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# Effects of Light for Callus Induction of Mangrove Plant (Rhizophora Apiculata Bi) by In Vitro

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*pencoklatan, induksi kalus, cahaya, rhizophora apiculata BI.*

*Abstract*

*Initiation of Rhizophora apiculata BI propagation in vitro can be done by callus culture. Induction of mangrove callus has the problem of browning emergence. The phenomenon of browning can be overcome by limiting the treatment of light. The purpose of this research were to study the effect of light duration on callus growth, to understand the duration of light treatment that can spur the optimal callus growth, and to understand the effect of the light treatment on the browning event. The explants used were leaf grown in Murashige and Skoog (MS) medium with addition of NAA 1 ppm, BAP 0.3 ppm and activated charcoal 12 g / l. The treatments included 24 hour dark treatment, 24 hours light, dark 16 hours light 8 hours, light 8 hours dark 16 hours with 4 repetitions each. The results showed that the light treatment could induce callus formation while 24 hour dark treatment could reduce browning. All explant grown in conditions exposed to light and dark spewn exudate. The best callus growth (0.1939 g) was obtained in the T16G8 treatment (light 16 hours, dark 8 hours) with the time of the emergence of callus 6 DAP. Research about tissue culture with mangrove plants is rarely conducted because of high browning possibility. Thus, the novelty of this research lies upon the process of browning prevention using light duration treatment so browning could be prevented and mangrove culture could produce callus.*

**Abstrak**

Inisiasi perbanyakan Rhizophora apiculata BI secara in vitro dapat dilakukan dengan kultur kalus. Induksi kalus mangrove memiliki masalah munculnya kecoklatan. Fenomena pencoklatan bisa diatasi dengan membatasi perawatan cahaya. Tujuan dari penelitian ini adalah untuk mempelajari pengaruh lama pencahayaan terhadap pertumbuhan kalus, mengetahui lama perlakuan cahaya yang dapat memacu pertumbuhan kalus yang optimal, dan mengetahui pengaruh perlakuan cahaya terhadap kejadian pencoklatan. Eksplan yang digunakan adalah daun yang ditanam pada media Murashige dan Skoog (MS) dengan penambahan NAA 1 ppm, BAP 0,3 ppm dan arang aktif 12 g / l. Perlakuan tersebut meliputi perlakuan gelap 24 jam, terang 24 jam, terang gelap 16 jam 8 jam, terang 8 jam gelap 16 jam dengan masing-masing 4 pengulangan. Hasil penelitian menunjukkan bahwa perlakuan cahaya dapat menginduksi pembentukan kalus sedangkan perlakuan gelap 24 jam dapat mengurangi pencoklatan. Semua eksplan tumbuh dalam kondisi terpapar eksudat yang dimuntahkan terang dan gelap. Pertumbuhan kalus terbaik (0,1939 g) diperoleh pada perlakuan T16G8 (terang 16 jam, gelap 8 jam) dengan waktu munculnya kalus 6 HST. Penelitian tentang kultur jaringan dengan tanaman mangrove jarang dilakukan karena kemungkinan kecoklatannya tinggi. Dengan demikian, kebaruan dari penelitian ini terletak pada proses pencegahan pencoklatan menggunakan perlakuan durasi yang ringan sehingga pencoklatan dapat dicegah dan kultur mangrove dapat menghasilkan kalus..



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## INTRODUCTION

*Rhizophora apiculata* BI is one of the most significant mangrove major groups in Indonesia, and has many benefits such as improving water quality, feeding ground, spawning ground and nursery ground, for species of fish, shrimp, shellfish and other marine biota. Mangrove forests also play a role in maintaining coastline and river banks from erosion as well as abrasion to remain stable, controlling sea water intrusion, protecting the area behind mengrove from waves, strong winds and reducing the risk of tsunami hazard (Noor *et al*., 2007). While the population of the last half century has decreased mangrove area by 30-50%. But to grow it takes a long time is about 2 months. This becomes an obstacle in meeting the needs of seeds required in mangrove conservation (Ng *et al*., 2001).

