, then stopped growing. Callus grew and showed differentiation by application of all the BAP concentration. Callus greowth was optimally at 2 ppm BAP treatment. The callus from 1 ppm BAP produced the most number of roots, shoots and leaves than another concentration. This experiment showed that different BAP concentrations affected callus development of S. tuberosum from stem explant. The conclusion was callus growth has obtained by the treatment of 2 ppm BAP, while the development of callus has obtained on addition of 1 ppm BAP. The novelty of this research is the callus induction method from potato sprout stems grown from potato seeds with plant growth regulators Benzyl Amino Purine. Callus induction method from potato sprout stems grown from potato seeds can be a guide for embryogenic callus induction.

Key words: buds; callus; cytokinin; potato; stem

How to Cite: Aprilia, M., Setiari, N., Nurchayati, Y. (2022). Callus Development from Potato (Solanum tuberosum, L.) Stem at Various Concentrations of Benzylaminopurine. *Biosaintifika: Journal of Biology & Biology Education*, *14* (2), 219-225.

DOI: http://dx.doi.org/10.15294/biosaintifika.v14i2.35703

INTRODUCTION

Solanum tuberosum is one of the plants as a source of carbohydrate (Buchory, 2008). The demand for seedling potatoes in Indonesia is increasing in large quantities. Upon this time, tubers used as a seedling in conventional potato propagation techniques. Potatoes seed is never used for propagation. Potatoes seed can be an alternative raw material for plant propagation. Plant propagation that uses tissue culture techniques or in vitro culture can solve the increasing potatoes demand.

In vitro culture is a technique that uses parts of a plant to be grown into whole plants in aseptic conditions. It can also produce plant seedling in large quantities in a short period (Kumlay and Ercisli, 2015). One of the in vitro cultures method for plant multiplication is callus culture. The callus is an important source of planting material to regenerate new plants (Das et al. (2018); Mohajer et al. (2012)). Tissues and organs meristematic were easier to proliferate form a callus. One of the meristematic explants that have vascular tissue is the stem of sprout explants. Potato's sprouts are chosen to be utilized potato seeds that have never been used for propagation

One of the important factors in in vitro multiplication is growth regulators added to the media. Plant growth regulators that are involved in callus induction are auxin and cytokinin. Dwiyani et al., (2013), Rivai et al., (2014) and Sugiyarto and Kuswandi, (2014) stated that callus could be formed with auxin and cytokinin in the right concentration ratio. Auxin is important for callus formation, while cytokinin promotes callus proliferation. In this study, plant growth regulators used for callus induction are 2,4-Dichlorophenoxyacetic and Benzylaminopurine (BAP).

Previous studies about potato tissue culture are callus induction which has an optimal concentration in 4 ppm 2,4-D (Asmono and Sari, 2016) and Benzyl Aminopurine (BAP) (Kumlay and Ercisli, 2015). BAP is a cytokinin that important in cell division. Therefore, it is necessary to research the callus induction from the stem of sprout potato explant derived from seeds on Murashige and Skoog (MS) medium with different BAP concentrations. The purpose of this research was to obtain the optimal concentration of BAP for callus developmen from stem seedling from seed. In the future, this tomato callus culture method is possible to be used for

embryogenic callus and somatic embryos production.

METHODS

This research was conducted from November to February 2019 at Plant Biology Structure and Function Laboratory of the Department of Biology, University of Diponegoro, Semarang, Central Java, Indonesia.

Plant materials and culture conditions

Potato fruits were obtained from Wonosobo, Central of Java. The seeds were separated from the pulp and then sterilized with a 5% fungicide, then 10% Sodium Hypochlorite (NaClO), and 25% alcohol, the last one was rinsed with sterile distilled water three times. Sterilized seeds were planted on ½ MS medium. Thereafter, the cultures were incubated in a culture bottle at a temperature of 25°C with 1000 lux continuous light. The stem of potato sprout which about 5 cm in height (Figure 1.) were used as explants.

