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Research Article
Callus Induction from Various Organs of Dragon Fruit, Apple and Tomato on some Mediums

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Abstract

Background and Objective: Dragon fruit (*Hylocereus* spp.), apple (*Malus sylvestris* Mill.) and tomato (*Solanum lycopersicum* L.) are high potential sources of antioxidant compounds such as phenolics. The compounds have the capability of protecting cells and tissues against free radicals. Secondary metabolite produced by callus cell culture from plant organs also acts as a source of antioxidants. This study aimed to determine the optimal ratio of sucrose and 2,4-D in Murashige and Skoog (MS) medium for callus induction from different plant organ explants. With all of characteristic, callus can be used further for the development of natural cell regeneration agent. **Methodology:** This study was conducted using analytical technique. Suitable explants were obtained. They were developed in various concentrations of combination between MS medium and 2,4-D. Callus growth, including their weight and surface was then measured and analyzed by using one-way analysis of variance (ANOVA). **Results:** Callus was able to grow from its explants in 5-7 days after induction process. They were clear in color and had friable texture. The highest value of fresh weight of dragon fruit callus was obtained through MS supplemented with 1 μL L^{-1} 2,4-D and 30 g sucrose. However, apple and tomato callus induction and growth maintenance reached optimal medium on MS supplemented with 30 g sucrose and 2 μL L^{-1} 2,4-D. **Conclusion:** Callus of apple, dragon fruit and tomato was maintained upon MS supplemented with 30-40 g sucrose and 1-2 μL L^{-1} 2,4-D for optimum induction and growth. The optimization of growth medium will give advantages for further development of natural cell regeneration agent.

Key words: Callus, dragon fruit, apple, tomato, explant, cell regeneration, Murashige and Skoog medium, plant tissue

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plant tissue culture has an important role in the production of secondary metabolite. The metabolite produced can then be developed further into agents that can prevent some degenerative diseases[1]. Reactive Oxygen Species (ROS) can cause cell damage that leads to the occurrence of a number of degenerative diseases such as cancer, diabetes and ageing. Compounds such as terpenoids, phenols and alkaloids from natural sources have been reported to have potentials to prevent some degenerative diseases, especially those caused by antioxidant activity[2].

Indonesia is rich in natural source, fruits and vegetables including dragon fruit, apple and tomato. The fruits have been grown and commonly consumed by Indonesians. Dragon fruit plant (*Hylocereus* spp.), also known as "Pitaya", originally comes from America and is widely distributed from coastal Florida to Brazil[3]. *Hylocereus polyrhizus* has been reported to contain betacyanin pigments, polyphenols and flavonoids[3]. The compounds were also known to have anti-tumor, antioxidant and anti-inflammatory activity[4-6]. Antioxidant compounds commonly have the ability to protect human body cells and tissues from the effects of free radicals. Another potential fruit grown in Indonesia, especially in Malang, is apple. Apple (*Malus sylvestris* Mill.) is an annual fruit and is a rich source of polyphenolic compounds, which acts as antioxidant for many diseases caused by reactive oxygen and oxidative stress[7]. Tomato is also an important vegetable grown in Indonesia. Tomato contains lycopene as its main secondary metabolite, which has been known to impede free radical activity[8].

Callus is a mass of irregular, unrecognized cells that can produce some metabolites like the parent plant[9]. Besides, the callus cell possesses totipotency and plasticity characteristics, which allow the cell to change their metabolism, adjusting their growth to environmental condition[10]. However, so far, there is only limited report on the efficient culture medium to support calluses growth, particularly from local dragon fruit, tomato and apple. Therefore, this study aimed to find which explants can be induced to form callus properly and to determine the optimal culture medium for its growth. By discovering the suitable organ and condition for callus growth, this study may be further used as a basis for subsequent researches of callus substance that can induce regeneration upon cell damage.

MATERIALS AND METHODS

Plant material: Dragon fruit (*Hylocereus* spp.) and tomato (*Solanum lycopersicum* L.) were collected from KP4 Universitas Gadjah Mada, Yogyakarta, while apple (*Malus sylvestris* Mill.) was collected from Malang, Indonesia.

Methods

Preparation of MS medium: The MS medium was prepared according to Osman *et al.*[11] with modifications. The MS medium were composed of macronutrient materials, i.e., NH_4NO_3, KNO_3, $CaCl_2.H_2O$, $MgSO_4.7H_2O$ and KH_2PO_4. The materials were dissolved in 500 mL of aquadest. Then added by zinc stock solution, micro stock solution and vitamin stock solution. A hundred milligrams of myo-inositol and 30-40 g of sucrose were added. Then, 2,4-D chemical substance was adjusted to various concentration levels. The pH of the solution must be between 5.60 and 6.30. The pH solution can be adjusted using HCl 1N and KOH 1N. The volume of the solution was expanded to up to 1000 mL using aquadest.

Two grams of gellan gum as solidifying agent was heated and 15 mL was poured to each culture bottle. They were sterilized using autoclave at 121°C, under a pressure of 1.5 atm for 15 min.