Constraints in multiplication of mangrove seedlings can be overcome by vegetative propagation that can provide mangrove seedlings in a shorter time, namely by using in vitro culture. In vitro culture is a modern plant propagation technique by isolating plant parts such as organs, tissues, cells and protoplasm and growing it in aseptic conditions until it becomes a perfect plant. This technique makes it possible to produce large quantities of plants derived from cuts of plant organs and in a short time (Nasution, 2013).

There are several obstacles in tissue culture techniques that need to be addressed. One of the obstacles encountered in mangrove propagation in vitro is the difficulty of regenerating callus from clonal plant explants due to the still high browning level in the callus induction phase (Hoesen *et al*., 2008). Browning happened because of oxidized phenolic compounds accumulation due to mechanical stress or lesions on the explants (Corduk & Aki, 2011). The wound caused by the explant draining results in an oxidative state so that the phenol compound will be produced as a form of self-defense. Synthesis of phenol will increase when the plants are exposed to direct light, therefore to reduce the presence of phenol which can cause the browning effort is done by the treatment of light restriction. Corduk and Aki (2011) argued that one of the effort to overcome browning on tissue culture explant can be done by placing it in a total dark space.

According to Putri (2008) light has an effect on cell metabolism and work effectiveness of plant grow regulator (PGR) in media. Light can damage auxin and can also cause transfer of auxin to organs that avoid light. Method of tissue culture in dark conditions is one way to streamline the work of auxin so as to accelerate the formation of callus (Hidayat, 2015). Chawla (2009) mentioned that the importance of light in tissue culture lies in its effect on photomorphogenesis. It shows that dark conditions and bright conditions affect the formation of callus. The state of a culture is influenced by photoperiodicity, the quality and intensity of light (Siregar *et al*., 2010).

The treatment of soaking clones of rubber (*Hevea brasiliensis* Muell) in ascorbic acid and incubation in dark conditions could prevent the appearance of browning. The treatment also supports callus formation (Admojo & Indrianto, 2016). Widoretno *et al.* (2013), reported on the effect of sucrose and photoperiode on somatic embryogenesis of tangerine citrus 55 (*Citrus reticulata* Blanco.) that 16 hours light photoperiode treatment was able to produce higher-sized planlets than 8 hours of light and

12 hours of light photoperiode. Pudyastuti *et al.* (2012) reported that interactions of auxin and lighting conditions are not effective to induce callus from soybean cotyledone.

The aim of this research was to understand the effect of light duration treatment on callus growth, understanding which duration of light treatment that spurs optimal callus growth and understanding the effect of light treatment on browning. The benefit of this research was to produce callus growth with browning prevention through light control of mangrove plants. The callus could then be regenerated into an acclimatized mangrove plantlet. This plant tissue culture technique on mangrove is one of conservation effort, especially on provision and propagation of pure mangrove seedlings. This makes Indonesia as a pioneer of mangrove ecosystem conservation and also as a supplier of complete and best mangrove seedlings in the world.

## METHODS

### Provision of explant materials

Explant was taken from the first sequence leaf of 7 years old *Rhizophora apiculata* BI trees growing in the area of mangrove forest, Mangkang, Semarang.

### Explant Sterilization

The explant was washed using detergent, followed by successive sterilization in antibiotic solution, fungicidal solution, bayclin solution, 70% alcohol solution.

### Medium Preparation

500ml aquadest was heated with hotplate, then MS 4.43g/L was put into a beaker with aquadest and then homogenized with magnetic stirrer. Furthermore, 0.1 g / L myo-inositol was put into the solution, stirred to homogenous, and then 30 g/L sucrose was added, stirred until homogeneous. NAA was added with concentration of 1 ppm, and BAP with concentration of 0.3 ppm. Activated charcoal as much as 12 g/L was added then up to 1000mL of distilled water was added. Then the pH was measured by pH-meter, to obtain an appropriate pH given the addition of HCl or KOH to achieve acidity of 5.6-5.8; the media solution was then added to 8 g/L, stirred until suspended and homogeneous, medium heated to boiling. The finished medium was poured into a culture bottle and covered with aluminum foil and plastic wrap then sterilized in autoclave for 15-20 minutes at 121 °C.