The explant in the form of stems from potato sprouts were cut transversely and put on ½ MS (Murashige and Skoog) medium supplemented with (0, 1, 2, 3) ppm BAP, and cultures were maintained for a months at temperature 25°C with 1000 lux continuous light.

Figure 1. Potato sprouts 30 days old after planting in vitro

Observation and data analysis

The observation of callus development includes the percentage of callus formation, length, and width of callus, the number of roots, shoots, and leaves were counted visually. The color and texture of the callus were observed with optilab. Observations are carried out every week for a month.

$$\text{Percentage of callus formation} = \frac{\text{Callus induced explant}}{\text{Total explant planted}} \times 100\%$$

This experiment examined 4 different levels of concentration of BAP (0, 1, 2, 3) with 4 replicates. The data were analyzed by ANOVA and followed by Duncan's test.

RESULTS AND DISCUSSION

The result showed that all the treatments used were able to induce callus formation (Table 1). Callus was yellowish-green with a compact, friable and watery texture. Callus at medium added 2 ppm BAP was the greatest than other concentrations (g). Callus from 1 ppm and 3 ppm BAP had a medium size. The smallest callus was formed at 0 ppm BAP. That callus was getting browning on the 14 days after planting, and brownish-yellow in color with a compact, friable and watery texture (Figure 2e). The callus was formed on the seventh day after planting. This is in accordance with Setiaji et al (2020) that callus from tomato hypocotyl explants was formed on days 5 -13.

The callus from potato stem of sprout showed that of potato stem were able to respond in the used combination of 2,4-D and BAP because 2,D and BAP the two Plant Growth Regulators (PGRs) are needed for callus formation. 2,4-Dichlorophenoxy-acetic acid (2,4D) is an auxin group and BAP is a cytokinin class. Mohajer et al., (2012) reported that a combination of plant growth regulator auxin and cytokinin were needed for callus formation. The colour of the yellowish-green callus shows the callus in good condition. According to Hariyati et al., (2016), the callus color was one indicator of callus quality. The green callus shows the formation of chlorophyll. Sugiyarto and Kuswandi, (2014), stated that the green colour of callus was a result of the influence of cytokinins (BAP) in the formation of chlorophyll. Based on Cortleven et al., (2016) cytokinins directly regulate the chlorophyll biosynthesis gene. Cytokinins give signals to regulate ARR1-type B receptors which will bind to the HEMA1 gene (Glutamyl-tRNA Reductase) in the chlorophyll biosynthesis pathway so that the level of chlorophyll formation is stable.

In this research, callus formed with a compact, friable and watery texture. It showed that callus gave a response to the PGR. Royani et al., (2015), stated that the characteristic of callus depends on the growth regulator used. This study used a combination of auxin with different BAP concentrations.

Table 1. Average potato callus formation, callus color, callus texture at ½ MS Medium added different BAP concentrations at 32 days after planting.

BAP Consentration (ppm)	Callus formation (%)	Callus Color	Callus Texture	Callus formation
0 (B0)	100	Brownish Yellow	Compact, friable & watery	+
1 (B1)	100	Yellowish green	Compact, friable & watery	++
2 (B2)	100	Yellowfish green	Compact, friable & watery	+++
3 (B3)	100	Yellowfish green	Compact, friable & watery	++

Description: + : Small callus | ++ : Medium callus | +++ : Large callus

Asmono and Sari, (2016) asserted that the watery-friable texture on potato callus caused by auxin, which increases the elasticity of cell walls. Based on Majda and Robert, (2018), auxin stimulates structural protein to pump H+ ions into the cell wall and activates enzyme expansin to cut off the hydrogen bonds of cellulose molecules which form the cell wall, thus water come into the cell by osmosis.

