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To cite this article: D Fitriana *et al* 2019 *J. Phys.: Conf. Ser.* **1217** 012166

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Effect of combination explant difference leaf part and concentration of active charcoal on callus initiation mangrove (*Rhizophora Apiculata* BI) by *in-vitro*

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Abstract. *Rhizophora Apiculata* BI is one of the mangroves that has many benefits, but to cultivate naturally takes a long time. The alternative to such problems is culture cultivation *in vitro*. One stage of *in vitro* culture is through callus formation. Initiation of callus in mangrove plants is inhibited because explant release phenol compound that causes *browning*. Thus, it takes explant and charcoal active in a medium that can support growth explant. The purpose of this research is to study the effect of the combination of leaf explant and different activated charcoal concentration on callus initiation, to analyze the color and texture of callus produced, and to study the effect of the combination of leaf explant and different concentration of activated charcoal on the growth of explant. The basal medium used was Murashige and Skoog (MS) formula with the addition of NAA 1 mg/L and BAP 0.3 mg/L, and activated charcoal at concentration 4, 12, 20 g/L as the medium treatments. A different section of explant between base and tip of leaves combined with active charcoal were grown in MS medium. Each treatment was conducted three replications. The results showed that the combination of explant section, i.e. base of the leaf as well as activated charcoal with a concentration of 12 g / L could induce callus formation. The color and texture of callus were brownish yellow and compact. The best growth (0.1685 g) of callus was obtained at the leaf tip section combined with 4 g / L activated charcoal.

1. Introduction

Mangrove plant is one of the natural resources that have value and importance both in terms of physical, biological and socio-economic. According to Pieter, et al. (2010), mangrove plants (*Rhizophora apiculata*) are able to grow and spread well from the swamp to the sea. The extent of the spread of this species indicates that this species has a high tolerance to the environment. However, the number of these species decreases as the number of mangrove forest ecosystems diminishes further. According to FAO (2007), the amount of mangrove forest in Indonesia in 2005 reached 3,062,300 ha or 19% of the world's mangrove forest area.

Rhizophora Apiculata population return can be done by cultivating mangroves *in-vivo*. Mangrove cultivation technique takes a relatively long time because it grows in extreme environments. In addition to cultivation *in-vivo*, there is also cultivation that uses a short time with a lot of results that is using cultivation *in-vitro*. According to Kumar (2015), the cultivation of plant tissue culture has several advantages. Among them is the time required is much shorter because of no need to wait for



the whole cycle of seed growth. This cultivation is suitable for species that have long dormancy time, low seed production, or seeds that are not easy to germinate.

One of the factors influencing callus initiation in plant tissue culture is the plant part used. In this study, the plant part used is explant the base and explant the tip of the leaf. This is because the base and the tip of the leaf have different meristematic properties. The base of the leaf is thought to have a more meristematic network because it is close to the SAM (*Shoot Apical Meristem*), while the tip is thought to have more mature cells. Kalve (2014) explains that he divides the leaf into two parts separating the two growth processes. These two parts are part of cell division located at the base of the leaf and the elongation of cells located in the tip of the leaf area.

The next factor affecting callus initiation is the type of plant. Mangrove plants are included in plants that produce phenolic compounds naturally with high concentrations that can inhibit their proliferation. In plant tissue culture, the amount of this phenolic compound will increase if explant is injured. The presence of these phenolic compounds can cause browning which can disrupt explant growth. According to Admojo (2016), mangrove culture is one culture that is difficult to be implemented because of the production of phenol naturally high enough. Phenol production will increase if the plants are injured. Phenol compounds are what causes browning so that the growth of tissue culture becomes disrupted. If culture growth is disrupted by the presence of browning, the growth of callus will be hampered.

One attempt to reduce the occurrence of browning in plant tissue culture is by giving active charcoal to the culture medium. Arumugam (2012) explains that the use of activated charcoal in tissue culture media can absorb polyphenols and reduce phenol synthesis to prevent browning on explant. Based on these problems, the combination of leaf explants and active charcoal concentration was done to initiate callus from the leaf part having different meristematic properties and to reduce phenol compounds that could inhibit callus initiation.