### Explant Planting

Leaf explant was cut with size of $1cm^2$, cutting was done in ascorbic acid solution 10mg/100ml and planted in medium according to amount of treatment.

### Culture Incubation

Culture was placed in the incubation room using lighting lamps with 478 lux light intensity and also dark treatment (no light bulbs) in accordance with the treatment. The culture was incubated for 30 days.

### Data Analysis

The data obtained were then analyzed descriptively in the form of explanatory responses such as browning, callus initiation (DAP), percentage of explant callus, initial weight and final weight of each explant, callus color, callus texture and exudates produced by the explant.

## RESULTS AND DISCUSSION

### Callus Initiation Time

The callus on *Rhizophora apiculata* BI explants was able to grow as much as 50% in the treatment of light 24 hours, 50% in $T_{16}G_8$ treatment (light 16 hours, dark 8 hours), 0% (not grow callus) on dark treatment 24 hours, 50% on treatment $T_8G_{16}$ (bright 8 hours, dark 16 hours). The time of callus appearance (callus initiation) was calculated with units of days after planting (DAP) on MS medium by treatment of the duration of light irradiation. The result of the light treatment effect on growth of callus on mangrove plant leaf explants, data obtained during the callus initiation, shown in Figure 1 as follows:

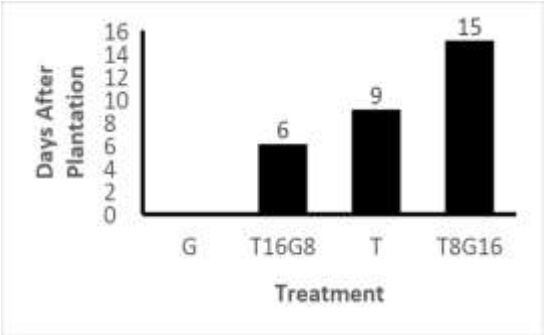


**Figure 1**. Initiation of *Rhizophora apiculata* leaf explant callus treated with light duration.

Callus induction from *Rhizophora apiculata* mangrove leaf explant could not take place in a 24 hours dark condition, because light is one of the important aspect for morphogenesis. The best light duration treatment is to combine light and dark conditions ie light 16 hours and dark 8 hours. It shows that bright conditions have a role in in vitro culture. Chawla (2009) mentions that the importance of light in tissue culture lies in its effect on photomorphogenesis. In the treatment of 16 hours dark followed by 8 hours of light the callus was still growing but it took a longer time that was 15 days. It shows that there is a dark optimum conditions required in tissue culture, because the longer the dark conditions the longer the callus initiation happen. Dark environment in tissue culture worked for organogenesis, but there is a certain time limit for dark conditions to function optimally.

The mechanism of light to spur induction of callus is to activate the action of enzymes that play roles in cell division. One such enzyme is the CDK enzyme (Cyclin dependent kinase). The darkness role in callus induction is to support the action of auxin because light can damage auxin and cause the transfer of auxin to organs that avoid light. According to Hidayat (2015) tissue culture method in dark conditions is one way to streamline the work of auxin so as to accelerate callus formation. Therefore,

the light conditions of 16 hours combined with dark conditions of 8 hours were able to induce faster callus induction than other treatments.

The developmental response of a culture is influenced by photoperiodisitas, quality and intensity of light (Siregar *et al*., 2010). Good light intensity for culture growth is 1000-4000 lux (Manuhara, 2014). Khumaida and Handayani (2010), reported that the light intensity required for induction and plankifion of embryonic callus in soybeans was 1500-2000 lux which resulted in the highest explosion rate of 93.94%. The intensity of light used in this study was 478 lux, and yielded a 37.5% cucumber explant. Allegedly the intensity of the light resulted in less optimal culture growth for mangrove callus. Light also affects the regulation of production of metabolites in cell suspension cultures, both primary metabolites such as enzymes, carbohydrates, lipids and amino acids or secondary metabolites such as anthocyanins, carotenoids, polyphenols, essential oils and terpenes. Suspected light intensity <1000 lux is not optimal for the work of several metabolic enzymes in mangrove explant