Callus formed at medium MS without BAP was browning and there was no developing in 14 days after planting (Figure 3a) due to insufficient endogenous auxin and cytokinin (BAP) required for callus formation and no addition of exogenous auxins and cytokinins. Cytokinin was needed in the development of callus. Following Jameson and Song, (2016), cytokinin played a role in cell division and callus regeneration. Cytokinins activate the phosphatase enzyme which will release phosphate from the CDK protein (cyclin dependent kinase). CDK protein which contains only one phosphate will become active and induce the cell to enter the mitotic phase (Schaller et al., 2014). Callus at 3 ppm BAP also turned into brown in 30 days after planting (Figure 3b). The browning is caused by aging in the callus. As stated by Fauzy et al., (2013), the browning callus was caused by the aging of the callus cells. Besides, according to Admojo & Indrianto, (2016), the cause of browning was the presence of phenolic compounds that appeared and accumulated due to organ injury.

The observation result of the BAP concentration effect was different toward the callus length and width. It showed that at B2, the largest callus formed with 1.23 cm in the length and 0.7 cm in the width (Figure 4). The average length and width callus at 0

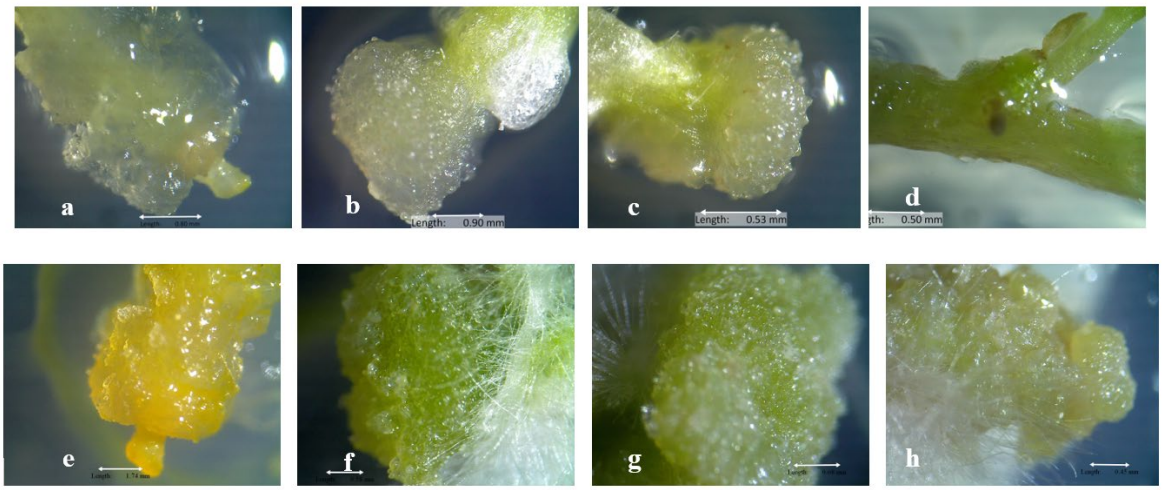

Figure 2. The development of callus at 7 days after planting (a-d) 32 days after planting (e-h), callus on MS medium added 0 ppm BAP (B0) (a & e), 1 ppm BAP (B1) (b & f), 2 ppm BAP (B2) (c & g), and 3 ppm BAP (B3) (d & h) treatment. Note: X: Absorbant hair formed at the callus