Sterilization of explants: Sterilization was carried out under laminar air flow. Different organ parts of apple, dragon fruit and tomato were cleaned thoroughly under running tap water. The explants were treated with sodium hypochlorite solution in a concentration range of 5.25-2.6% and tween solution by shaking for 5-10 min, followed by washing using distilled water three times, each for 5 min.

Callus induction: Sterilized explants were cut into small pieces (0.5-1.0 cm) and transferred to MS medium, supplemented by 3% (w/v) sucrose. The auxin of 2,4-D at four different concentrations (0.5, 1.0, 1.5 and 2.0 mg L^{-1}) and in combination with cultures were maintained in the dark for 2 weeks, followed by incubation at 16 h under the light (2000 lx; 25±1°C) and 8 h in the dark (16±1°C). Leaf explants cultured on the MS medium without any growth regulators were used as the control for callus induction. All treatments were conducted thrice with 25 replicates in each treatment. The following callus characteristics were measured.

Callus growth analysis: Callus obtained after 4 weeks of culture were used for callus growth study. Based on Chen *et al.*[12], with a few modifications, callus was weighed before being transferred to the fresh callus induction medium (W1). They were weighed again after 4 weeks of sub-culture

(W2). The Relative Fresh Weight Growth (RFWG) was calculated as (W2–W1)/W1. The measurement of surface area was done by placed the harvested callus upon millimeter block area, then measured its length and width. All data was collected and compared relatively among others.

Statistical analysis: The data were analyzed statistically by one-way analysis of variance (ANOVA) with Duncan test using IBM SPSS version 17.0 with 95% confidence level.

RESULTS

Callus induction: Callus induction was carried out in MS medium. Some organs of dragon fruit, tomato and apple plants, for example the leaf, cotyledon and stem were used for induction of callus. The result showed that explants for callus induction differed between dragon fruit, apple and tomato, respectively as shown in Fig. 1. Cotyledon was used for apple and tomato callus induction, while stem is used from dragon fruit as an explant to grow callus.

Development of apple callus: At the beginning, callus induction on apple was carried out using some plant organs, i.e., cotyledon, leaf and stem. The wet weight and surface area of callus during growing process was measured on 28 days. The development of apple callus from cotyledon is shown in Fig. 2. Its texture and color on 20 days had changed to soft orange-brownish color. Meanwhile, the graphic in Fig. 3 illustrated the wet weight of apple callus. Cotyledon explant showed callus growth on MS medium, while leaf and stem did not show callus growth at all. Apple callus from cotyledon explant had the heaviest weight, 326,5 mg, on MS supplemented with 30 g sucrose and 2,4-D 2 μL L^{-1}. Meanwhile, lower concentration of 1 μL L^{-1} of MS 30 and 40 g sucrose produced callus which had half weight from 2 μL L^{-1} callus.

The measurement of surface area on 28 days in Fig. 4 also showed that MS supplemented with 30 g sucrose and 2,4-D 2 μL L^{-1} induced the broadest area of callus, 62.4 mm^2. In contrast, MS 40 g sucrose with the same concentration of 2,4-D only induced 1.6 mm^2 surface area of callus. This result of surface measurement was relevant to the weight measurement. Although the callus from MS 30 and MS 40 had nearly the same weight, the callus from MS 30 was relatively broader than the callus from MS 40.

Development of dragon fruit callus: Callus was grown from a star-shaped stem of dragon plant. The development of dragon fruit callus within 28 days is shown in Fig. 5. On 18 days, the callus emerged at the surface area. The texture was friable with dark orange-brown color. On 20 days, the wet weight and surface area were measured and the result is shown in Fig. 6. Concentration of 2,4-D at 1 μL L^{-1} in both mediums of MS supplemented with 30 g sucrose and 40 g sucrose induced the heaviest callus, i.e., 420 and 152 mg, respectively, compared to other concentration levels. On the other hand, the callus obtained from MS supplemented with 30 g sucrose comparatively had less surface area to the callus from MS supplemented with 40 g sucrose (Fig. 7). However, this result was not significantly different in statistic analysis.

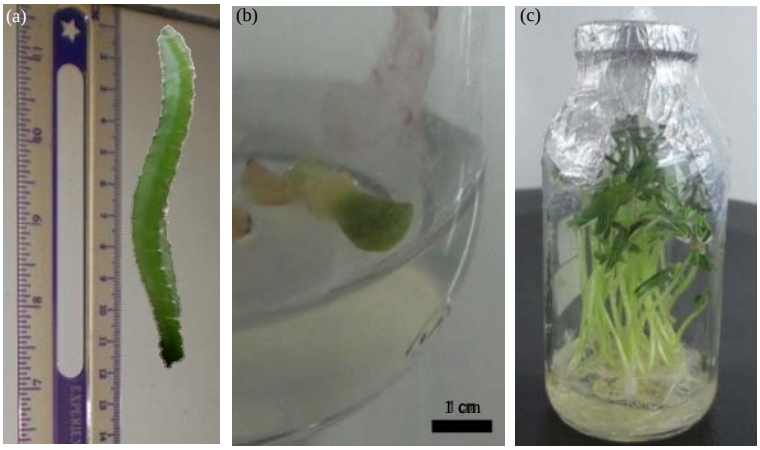

Fig. 1(a-c): Explants for callus induction, (a) Stem for dragon fruit, (b) Cotyledon for apple and (c) *In vitro* plant culture for tomato
Explants were carried out in MS medium at different concentrations of 2,4-D