The addition of auxin in the medium will stimulate cell division and enlargement in explant so that spur the formation and growth of callus (Rahayu & Solichatun, 2003). BAP has a structure similar to kinetin, active in growth and callus poliferation (Sari *et al*., 2013). According to Santoso and Nursandi (2003) the effect of cytokinins in plant tissue culture is related to the process of cell division and callus proliferation. Cell division occurs with the help of cytokinins, cytokinins primarily play a role in the formation of threads at the metaphase stage (Santoso & Nursandi, 2003). BAP enhances regeneration by stimulating cell division within the tissues. The performance of this BAP is related to the control of CDKs enzyme activity (Cyclin Dependent Kinase) at the end of the S, M, G phase of the cell cycle. According to Kader *et al*. (2015), the combination between NAA and BAP can spur the initiation of callus in mangrove species.

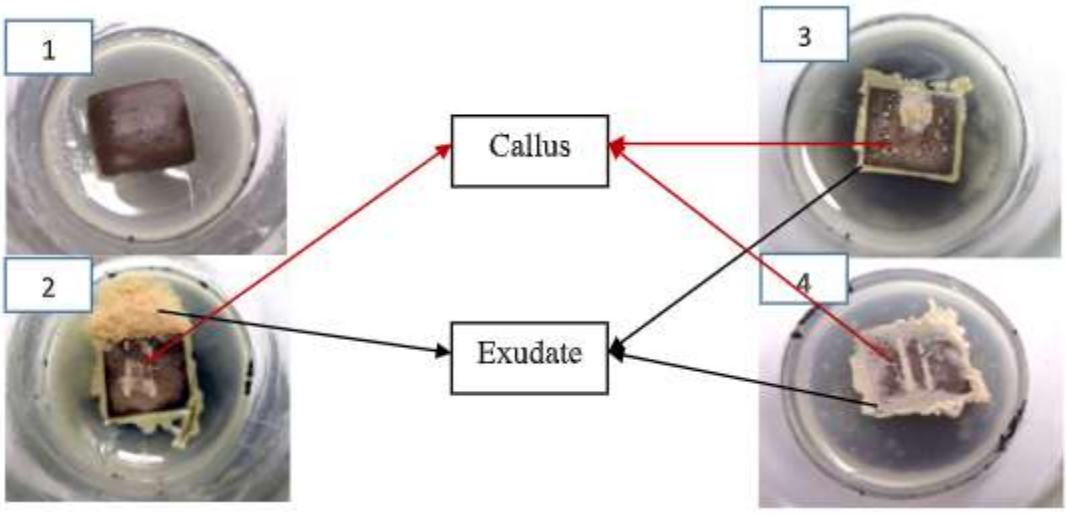


**Figure 2**. Morphology of *Rhizophora apiculata* BI Leaf Explants on the 30th day after planting. 1) 24 hour dark treatment, 2) light 16 hours dark 8 hours treatment, 3) 24 hours light treatment, 4) light 8 hours dark 16 hours treatment.

**Color and texture Callus**

The explant growth indicator on in vitro culture in the form of callus color depicts the visual appearance of callus so that the morphology of actively splitting or dead cells can be noticed. Callus tissue generated from a leaf explant usually bring up different colors. Colors on cells or tissues show the development of pigments and plastids in living cells. Callus color indicates its pigment content. Callus color produced on the treatment of the light duration can be seen in Table 1.

**Table 1.** Color and texture of callus on *Rhizophora apiculata* BI leaf explant after 30 days of light treatments

| Treatment | Callus Color | Callus Texture |
|---|---|---|
| $G_{24}$ | - | - |
| $T_{16}G_8$ | Brownish | Compact |
| $T_{24}$ | Brownish | Compact |
| $T_8G_{16}$ | White | Compact |

The brownish color of the callus is known as the browning phenomenon, which is caused by the metabolism of phenol compounds that are toxic, and often arise due to explant sterilization process. Phenol compounds are generally inhibit the growth or even cause tissue death (Andaryani, 2010). Zulkarnain (2009) stated that the browning event is actually a natural event and adaptive change process of plant parts due to physical effects such as stripping, and cutting.