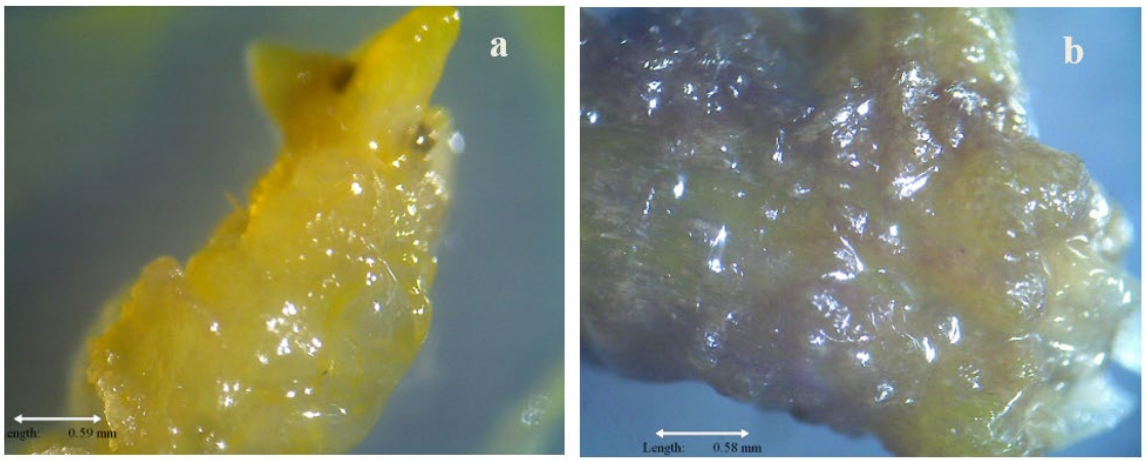

Figure 3. Potato callus got browning a) B0 in 14 days after planting and b) B3 in 30 days after planting

ppm BAP was the smallest, with 0.66 cm and 0.26 cm. These cases were caused by a callus at 0 ppm BAP that get browning and not developing. The smallest callus among the other growth was in 1 ppm BAP, with 0.96 cm in length and 0.36 cm in width. Meanwhile, the callus in 3 ppm BAP media was 1.06 cm in length and 0.36 cm in width.

Callus formed at 1 ppm BAP was the smallest, but it had more buds, roots and leaves compare with the other concentrations (Figure 5). The least number of buds and roots formed in the callus growing in media was in the 3 ppm BAP. The number of them was 0.67 and 2.67. Whereas, at 2 ppm BAP, the total buds and roots were 1.00 and 6.00 (Figure 5). The fewest leaves were formed at 3 ppm BAP with 6.33 strands, while at the B3 produced 6.67 strand leaves (Figure 5).

Callus at 2 ppm BAP concentration was larger than callus from other treatments. It showed that the concentration of 2,4-D 0.1 ppm with the BAP concentration of 2 ppm was the optimal concentration in the formation explant callus of potato sprout stem (Figure 6). As stated by Shah and George, (2019), the combination of auxin and cytokinin produced a better callus induction and biomass than adding only one growth regulator. Cytokinins are hormones involved in cell division. This is supported by Lipavská et al., (2011) which stated that in the G2 phase of cell division, cytokinins induce cyclin-dependent protein kinase (CDKs) which play a role in gene transcription at the stage of DNA replication. These kinases depend on cyclins as catalysts. There are two types of cyclin-dependent kinases, type A (CDKA) and type D (CYCD). This

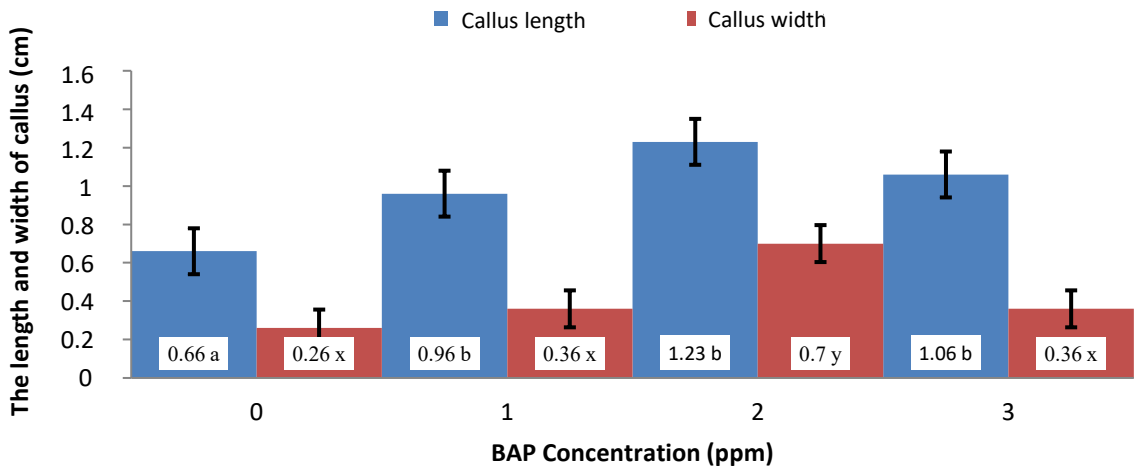

Figure 4. The development of the length and width callus potato by different concentration of BAP