2. Material and Methods

2.1. Provision of explant materials

Leaves were picked from *Rhizophora Apiculata* BI trees grown in the mangrove forest of Mangkang, Semarang. Leaves were selected from mangrove that is about seven years old and taken on the first branch of the 1st strand of the apical bud.

2.2. Media preparation

500 ml distilled water, then MS 4.43 g / L added and homogenized. Furthermore, 0.1 g / L myoinositol was incorporated into a common solution and then added sucrose 30 g / L and stirred until homogeneous. Then added NAA 1 ppm and BAP 0.3 ppm. Furthermore, I was added to the volume of 1000 ml. Then the medium was given active charcoal treatment with concentrations of 4, 12, and 20 g / L. pH is measured to achieve acidity of 5.6 to 5.8; the aqueous solution was then added to 8 g / L, stirred until suspended and homogeneous, medium heated to boiling. The complete medium was poured into a bottle, and the media was sterilized in the autoclave for 15-20 minutes at 121 ° C.

2.3. Induction of callus

Sterilization of explants from the field as using detergent and bayclin subsequently sterilized of explant in laminar water flow immersed in an antibiotic solution, a fungicidal solution, a bayclin solution, an alcohol solution. Leaf explant cut between the base and the tip of the leaf. The base part was the portion close to the stalk while the tip portion was the part located below the base. Then the parts were cut into explant with size 1 cm²; cutting was done in ascorbic acid solution 10 mg / 100 ml and planted in a medium according to the amount of treatment. The culture was placed in the incubation room for 30 days.

3. Result and Discussion

3.1. Callus Initiation Time

The result of observation of callus initiation time on the treatment of combination of leaf explant difference and active charcoal concentration on mangrove callus initiation (*Rhizophora Apiculata* BI) *in-vitro* determined by observing explant since planting until the first callus expressed in DAP (Day After Planting). The fastest initiation time of callus was shown in the explant treatment of the base

with the active charcoal concentration of 4 g / L of 8 HST while the longest callus initiation time was the tip explant treatment with active charcoal concentration 4 g / L that is 17,5 HST.

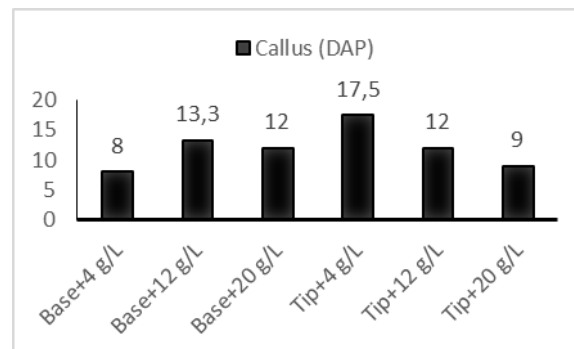


Figure 1. *Rhizophora Apiculata* BI leaf explant initiation after different leaf parts explant and activated charcoal concentrations.

Base part explant treatment with activated charcoal 4 g / L having the fastest initiation time, despite having the smallest growing frequency. Contrary, base explant with active charcoal 12 g / L took 13.3 DAP initiation but had the highest growing frequency of 3 replications. This was caused by the base explant of the leaf that had more meristematic cells contacted with a medium that could absorb phenol compounds thus increasing callus growth frequency.

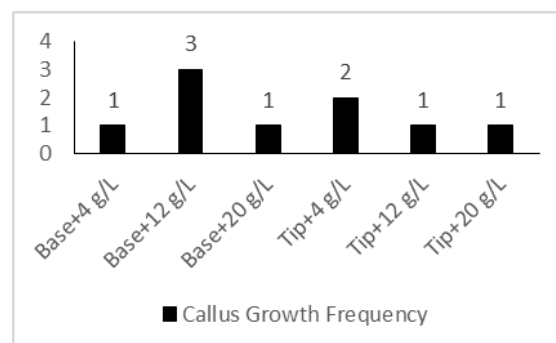


Figure 2. The frequency of callus growth from *Rhizophora Apiculata* BI leaf treated with a combination of different leaf part explant and active charcoal concentration.