Callus texture is one of the markers used to assess the quality of a callus. The compact callus texture is considered good because it can accumulate more secondary metabolites (Yelnititis, 2012). Andaryani (2010), stated that the callus texture may vary from compact to weaker depending on the type of plant used, the nutrient composition of the media and the growth regulator.

According to Andaryani (2010), the formation of compact textured callus spurred by the presence of endogenous auksin hormone produced internally by explant that has grown to form the callus. Provision of growth regulators may affect secondary metabolite production. This is due to the added PGR can cause physiological and biochemical changes of plants through the regulation of enzyme work. PGR plays a role in the binding of protein membranes that have the potential for enzyme activity. The result of this binding activates the enzyme and converts the substrate into several new products. This new product is formed causing a series of secondary reactions one of which is the formation of secondary metabolites. According to Putri (2008), the dark and light conditions in the treatment medium have no effect on the texture of the callus.

**Initial and Final Weight Difference of Explant**

Growth is characterized by increased irreversible weight, so that the measurement of the initial weight and the late weight of callus may represent callus growth variables derived from mangrove leaf explants (*Rhizophora apiculata* BI). According to Kartika *et al*. (2013), the biomass produced in tissue cultures is highly dependent on the speed of the cells dividing, multiplying, which is continued by cell enlargement. The speed of the split cell can be affected by the presence of certain combinations of auxin-

cytokines in certain concentrations depending on the plant, as well as external factors such as light intensity and temperature.

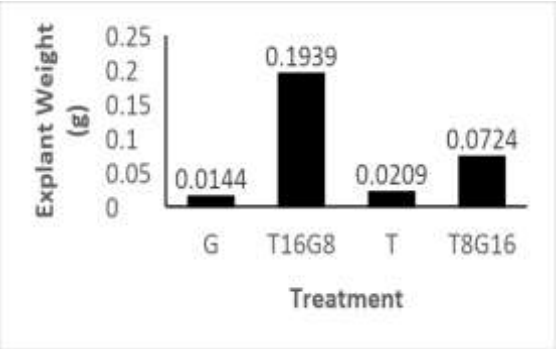


**Figure 3**. Average initial weight and final weight of *Rhizophora apiculata* BI leaf explants grown in bright dark environments

The increase of explant weight from the first time of planting until day 30 was caused by the explant of mangrove leaf growing callus and exudate secretion. The highest increase in explant weight was in the $T_{16}G_8$ treatment (light 16 hours, dark 8 hours), with weight gain of 0.1939 g, followed by $T_8G_{16}$ (0.0724 g) treatment and $T_{24}$ treatment (0.0209 g). The increase in weight was due to cell division, cell enlargement and cell differentiation. The lowest increase in explant weight was experienced by $G_{24}$ treatment (0.0144 g), this was because at the treatment of $G_{24}$ (dark 24 hours) there was no callus growth and only produced exudate.

Rahmaniar (2007) stated that the large weight of the large callus is due to its high water content. The resulting wet weight is highly dependent on the speed of the cells dividing, multiplying and continued with enlargement of callus. Callus growth in one plant species may differ depending on factors such as the original explant position in plants and growth conditions. According to Zulkarnain (2009), growth and morphogenesis in vitro is influenced by the interaction and the ratio of growth regulator substances added in media and PGR produced endogenously by cultured cells.

Kartika (2013) stated that auxin can alter the activity of enzymes that play a role in the synthesis of cell wall components and rearrange them in an intact cell wall matrix that will affect cell weight. Auxins can encourage cell elongation followed by cell enlargement and increased wet weight. The increase in wet weight is mainly due to the increased absorption of water by these cells. According Zulkarnain (2009), the provision of cytokinin into the tissue culture medium is important to induce the development and growth explant. If the availability of cytokinin in the culture medium is so limited, cell division in the tissue will be obstructed.