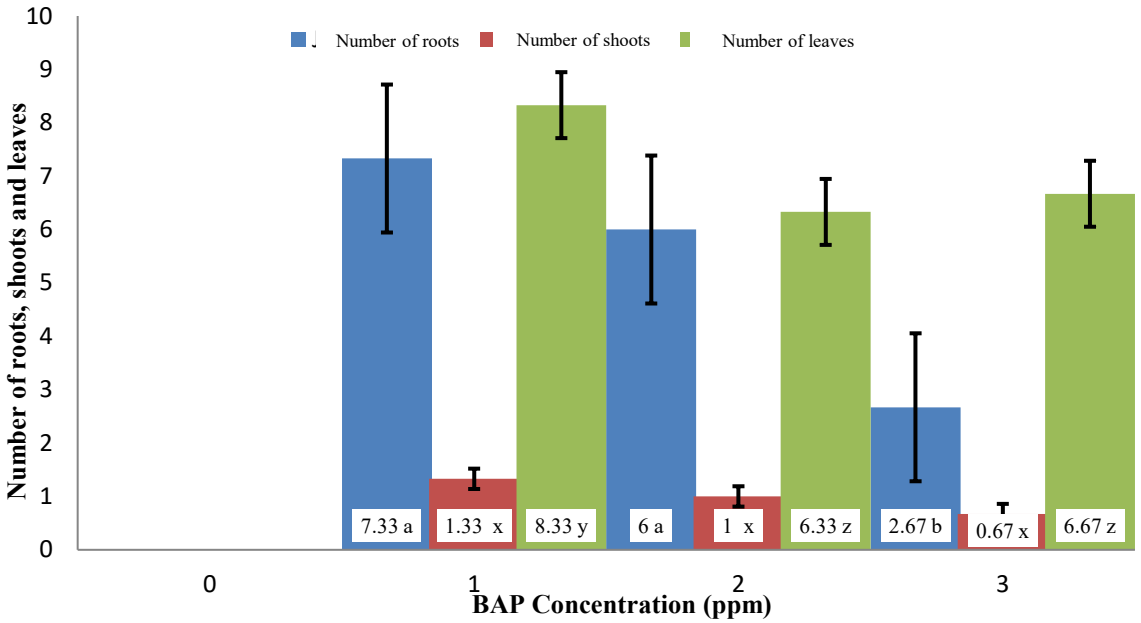

Figure 5. The number of roots, shoots, and leaves potato in different concentration of BAP Note: The following number with the same letters showed there is no significance of the "Duncan" follow-up test. The bar is the SD grades

kinase is central to the G1/S phase transition in cell division. CDKA is a key protein for controlling cell division in A. thaliana, and is present at constant levels throughout the cell cycle. Furthermore, cytokines got involve in the activation of the enzyme phosphatase to reduce phosphate. Phosphate was reduced because it can inhibit the protein CDKs toward the mitosis phase (M) cell division, thus cytokinins can accelerate the G2 phase in cell division.

Callus at 3 ppm developed in small size, it is suggested by the high BAP to the explant. The growth regulator was effective in the low concentration, with a too high concentration that would inhibit cell division. This is supported by Hariyati et al., (2016) that, the higher BAP concentration will inhibit the callus formation in the chrysanthemum plant. In line with Kieber and Schaller (2014) in plant tissues contain cytokinin oxidase enzymes. This enzyme cleaved the N6 side chain from a subset of cytokinins. The oxidation cytokinin enzyme can limit the effect of cytokinin if the concentration given was too high.