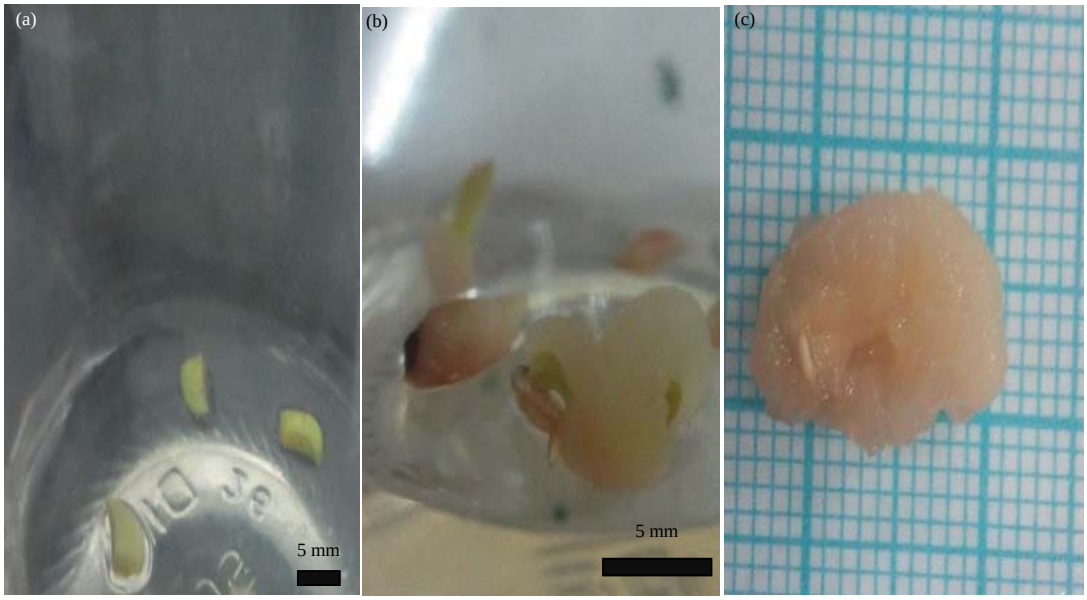

Fig. 2(a-c): Development of apple callus within 28 days, (a) 1 day, (b) 20 days and (c) 28 days on MS medium with 30 g sucrose and 2 μL L^{-1} 2,4-D, (a) On the 1 day, cotyledon were cut into pieces and cultured in MS medium, (b) Callus grew from the cut area in cotyledon at 20 days. The color of callus and explant also had changed to soft orange-brownish color and (c) 28 days callus had formed before shoot appeared and it was cleaned from media and then measured

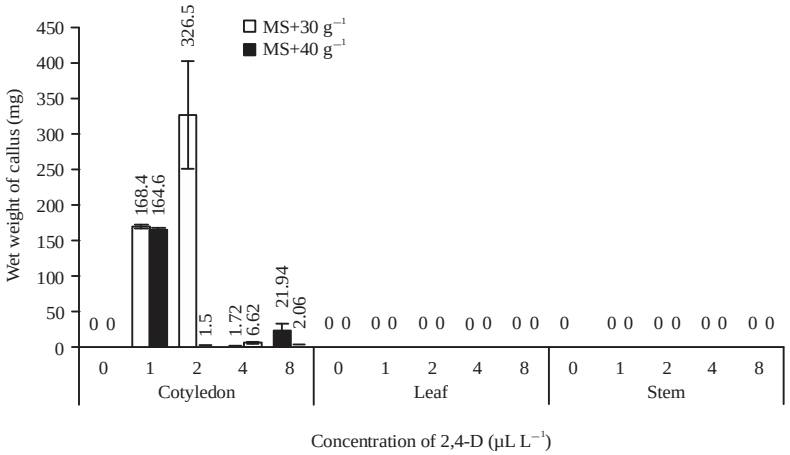

Fig. 3: Wet weight of apple callus from different organ plants on MS medium at different concentrations of 2,4-D (μL L^{-1}) in milligram

The white bar showed the addition of 30 g L^{-1} sucrose in MS medium, while the black bar showed 40 g L^{-1} sucrose in MS medium

Development of tomato callus: The result showed that the hypocotyl and cotyledon of tomato were the appropriate organ plants to form callus. The callus development from the hypocotyl and cotyledon of tomato showed in Fig. 8a-f. In 0 week, the callus had not developed. However, after 6 weeks, hypocotyl began to swollen and more expanded. Then, in 12 weeks, it was differentiated to friable brown callus (Fig. 8a-c). Cotyledon was also transformed to callus (Fig. 8d-f). Although no callus was formed in 0 week, it started to grow in 6 weeks. The tomato callus from cotyledon formed more texture with brown color.