**Browning**

Browning occured in all treatments after 30 days of planting. The thing that distinguishes it from each treatment was the day when the leaf explant browning as a whole. The fastest explant browning was explant on the $T_{24}$ (light 24 hour) treatment on 7.5 days after planting, while the longest explant browning was explants treated with $G_{24}$ (dark 24 hours) ie on day 11.75 after planting (Figure 4). Based on

observations, the most appropriate method to reduce the occurrence of browning is to use dark conditions, but this condition does not spur induction of callus on mangrove leaf explants.

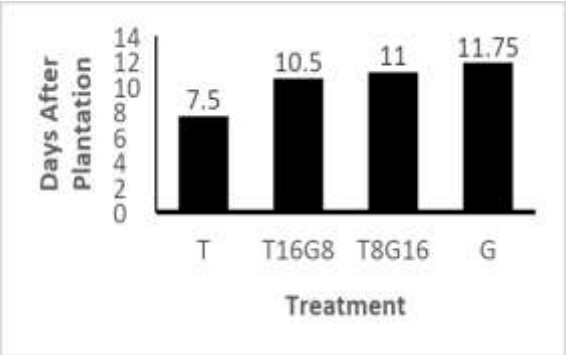


**Figure 4**. Days average when *Rhizophora apiculata* BI leaf explant underwent *browning* throughly.

Ru *et al*. (2013), explained that browning in the tissues is one of the problems that often occur in woody plant culture. Browning is generally caused by phenolic compounds that usually appear and accumulate when the explants are injured, which is usually caused by the activation of the polyphenol oxidase (PPO) enzyme. An organ opening can cause metabolic imbalance from ROS (Reactive Oxygen Species), peroxidation of the lipid membrane, and loss of integrity of cell membranes that can trigger over accumulation of phenolic compounds and cause browning.

The oxidation of phenol will increase with the presence of light (Hutami, 2008). The oxidation occurring in phenolic compounds may be via autoxidation or enzymatic oxidation reactions. Autoxidation is an oxidation reaction caused by the presence of light and oxygen (Andarwulan & Faradilla, 2012). Polyphenol oxidase enzyme, when there is widespread in the presence of oxygen and light will convert the monophenol group into o-hydroxy phenol then converted again into o-quinone groups. This o-quinone group forms a brown color. Dark treatment ($G_{24}$) in mangrove leaf explant isolation inhibits browning time compared with other treatments. The lightless conditions resulted in slow oxidation of phenol. This is in accordance with the opinion of Corduk & Aki (2011), that the increase of phenol occurs when plants are exposed to direct light. Therefore, 24 hours dark treatment ($G_{24}$), blocking the existence of light that can spur the production of phenol that causes browning.

**Exudate**

Explant slices grown in MS medium to give another response that exudate production. When plant tissue is injured, because of the cutting effect, there will be physiological reactions around the wound. One of the physiological reactions is the appearance of exudate. Exudate secreted out of sliced mangrove leaf because the leaf of the family Rhizophoraceae has water storage (Lechtaler *et al*., 2016). According to Widyati (2013), exudates include compounds with low molecular weight, such as sugars, amino acids and aromatics. The exudates do not exert a lethal effect on the growth of culture. Exudates produced by explants can be seen in Figure 5.

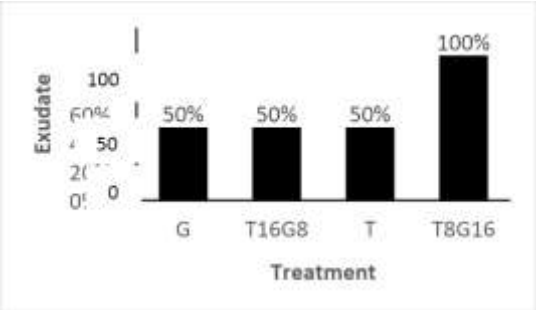


**Figure 5.** The percentage of exudate released by explant leaves *Rhizophora apiculata* BI due to the treatment of light duration after day 30.

The most exudate secreted was in the $T_8G_{16}$ treatment (light 8 hours, dark 16 hours) of 100%, while in $T_{24}$ treatment (light 24 hours), $T_{16}G_8$ (light 16 hours, dark 8 hours), and $G_{24}$ (dark 24 hour) of exudate produced by 50%, and 100% in T8G16 treatment (light 8 hours, dark 16 hours). The resulting exudate had no effect on callus growth, because the most exudate was in the $T_8G_{16}$ treatment (light 8 hours, dark 16 hours), whereas the most grown callus was in the $T_{16}G_8$ treatment (light 16 hours, dark 8 hours).