Leaf base explant tends to be better than the leaf tip explant because the location of the section adjacent to the point of growing the leaves can cause the base has a speed of splitting faster than the tip. According to Kalve (2014), the process of leaf development path in the leaf comes from SAM (*Shoot Apical Meristem*) which is adjacent to the base of the leaf. After that, the cells will migrate to the lateral region and conduct further growth processes.

The location of the SAM adjacent to the base area causes the area to be an active area of cleavage for leaf formation as well as to repair damaged cells. This is as described by Kalve (2014) through the illustration of the leaf growth area that the leaf growth area is divided into two. The two parts are the cell division area located at the base of the leaf and the elongation area of the cell located at the tip of the leaf while the meristem function is explained by Ayulina (2004), that in meristem tissue, every

time the division, one of the sapling cells will remain meristem, while the other tiller cells will be modified.

Activated charcoal also has a role in initiating callus. Activated charcoal treatment with a concentration of 12 g / L is thought to be the optimal concentration for the growth of callus. This is because the active charcoal can absorb the phenol produced by explant. Here is an explanation of the adsorption process according to Adli (2012), which begins with an active group located on the surface of activated charcoal interacting with adsorbate in the form of chemical compounds. The influence of Van Der Waals forces between the surface of the activated charcoal and the adsorbate causes the adsorbate to be adsorbed into active charcoal pores.

In addition, the accumulation of phenol compounds lies at the tip, so that the growth frequency of callus at the base of the leaf treatment is greater. This is reinforced by the opinion of Chauser (2012) that the higher the phenol leads to an unpleasant taste when eaten by herbivores. Therefore, phenols are collected in areas easily accessible by herbivores. The area is located on the tip of the leaf, both on young leaves and old leaves. Moreover, based on observations under UV light, the more bundles, the more phenols will accumulate. The carrier file is present on the leaf bone. So at the edge of the leaf or the tip of the leaf has a more phenol because in that part the bone leaves empty.

3.2. Color and texture Callus

Observation of color and texture of *Rhizophora Apiculata* leaf explant done visually. The observation of color and texture of *Rhizophora Apiculata* leaf explant is brownish yellow and compact. Callus is brownish yellow due to the content of phenol compounds that exist in explant. As described by Hutami (2008) that the brownish color of the callus is a result of the excess metabolism of phenol compounds, which are often aroused by the explant sterilization process. From these statements can be concluded that the use of leaf explant can affect the color of callus to brownish yellow because of phenol produced by explant. While the provision of activated charcoal does not affect the color of callus.

Table1. The color and texture of callus from *Rhizophora Apiculata* BI leaves treated with a combination of explant of different leaf part and active charcoal concentration.

Treatment	Callus Color	Callus Texture
BA1	Brownish yellow	Compact
BA2	Brownish yellow	Compact
BA3	Brownish yellow	Compact
TA1	Brownish yellow	Compact
TA2	Brownish yellow	Compact
TA3	Brownish yellow	Compact

The callus produced in the present study included a compact callus. Callus texture produced by explant is influenced by the type of explant used. This is consistent with Yelnitis (2012) statement that callus texture is one of the markers used to assess the quality of a callus. The compact callus texture is good because it accumulates more secondary metabolites. Secondary metabolites are only produced by certain plants, so the texture of this callus is influenced by the type of explant used.

3.3. Weight Difference Beginning and End of Explant

The result of observation of initial and final weight difference was measured by weighing the explants at the beginning of planting (0 days) and end of observation (day 30). The final weight difference and the highest initial weight were shown in the leaf tip explant treatment and activated charcoal 4 g / L with 0.1685 while the final weight difference and the smallest initial weight were shown in the explant treatment of the leaf base and the activated charcoal 4 g / L with 0.0058.