According to the result, tomato callus from both cotyledons had wet weight over 350 mg, in MS supplemented with 40 g sucrose and 2,4-D 2 μL L^{-1} (Fig. 9). On the contrary to its wet weight, the callus from cotyledon in MS supplemented with 30 g sucrose and 2,4-D 2 μL L^{-1} had area around 148 mm^2 (Fig. 10).

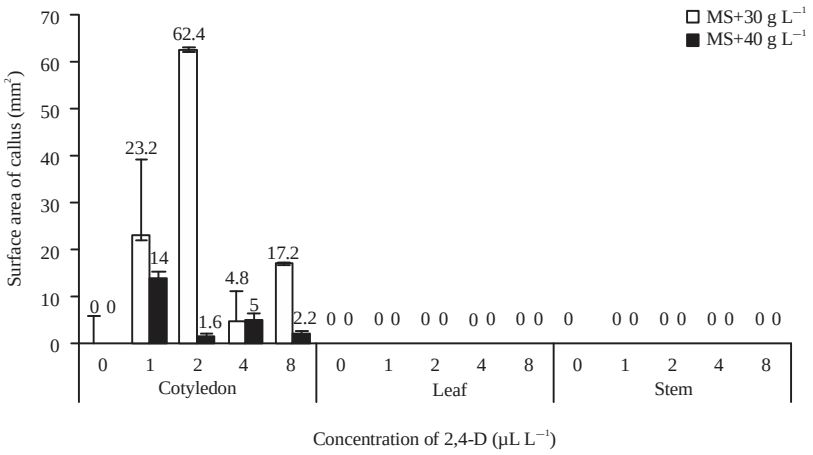

Fig. 4: Surface area of apple callus developed from cotyledon, leaf and stem on MS medium at different concentrations of 2,4-D (µL L^{-1}) in millimeter square

The white bar showed the addition of 30 g L^{-1} sucrose in MS medium, while the black bar showed 40 g L^{-1} sucrose in MS medium

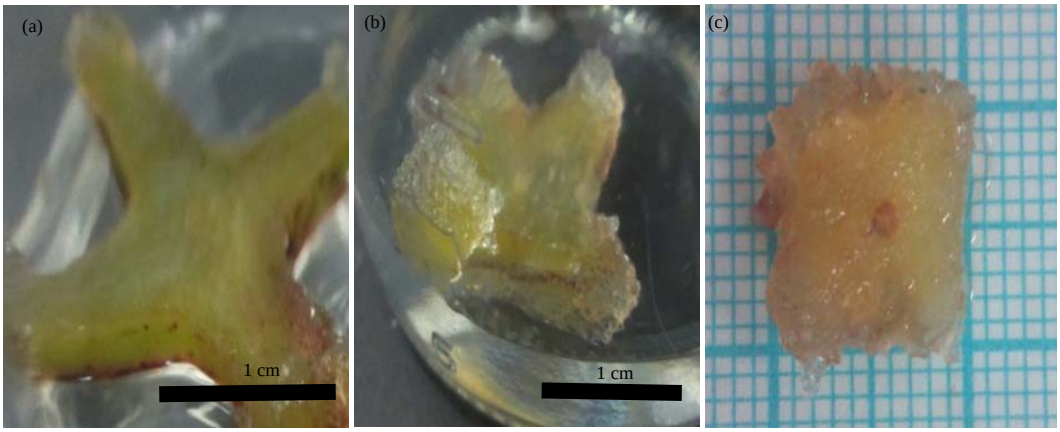

Fig. 5(a-c): Development of dragon fruit callus within 28 days, (a) 1 day, (b) 18 days and (c) 28 days on MS medium with 30 g sucrose and 2 µL L^{-1} 2,4-D, (a) On 1 day stem were cut into pieces and cultured in MS medium, (b) Callus appeared from surface explant in cut area on 20 days and (c) 28 days callus had formed before shoot appeared and it was cleaned from media

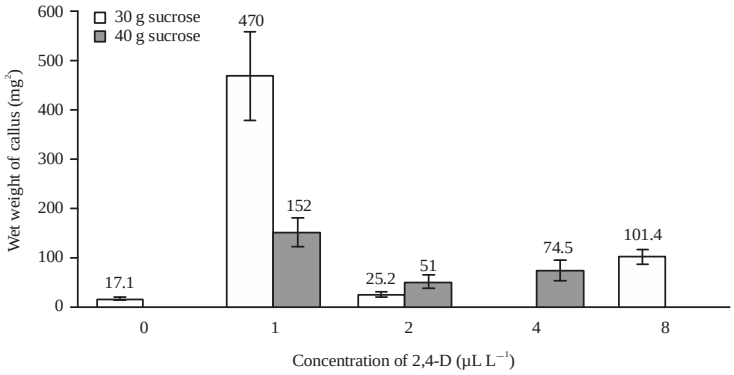

Fig. 6: Wet weight of dragon fruit callus developed from its stem on MS medium at different concentrations of 2,4-D (µL L^{-1}) in milligram