The most buds, roots, and leaves are formed at the callus in the media added 1 ppm BAP (B1) (Figure 6). This showed that the combined concentration of 0.1 ppm 2,4-D and 1 ppm BAP were optimal for the formation of buds, roots, and leaves. The formation buds at certain BAP concentrations are following the research by Kumlay and Ercisli (2015) and Rizal et al., (2017) who reported that the addition of different BAP concentrations to the media would affect the induction of shoots in vitro. There were not many buds formed at the callus in the media added 3 ppm BAP (B3) (Figure 6). This is supported by Wartina (2011), PGR which is used excessively will inhibit plant morphogenesis.

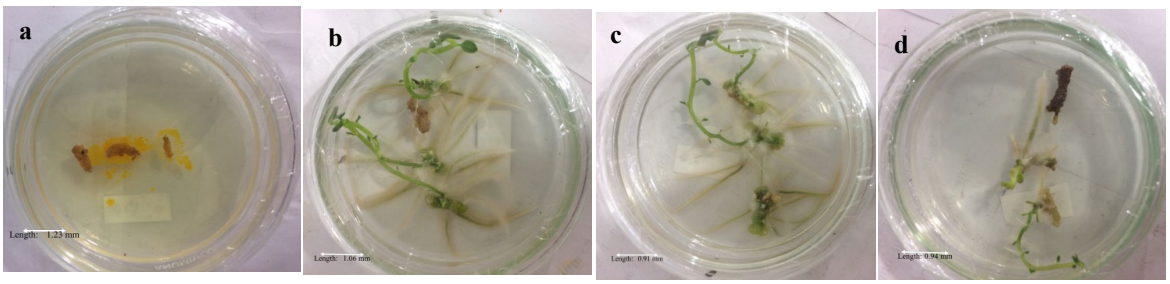

Figure 6. Callus formed in concentration a) without BAP (B0), b) 1 ppm BAP (B1), c) 2 ppm BAP (B2), d) 3 ppm BAP (B3)

In this research, the most root formation occurred at MS medium added 1 ppm BAP (B1) treatment. Root formation usually occurred in the media with high auxin and low cytokinin concentrations. This is supported by Das et al. (2018) which stated that the higher concentration of auxin, the more effective the root induction. This study used a lower auxin concentration than cytokinin. However, the roots could form on the all given concentration (Figure 6). This was caused by the 1 ppm BAP was in accordance with the endogenous auxin hormone within the explant, so it was needed in root induction. This is supported by the statement of Indah and Ermavitalini (2013) that the effectiveness of exogenous hormones depends on the endogenous hormones concentration in plant tissue. Following Sugimoto et al. (2011) which stated that in the roots formation, pericycle cells adjacent with the xylem had to split, differentiate and all the cells layers in the roots formed a lateral root primordium.

In this research, the most leaves formation occurred at 1 ppm BAP. This was because the number and length of shoots at 1 ppm BAP affecting the number of stem nodes, therefore more leaves were formed. There were more buds at 2 ppm BAP than 3 ppm BAP (Figure 6). However, more leaves were formed at 3 ppm BAP. This was caused by the BAP role in shortening the nodes, thus the more nodes formed, the more leaves would be formed. This was in line with Pratama et al. (2014), stated that cytokinins with too high concentrations could cause dense stem segments. Besides the BAP, the given auxin could induced leaves. This is supported by Iqbal et al. (2017) which stated that auxin was important in leaf development. Schepetilnikov and Ryabova (2017) asserted that auxins bound by the transporter Auxin Binding Protein 1 (ABP1), then auxin stimulates the TIR / AFB F-box protein and Aux / IAA receptors. Furthermore, the Aux / IAA protein activated ubiquitin ligase enzymes so the concentration of Auxin Response Factor (ARF) was higher and increased the transcription of genes that could develop leaves.

The novelty of this research is the callus induction method from sprouted stems grown from potato seeds. Furthermore, the formed callus can be induced to become embryogenic callus which is important for the formation of somatic embryos. Somatic embryos that grow further can be induced with growth regulators to form shoots. This callus induction method until it grows into shoots can be used as a micropropagation method.