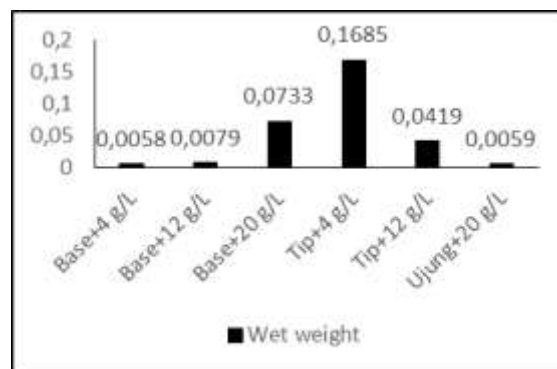


Figure 3. The initial weight difference and final weight of the *Rhizophora Apiculata* BI leaves were treated with a combination of explant differences in the leaf portion and the concentration of activated charcoal.

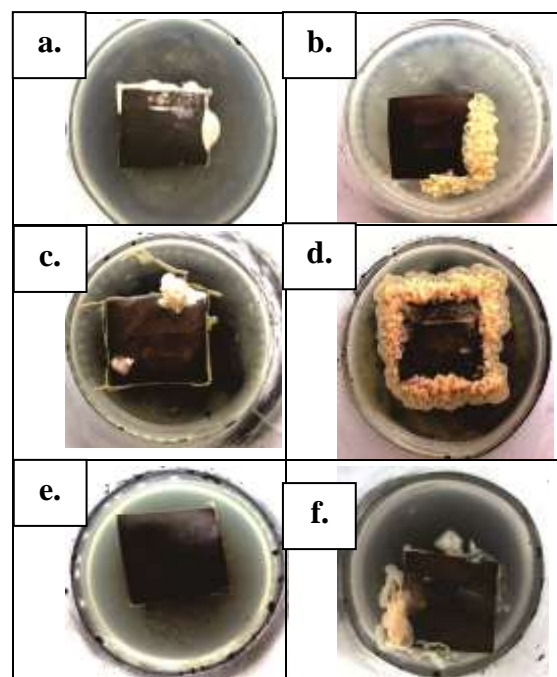


Figure 4. The number of calluses from the combination treatment of explant of different leaf part and the concentration of activated charcoal. (a) leaf base explant and active charcoal 4 g / L (b) leaf tip explant and active charcoal 4 g / L (c) Explant leaf base and active charcoal 12 g / L (d) Explant leaf tip and active charcoal 12 g / L (e) Explant leaf base and activated charcoal 20 g / L (f) Explant leaf tip and activated charcoal 20 g / L.

The increase of explant weight from the first time of planting until day 30, is caused by the explant of mangrove leaves grow callus and exudate exit. Exudate out of sliced mangrove leaves because on Rhizophoraceae family leaves there is water storage (Lechtaler, 2016). According to Widyati (2013), exudates include compounds with low molecular weight, such as sugars, amino acids, and aromatics. Exudates do not exert a lethal effect on culture growth (Sito, 2014).

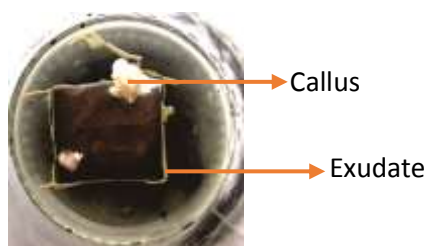


Figure 5. Explant *Rhizophora Apiculata* BI that grown callus and secreted exudate.

The treatment of leaf tip and activated charcoal 4 g / L explant had higher wet weight because the cells contained at the base were the cells that tasked for elongation so that the cells absorbed more nutrients and produced more wet weight also. In addition, the concentration of the charcoal provided could also reduce the phenol compounds produced by explants.

According to Kalve (2014) through the illustration drawing of the leaf area division explains that there is a dashed line which is the boundary separating the two growth processes. The two parts are the cell division area located at the base of the leaf and the elongation area of the cell located at the end of the leaf. Displaying the cells that occur at the tip resulted in the absorption of nutrients and water increases with increased wet weight as well. According to Wijayati (2005), cell lengthening will be followed by cell enlargement and increased wet weight. The increase in wet weight is mainly due to increased water retrieval by the cell

In addition, when in the mother tree, the tip of the leaf is used as a stockpile of nutrients. According to Prado (2013) in the illustration of water transport mechanism from root to leaf it is explained that nutrients are brought to all leaf lamina through leaf bone. This stream starts from the mother of the leaf bone that is in the stalk and ends at the tip of the leaf. After the mother leaves the bone, nutrients flow in the branch bone and the leaf veins which is the last branch on the leaves. Based on the explanation, it shows that the flow of nutrients ends at the tip of the leaf so that the nutrients that are carried accumulate in the area. Thus, when the leaves are cut, the leaves still contain nutrients such as when in the mother tree.