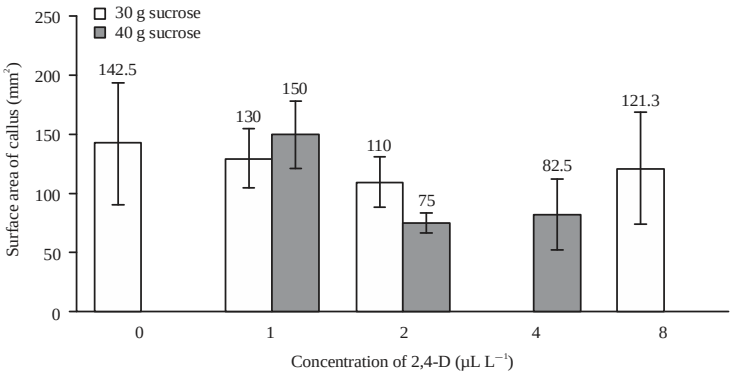

Fig. 7: Surface area of dragon fruit callus developed from its stem on MS medium at different concentrations of 2,4-D (µL L^{-1}) in millimeter square

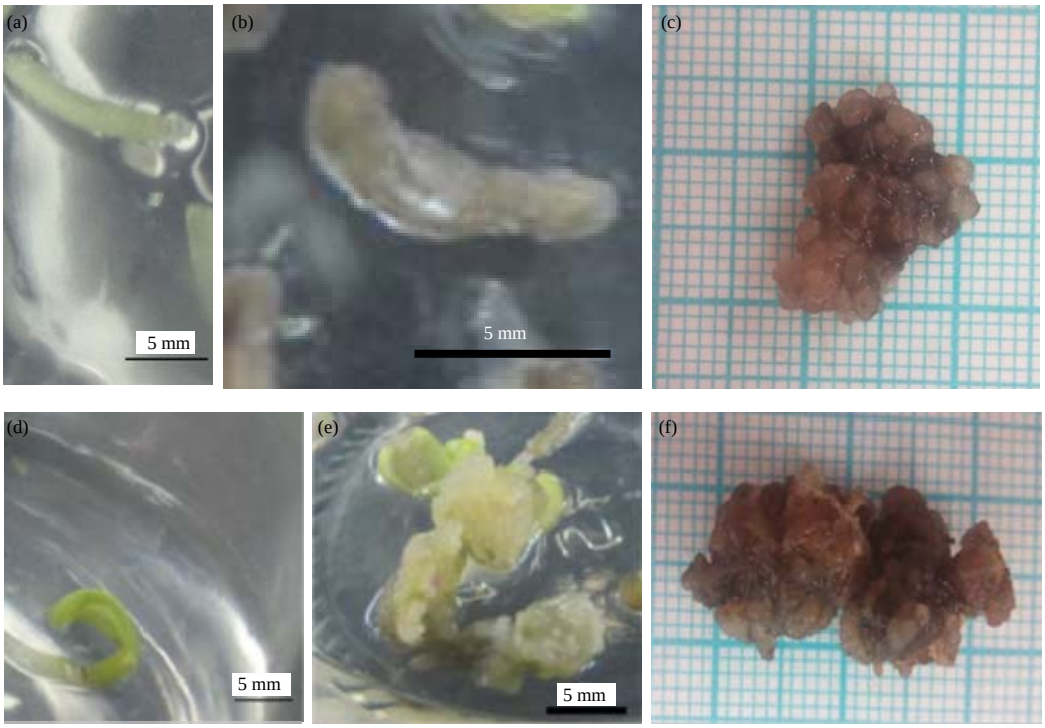

Fig. 8(a-f): Development of tomato callus from (a-c) Hypocotyl and (d-f) Cotyledon, in (a, d) Week 0, (b, e) Week 6 and (c, f) Week 12 on MS medium combination with sucrose and 2,4-D

In week 0 hypocotyl and cotyledon were cut into pieces and cultured in MS medium. Callus appeared from surface explant in cut area in week 6. The color of callus turned darker in hypocotyl, while callus from cotyledon colored light green. Week 12, callus was harvested before the shoot appeared

All data measurement including wet weight and surface area, of apple callus, dragon fruit callus and tomato callus was analyzed statistically using ANOVA. Data of wet weight measurement were compared among three samples as well as surface area data. The statistic result of wet weight represented that the experiment among sample of calluses were significantly different with p-value 95%. Likewise, the significance result of surface area among samples was less than 0.05.