CONCLUSION

Stem explant from the shoot of potatoes has been able to form callus in all of BAP concentrations treatment. MS medium without BAP has caused callus browning and callus has not developed in 14 days after planted. Optimal callus growth has obtained in the treatment of media with 2 ppm BAP, while the optimal formation of roots, shoots, and leaves has obtained on the media with the addition of 1 ppm BAP.

Suggestions for further research from the results of this study is to grow callus on Murashige and Skoog medium plus growth regulator thidiazuron for induction of embryogenic callus and somatic embryos.

ACKNOWLEDGEMENT

The author would like to thank the Development and Implementation Research (RPP) and Diponegoro University (329-51/UN7.P4.3/PP/2019) for the financial support

REFERENCES

Aritonang, Admojo, L., Indrianto, A. (2016). Pencegahan Browning Fase Inisiasi Kalus pada Kultur Midrib Daun Klon Karet *(Hevea brasiliensis)* PB 330. Indones. J. Nat. Rubber Res. 34, 25–34.

Asmono, S.L., Sari, V.K. (2016). Induksi Kalus Dari Beberapa Kultivar Tanaman Kentang (*Solanum Tuberosum* L.) Dataran Medium Secara In Vitro Menggunakan Variasi Konsentrasi 2,4-D. J. Ilm. Inov. 16, 116–121. https://doi.org/10.250 47/jii.v16i2.295

Buchory, A.K.A. (2008). Pengaruh Auksin dan Sitokinin terhadap Pertumbuhan dan Perkem bangan Jaringan Meristem Kentang *Kultivar Granola*. J. Hortik. 18, 380–384.

Cortleven, A., Marg, I., Yamburenko, M. V., Sch licke, H., Hill, K., Grimm, B., Schaller, G.E., Schmülling, T. (2016). Cytokinin regulates the etioplast-chloroplast transition through the two-component signaling system and activation of chloroplast-related genes. Plant Physiol. 172, 464–478. https://doi.org/10.11 04/pp.16.00640

Das, P., Tanti, B., Borthakur, S.K. (2018). In vitro callus induction and indirect organogenesis of Brucea mollis Wall. Ex Kurz – A potential medicinal plant of Northeast India. South African J. Bot. 119, 203–211. https://doi.org /10.1016/j.sajb.2018.09.012

Dwiyani, R., Yuswanti, H. & Darmawati, I.A.P. (2013). Menciptakan Kultivar Baru Anggrek *Vanda tricolor Lindl. var. suavis* forma Bali yang Cepat Berbunga melalui Rekayasa Genetika Tahun ke 1 dari rencana 3 tahun Universitas Udayana.

Fauzy, E., Mansyur, Husni, A. (2013). Pengaruh Penggunaan Media Murashoge dan Skoog (Ms) dan Vitamin terhadap Tekstur, Warna dan Berat Kalus Rumput Gajah (*Pennisetum purpureum*) Cv. Hawaii Pasca Radiasi Sinar Gamma pada Dosis LD50 (In Vitro). J. Chem. Inf. Model. https://doi.org/10.1017/CBO9781107415324.004

Hariyati, M., Bachtiar, I., Sedijani, P. (2016). Induksi Kalus Tanaman Krisan (*Chrysanthemum Morifolium*) Dengan Pemberian Benzil Amino Purin (Bap) Dan Dichlorofenoksi Acetil Acid (2,4 D). J. Penelit. Pendidik. IPA 2. https://doi.org/10.29303/jppipa.v2i1.37

Indah, P.N., Ermavitalini, D. (2013). Induksi Kalus Daun Nyamplung (*Calophyllum inophyllum Linn.*) pada Beberapa Kombinasi Konsentrasi 6-Benzylaminopurine (BAP) dan 2,4-Dichlorophenoxyacetic Acid (2,4-D). Sains dan Seni Pomits 2, 1–6.