Research about tissue culture with mangrove plants is rarely conducted because of high browning possibility. Thus, the novelty of this research lies upon the process of browning prevention using light duration treatment so browning could be prevented and mangrove culture could produce callus. The benefit of this research was to produce callus growth with browning prevention through light contro1 of mangrove plants. The callus could then be regenerated into an acclimatized mangrove plantlet. This plant tissue culture technique on mangrove is one of conservation effort, especially on provision and propagation of pure mangrove seedlings. This makes Indonesia as a pioneer of mangrove ecosystem conservation and also as a supplier of complete and best mangrove seedlings in the world.

## CONCLUSION

The treatment of light duration affects callus growth in in vitro culture of mangrove plants. The fastest treatment in callus exchant of *Rhizophora apiculata* BI leaf callus was $T_{16}G_8$ treatment (light 16 hours, dark 8 hours), while the most optimal treatment to inhibit the occurrence of browning was $G_{24}$ treatment (dark 24 hours), although the condition was not spur induction of callus. Dark conditions have the effect to reduce browning in in vitro culture of mangrove plants.

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## REFERENCES

Admojo, L. & Indrianto, A. (2016). Browning prevention on callus initiation phase on leaf midrib of PB 330 rubber clone culture *(Hevea brasiliensis* Muell. Arg*). Indonesian Journal of Natural Rubber Research,* 34(1): 25-34.

Andaryani, S. (2010). Kajian penggunaan berbagai konsentrasi BAP dan 2,4-D terhadap induksi kalus jarak pagar *(Jatropha curcas L.)* secara *in vitro.* Skripsi Universitas Sebelas Maret Surakarta.

Andarwulan, N. &Faradilla, R.H.F. (2012). Senyawa fenolik pada sayuran indigenous. Bogor: Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center Research and Community Service Institution Bogor Agricultural University.

Corduk, N. & Aki, C. (2011). Inhibition of browning problem during micropropagation of Sideritis trojana Bornm. an endemic medicinal herb of Turkey. *Romanian Biotechnological Letters,* 16(6): 6760-6765.

Chawla, H.S. (2009). *Introduction to Plant Biotechnology* Third Edition. India: Genetics & Plant Breeding Department, G.B. Pant University of Agriculture & Technology.

Eddy, S. (2008). *Pengelolaan Potensi Hutan Mangrove secara Berkelanjutan*. Palembang: Jurusan Biologi FMIPA Universitas PGRI Palembang.

Hoesen, D.S.H., Witjaksono, & Sukamto, L.A. (2008). Callus induction and organogenesis of in vitro culture *Dendrobium lineale* Rolfe. *Biology News,* 9(3): 333-341.

Hutami, S. (2008). Masalah pencoklatan pada kultur jaringan. *Jurnal Agro Biogen,* 4(2): 83-88.

Kader, A., Sinha, S.N., & Ghosh, P. (2015). Contribution of environmental factors on in vitro culture of an endangered and endemic mangroves *Heritiera fomes* Buch.-Ham. and *Bruguiera gymnorhiza* (L.) Lam. *International Journal of Tropical Plant Research,* 2(3): 192–203

Kartika, L., Atmodjo, P.K., & Purwijantiningsih, L.M.E. (2013). Callus induction rate and eugenol content of red betel (*Piper crocatum* Ruiz and Pav.) treated using a variation of the type and concentration of auxin http://e-journal.uajy.ac.id/4836/1/naskah%20jurnal.pdf.

Khumaida, N. & Handayani, T. (2010). Embryogenic callus induction and proliferation on several soybean genotypes. *Journal Agronomy Indonesia*, 38(1): 19- 24.