DISCUSSION

The formation process of apple callus was evaluated in the end of the study period. The callus from leaf and stem underwent the process of browning. As reported by Jones and Saxena[13], callus browning might be caused by the accumulation and subsequent oxidative reaction of phenolic compounds and culture media. Furthermore, it is probably

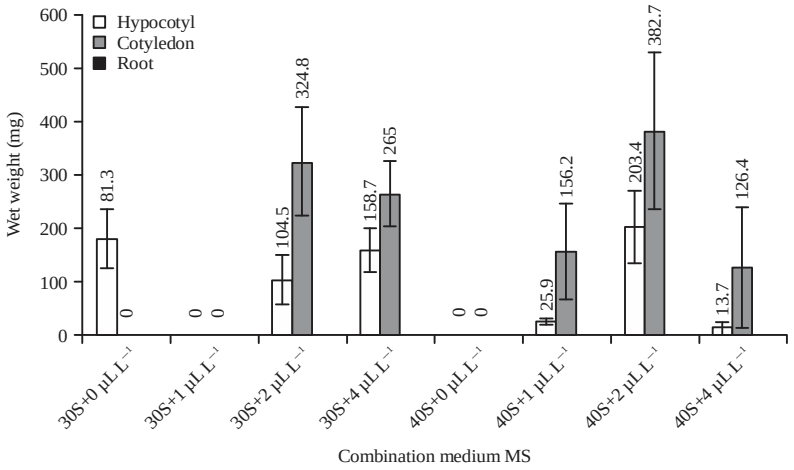

Fig. 9: Wet weight of tomato callus developed from hypocotyl, cotyledon and root on MS medium, sucrose (g L^{-1}) and 2,4-D (µL L^{-1}) in milligram

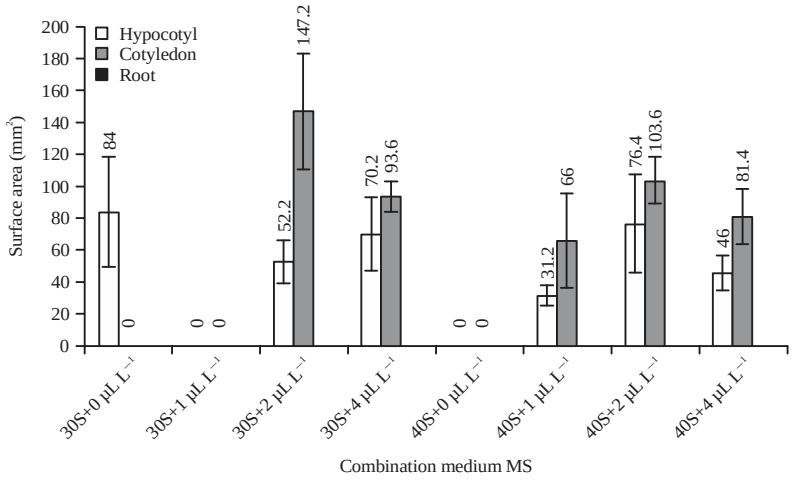

Fig. 10: Surface area of tomato callus developed from hypocotyl, cotyledon and root on MS medium sucrose (g L^{-1}) and 2,4-D (µL L^{-1}) in millimeter square

because the explants produced secondary metabolites that contributed to the brown color.

Apple callus from cotyledon explant showed optimum growth on MS supplemented with 30 g sucrose and 2,4-D 2 µL L^{-1}. The 2,4-D is the hormone that regulates and induces callus growth[14]. The amount of 2,4-D in the medium affected the callus growth. The concentration of 2 µL L^{-1} of 2,4-D showed optimum callus growth (Fig. 3) because it worked efficiently in lower concentration. Higher concentration would lead to toxicity in the explant.

Based on the surface area on 28 days (Fig. 3), the cotyledon on medium MS supplemented with 30 g sucrose and 2,4-D 2 µL L^{-1} induced callus with the broadest area compared to the others. If we referred to the statistic result, the data could be interpreted as a comparison due to significance results. However, it was not supposed to detract from the biological significance or other key consideration[15].

As reported by Lee and Huang[16], sucrose is the main exogenous source of carbohydrate as well as an osmotic regulator in plant tissue culture. Sucrose from medium is hydrolyzed into glucose and fructose, then resulting the increase of osmotic medium level. High concentration of sucrose will cause the release of water from cells, leading to inhibition of callus growth.

Dragon fruit callus was formed from dragon fruit's stem. Based on the measurement of wet weight, the concentration of 2,4-D 1 µL L^{-1} was possibly the optimum ratio of media to regulate the callus growth. Although the widest callus

appeared on medium MS supplemented with 40 g sucrose and 2,4-D 1 μL L^{-1}, but in general view, concentration showed that most callus developed from medium MS supplemented with 30 g sucrose are better.