Iqbal, N., Khan, N.A., Ferrante, A., Trivellini, A. (2017). Ethylene Role in Plant Growth, Development and Senescence : Interaction with Other Phytohormones 8, 1–19. https://doi.org/10.3389/fpls.2017.00475

Jameson, P.E., Song, J. (2016). Cytokinin: A key driver of seed yield. J. Exp. Bot. 67, 593–606. https://doi.org/10.1093/jxb/erv461

Kieber, J.J., Schaller, G.E. (2014). Cytokinins. Arab. B. 12, e0168. https://doi.org/10.1199/tab.0168

Kumlay, A.M., Ercisli, S. (2015). Callus induction, shoot proliferation and root regeneration of potato (*Solanum tuberosum* L.) stem node and leaf explants under long-day conditions. Biotechnol. Biotechnol. Equip. 29, 1075–1084. https://doi.org/10.1080/13102818.2015.1077685

Lipavská, H., Mašková, P., Vojvodová, P. (2011). Regulatory dephosphorylation of CDK at G2/M in plants: Yeast mitotic phosphatase cdc25 induces cytokinin-like effects in transgenic tobacco morphogenesis. Ann. Bot. 107, 1071–1086. https://doi.org/10.1093/aob/mcr016

Majda, M., Robert, S. (2018). The role of auxin in cell wall expansion. Int. J. Mol. Sci. 19. https://doi.org/10.3390/ijms19040951

Mohajer, S., Taha, R.M., Khorasani, A., Yaacob, J.S. (2012). Induction of different types of callus and somatic embryogenesis in various explants of Sainfoin (*Onobrychis sativa*). Aust. J. Crop Sci. 6, 1305–1313.

Pratama, A.R., Sugiyono, S., Prayoga, L., Husni, A. (2014). Upaya Memacu Pertumbuhan Tu nas Mikro Kentang Kultivar Granola dengan Jenis dan Konsentrasi Sitokinin Berbeda. Scr. Biol. 1, 209. https://doi.org/10.20884/1.sb.2014.1.3.553

Rivai, R.R., Husni, A., Purwito, A. (2014). Induksi Kalus dan Embrio Somatik Tanaman Jambu Biji Merah (*Psidium guajava* L.). Bul. Agrohorti 2, 49. https://doi.org/10.29244/agrob.2.1.49-58

Rizal, S., Murdiono, W.E., Nihayati, E. (2017). Pengaruh pemberian beberapa konsentrasi kinetin terhadap induksi tunas aksilar tanaman kakao (*Theobroma cacao* L.) secara in vitro. J. Produksi Tanam. 5, 1512–1517.

Royani, I., Zulkifli, L., Prapti, S. (2015). Jurnal penelitian pendidikan IPA. Penelitian-Pendidikan 1, 69–76.

Schaller, G. Eric, Ian H. Street, and Joseph J. Kieber. "Cytokinin and the cell cycle." Current opinion in plant biology 21 (2014): 7-15.

Schepetilnikov, M., Ryabova, L.A. (2017). Auxin signaling in regulation of plant translation reinitiation. Front. Plant Sci. 8, 1–15. https://doi.org/10.3389/fpls.2017.01014

Setiaji, A., Annisa, R. R., Rumiyati, R., & Semiarti, E. (2020). Induction and Growth Kinetics Callus of Tomato (*Solanum lycopersicum*). Biosaintifika: Journal of Biology & Biology Education, 12 (1), 35-41

Shah, M.R., George, I.A. (2019). Increased bio mass and pigment production from *Cassia alata* L. callus cultures and their potential as a textile dye. Ind. Crops Prod. 128, 346–353. https://doi.org/10.1016/j.indcrop.2018.11.018

Sugimoto, K., Gordon, S.P., Meyerowitz, E.M. (2011). Regeneration in plants and animals: Dedifferentiation, transdifferentiation, or just differentiation? Trends Cell Biol. 21, 212-218. https://doi.org/10.1016/j.tcb.2010.12.004

Sugiyarto, L., Kuswandi, P. (2014). Pengaruh 2, 4-Diklorofenoksiasetat (2, 4-D) Dan Benzyl Aminopurin (BAP) Terhadap Pertumbuhan Kalus Daun Binahong (*Anredera).* J. Penelitian Saintek 23–30.