Besides as nutrient storage, the tip of the leaf is also a place of phenol accumulation. However, on the treatment of leaf tips, active charcoal is needed in small concentrations because if the concentration of activated charcoal is added, then the nutrients in the media will be absorbed. This is consistent with that described by Widiastoety (2004) that all types of activated charcoal absorb not only toxic compounds but also absorb other organic materials such as auxin and cytokines. Lack of nutrients can disrupt cell metabolic processes because the resulting energy is very low then the hormonal biosynthesis that regulates cell division and development work is not optimal. As a result growth and development of plants become obstructed. Therefore, the active charcoal treatment of 4 g / L is presumed to be the optimal treatment to produce high wet weight because if the concentration of activated charcoal is added, the nutrient needed explant will be absorbed by the active charcoal.

4. Conclusion

1. Treatment of leaf base and active charcoal 12 g / L combination was the optimal treatment to initiate callus in mangrove (*Rhizophora apiculata*) plant tissue culture.
2. Callus color resulting from plant tissue culture mangrove (*Rhizophora apiculata*) was brownish yellow. Callus texture produced from mangrove plant tissue culture (*Rhizophora apiculata*) was compact.
3. The combination treatment which produces the highest growth was the treatment of leaf tip and activated charcoal 4 g / L with 0.1685 g.

Acknowledgment

Thanks to the pnbp Faculty of Science and Mathematics, Diponegoro University who has helped the research so this research can be completed properly.

References

- [1] Admojo, L. and Indrianto, A 2016 *Jurnal Penelitian Karet* **330** 25
- [2] Arumugam, M., Panneerselvam, R 2012 *Asian Pacific Journal Of Tropical Biomedicine* 1096
- [3] Chauser 2002 *Botanical Journal of the Linnean Society* **138**.107
- [4] Hutami, S 2008 *Jurnal Agrobiogen* 83
- [5] Kalve, S., Vos, D.D., Beemster, G.T.S 2014 *Leaf Development: A Cellular Perspective* (Belgium: University Of Antwerp)
- [6] Kumar, G., Dashora, R., Jagetiya, S 2015 *General Techniques of Plant Tissue Culture* (North Carolina: Lulu Press Inc. Raleigh)
- [7] Lechtaler, S., Robert, E.M.R., Tonne, N., Prusova, A., Gerkema, E., As, H.V., Koedam, N., and Windt, C.W 2016 *Front Plant Sci* **7** 895
- [8] Pieter, O., Djoko, M., Ritohardoyo, S 2010 *Keanekaragaman Dan Pola Komunitas Hutan Mangrove Di Andai Kabupaten Manokwari* (Yogyakarta: UGM)
- [9] Prado, K. and Maurel, C 2013 *Regulation Of Leaf Hydraulics: From Molecular To Whole Plant Levels* (Perancis: Université Montpellier 2)
- [10] The Power of THLTBB Totally for Indonesia [www.penyuluhthl.wordpress.com], accessed date November 24th, 2017
- [11] Widiarti 2003 *Pembiakan Tanaman Melalui Kultur Jaringan* (Jakarta: Gramedia)
- [12] Widiastoety 2004 *J. Hort* **14** 1
- [13] Widyati, E 2013 *Dinamika Komunitas Mikroba Di Rizosfir Dan Kontribusinya Terhadap Pertumbuhan Tanaman Hutan. Bogor Pusat Penelitian dan Pengembangan Peningkatan Produktivitas Hutan*
- [14] Wijayati, A; Solichatun; Sugiyarto 2005 *Biofarmasi* **3** 16
- [15] Yelnitis 2012 *Jurnal Pemuliaan Tanaman Hutan* **6** 181