The surface area of callus growth is not often directly proportional to the fresh weight of the callus. For example, in the treatment using MS supplemented with 30 g sucrose and 0 μL L^{-1} 2,4-D, the callus fresh weight was low, but it had a relatively high extensive growth. This is caused by the growth of thin callus which results in lower fresh weight although it has broad surface area.

Tomato callus could be induced from its stem and cotyledon. The result showed that cotyledon is the most suitable plant organ to form callus. It can produce maximum weight compared to hypocotyls and roots. Cotyledonary leaf explants (leaf cotyledon that shaped and structured like leaves) has been using for plant regeneration in *Jatropha curcas*[17]. Cotyledonary leaves explant induced callus from the edges and led the absorption of nutrients in medium. Thus, it has good ability of callus formation. The explant has been mostly studied for *de novo* shoot formation[17]. On root explant, callus is not formed in a combination of any medium (Fig. 9).

The result showed that the fresh weight of callus is not directly proportional to the surface area of the callus itself (Fig. 10). This might be because the thickness of heavy callus was thin, while on the other hand the narrow callus is thicker, which affected the volume and weight of the callus.

This study used a variety of concentrations of sucrose and 2,4-D growth regulator MS medium. High concentration of mineral salts and great amount of compounds N in the form of ammonium and nitrate became the main reason using MS medium[18], thus the growth of cultured explants may be optimum in accordance with the objectives to be achieved. MS medium was added with a combination of sucrose concentration and 2,4-D to obtain optimum callus growth. The combination of sucrose used in this study is 30 g L^{-1} and 40 g L^{-1}. Sucrose is a sugar that acts as the source of carbon to produce energy which is used for the process of cell metabolism[19]. According to Yaacob[19], the higher the level of sucrose in the medium, the higher the size of the callus obtained. Based on this research, 40 g $L^{-1}$of sucrose induced callus optimally, meaning that it is relevant with the theory which stated that the higher the level of sucrose, the higher the productivity of the callus as the source of energy is available in large quantity.

Hormone of 2,4-D growth regulator are also added to the combination of MS medium as 2,4-D is a synthetic auxin hormone that is often used in callus culture due to its strong activity to stimulate the process of de-differentiation of cells, press organogenesis and maintain callus growth. 2,4-D compound showed stronger activity because the carboxyl groups are separated by carbon or carbon and oxygen[20]. Concentrations of 2,4-D used in this study were 0, 1, 2 and 4 μL L^{-1}. The 2,4-D is a synthetic auxin hormone that works effectively in low concentrations and when present in concentrations that are too high, it will inhibit growth[21]. This can be seen from the results of the research that has been conducted. In the control (without 2,4-D), there is only a slight callus formation because the one that plays a role in the induction of callus is only endogenous hormones, while the 2,4-D at the concentration of 1 μL L^{-1} increased the amount of callus fresh weight and at the concentration of 2 μL L^{-1} the maximum fresh weight of callus is obtained. Then, the fresh weight of callus with 4 μL L^{-1} concentration reduced. Thus, callus growth produced optimally within concentration of 2,4-D 2 μL L^{-1}. Any concentrations greater than 2 μL L^{-1} would inhibit the growth of callus.

According to Delporte *et al.*[22], a complex relationship between endogenous and external factors, such as age degree of differentiation, genotype, physiological condition and hormone concentrations affect the response of the explants to callus produced condition. Based on the results of the study, most of the good tomato callus derived from hypocotyl and cotyledon explants have friable texture and brownish-black color, similar to the results of the research conducted by Chandra *et al.*[8].

CONCLUSION

It is concluded that callus of dragon fruit (*Hylocereus* spp.), apple (*Malus sylvestris* Mill) and tomato (*Solanum lycopersicum* L.) was maintained upon MS supplemented with sucrose range of 30-40 g and 2,4-D range of 1-2 μL L^{-1} for optimum induction and growth. This finding provides potential scientific information for natural cell regeneration agent.

SIGNIFICANCE STATEMENTS

- This study was about finding the suitable explants from dragon fruit, apple and tomato for callus growth and optimizing the ratio of medium concentration for ideal growth
- Research on fruit callus growth had been conducted but information about local Indonesian fruit for callus and the optimum growth environment are still limited
- The result provides scientific information about the right part of plant organs for callus growth and the optimum range of concentration for its growth

- The finding of this study is a potential scientific information for the development of natural cell regeneration

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REFERENCES

1. Pandey, K.B. and S.I. Rizvi, 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Med. Cell. Longevity, 2: 270-278.
2. Hariprasath, L., R. Jegadeesh, P. Arjun and N. Raaman, 2015. *In vitro* propagation of *Senecio candicans* DC and comparative antioxidant properties of aqueous extracts of the *in vivo* plant and in vitro-derived callus. S. Afr. J. Bot., 98: 134-141.
3. Ortiz-Hernandez, Y.D. and J.A. Carrillo-Salazar, 2012. Pitahaya (*Hylocereus* spp.): A short review. Commun. Sci., 3: 220-237.
4. Rebecca, O.P.S., A.N. Boyce and S. Chandran, 2010. Pigment identification and antioxidant properties of red dragon fruit (*Hylocereus polyrhizus*). Afri. J. Biotechnol., 9: 1450-1454.
5. Bakar, J., C.E. Shu, S. Muhammad, S. Kharidah and D.M. Hashim, 2011. Physico-chemical characteristics of red pitaya (*Hylocereus polyrhizus*) peel. Int. Food Res. J., 18: 279-286.
6. Luo, H., Y. Cai, Z. Peng, T. Liu and S. Yang, 2014. Chemical composition and *in vitro* evaluation of the cytotoxic and antioxidant activities of supercritical carbon dioxide extracts of pitaya (dragon fruit) peel. Chem. Central J., Vol. 8.
7. Stojiljkovic, D., I. Arsic and V. Tadic, 2016. Extracts of wild apple fruit (*Malus sylvestris* (L.) Mill., Rosaceae) as a source of antioxidant substances for use in production of nutraceuticals and cosmeceuticals. Ind. Crops Prod., 80: 165-176.
8. Chandra, I., P. Singh, A. Bhattacharya, P. Singh, S. Javed and A. Singhamahapatra, 2013. *In vitro* callus induction, regeneration and micropropagation of *Solanum lycopersicum*. Int. J. Curr. Microbiol. Applied Sci., 2: 192-197.
9. Ikeuchi, M., K. Sugimoto and A. Iwase, 2013. Plant callus: Mechanisms of induction and repression. Plant Cell, 25: 3159-3173.
10. Barbulova, A., F. Apone and G. Colucci, 2014. Plant cell cultures as source of cosmetic active ingredients. Cosmetics, 1: 94-104.
11. Osman, N.I., N.J. Sidik and A. Awal, 2016. Effects of variations in culture media and hormonal treatments upon callus induction potential in endosperm explant of *Barringtonia racemosa* L. Asian Pacific J. Trop. Biomed., 6: 143-147.
12. Chen, L., A. Bhagsari and J. Carter, 2006. Effects of medium composition and culture duration on *in vitro* morphogenesis of sweet potato. Biol. Plantarum, 50: 114-117.
13. Jones, A.M.P. and P.K. Saxena, 2013. Inhibition of phenylpropanoid biosynthesis in *Artemisia annua* L.: A novel approach to reduce oxidative browning in plant tissue culture. PLoS ONE, Vol. 8.
14. Guan, C.M., S.S. Zhu, X .G. Li and X.S. Zhang, 2006. Hormone-regulated inflorescence induction and TFL1 expression in *Arabidopsis callus in vitro*. Plant Cell Rep., 25: 1133-1137.
15. Vaux, D.L., 2012. Research methods: Know when your numbers are significant. Nature, 492: 180-181.
16. Lee, S.T. and W.L. Huang, 2014. Osmotic stress stimulates shoot organogenesis in callus of rice (*Oryza sativa* L.) via auxin signaling and carbohydrate metabolism regulation. Plant Growth Regul., 73: 193-204.
17. Mazumdar, P., A. Basu, A. Paul, C. Mahanta and L. Sahoo, 2010. Age and orientation of the cotyledonary leaf explants determine the efficiency of de novo plant regeneration and *Agrobacterium tumefaciens*-mediated transformation in *Jatropha curcas* L. S. Afr. J. Bot., 76: 337-344.
18. Chaffey, N., 2010. News in botany: Nigel Chaffey presents a round-up of plant-based items from the world's media. Ann. Bot., 105: 3-6.
19. Yaacob, J.S., N. Mahmad, R.M. Taha, N. Mohamed, A.I.M. Yussof and A. Saleh, 2014. Optimization of culture conditions (sucrose, pH and photoperiod) for in vitro regeneration and early detection of somaclonal variation in ginger lime (citrus assamensis). Sci. World J., 10.1155/2014/262710.
20. Indah, P.N. and D. Ermavitalini, 2013. Induksi kalus daun nyemplung (*Calophyllum inophyllum* Linn.) pada beberapa kombinasi konsentrasi 6-Benzylaminopurone (BAP) dan 2,4-Dichlorophenoxyacetic Acid (2,4 D) [Callus induction of Nyamplung leaf (*Calophyllum inophyllum* Linn.) on some combination of the concentration of 6-Benzylaminopurone (BAP) and 2,4-Dichlorophenoxyacetic acid (2.4 D)]. J. Sains Seni Pomits, 2: 1-6.
21. Tahir, S.M., K. Victor and S. Abdulkadir, 2011. The effect of 2, 4-Dichlorophenoxy acetic acid (2, 4-D) concentration on callus induction in sugarcane (*Saccharum officinarum*). Niger. J. Basic Applied Sci., 19: 213-217.
22. Delporte, F., A. Pretova, P. Du Jardin and B. Watillon, 2014. Morpho-histology and genotype dependence of in vitro morphogenesis in mature embryo cultures of wheat. Protoplasma, 251: 1455-1470.