Lechtaler, S., Robert, E.M.R., Tonne, N., Prusova, A., Gerkema, E., As, H.V., Koedam, N., & Windt, C.W. (2016). Rhizophoraceae mangrove saplings use hypocotil and leaf water storage capacity to cope with soil water salinity changes. *Front Plant Sci,* 7: 895. DOI*:* 10.3389/fpls.2016.00895

Manuhara, Y.S.W. (2014). *Kapita Selekta Kultur Jaringan Tumbuhan*. Surabaya: Airlangga University Press.

Nasution, S.S. (2013). Pengaruh teknik sterilisasi terhadap keberhasilan eksplan paulownia *(Paulownia elongata* SY. Hu*)* secara *in vitro. Bogor : Institut Pertanian Bogor*.

Ng, P.K.L. and Sivatoshi, N . (2001). A Guide to mangroves of Singapore. *Volume 1: The Ecosystem and Plant Diversity and Volume 2: Animal Diversity*. *Singapore: The Singapore Science Centre.*

Noor, Y.R., Khazali, M., & Suryadiputra, I.N.N. (2007). *Panduan Pengenalan Mangrove di Indonesia.* Bogor: PHKA/WI-IP.

Pudyastuti, S., Habibah, N.A., & Sumadi. (2012). Efektivitas ZPT 2,4-D pada medium MS dan lama pencahayaan untuk menginduksi kalus dari kotiledon kedelai. *Biosaintifika Journal of Biology & Biology Education,* 4(1): 42-46.

Putri, N.I. (2008). Kajian berbagai komposisi media serta kondisi gelap dan terang terhadap induksi kalus tanaman jati belanda *(Guazuma ulmifolia* Lamk.). Skripsi Universitas Sebelas Maret Surakarta.

Rahayu, B. & Solichatun, A. (2003). Pengaruh 2,4-D terhadap pembentukan dan pertumbuhan kalus serta kandungan flavonoid kultur kalus *Acalypha indica* L., *Biofarmasi* 1(1): 1-6.

Rahmaniar, A. 2007. Pengaruh macam eksplan dan konsentrasi 2,4-d-dichlorophenoxyacetic acid (2,4-D) terhadap pertumbuhan anthurium (*Anthuriumm plowmanii* Croat) pada medium MS. Skripsi. Fakultas Pertanian UNS. Surakarta.

Ru, Z., Lai, Y., Xu, C., and Li, L. (2013). Polyphenol oxidase (PPO) in early stage of browning of *Phalaenopsis* leaf explants. *Journal of Agricultural Science,* 5(9): 57 - 64. DOI*:* 10.5539/jas.v5n9p57

Santosa, U. & Nursandi, F. (2004). *Kultur Jaringan Tanaman*. Malang: Penerbit UMM.

Sari, N., Ratnasari, E., & Isnawati. (2013). Pengaruh penambahan berbagai konsentrasi 2,4-dikhlorofenoksiasetat (2,4-D) dan 6-bensil aminopurin (BAP) pada media MS terhadap tekstur dan warna kalus eksplan batang jati (*Tectona grandis* Linn. F.)" JUL". *Lentera Bio*, 2(1): 69-73.

Siregar, L.A.M., Lai-Keng, C., & Peng-Lim, B. (2010). Effects of casein hydrolisate and light intensity on production of biomass and canthinone alkaloid in cell suspension cultures of pasak bumi *(Eurycoma longifolia* Jack*). Malaysia: Makara, Sains,* 14(1): 21-26

Widyati, E. (2013). *Rhizosphere Microbes Community Dinamic and It Contribution on Plants Growth*. Bogor: Pusat Penelitian dan Pengembangan Peningkatan Produktivitas Hutan.

Widoretno, W., Martasari, C., &Nirmala, F.D. (2013). Effects of sucrose and photoperiod on somatic embryogenesis of citrus keprok batu 55 *(Citrus reticulata* Blanco*.). Journal of Horticulture Indonesia,* 4(1): 44-53

Yelnititis. (2012). Friable callus induction from leaf explant of ramin *(Gonystylus bancanus (*Miq) Kurz.). *Jurnal Pemuliaan Tanaman Hutan,* 6: 181–194.

Zulkarnain. (2009). *Kultur Jaringan Tanaman. Solusi Perbanyakan Tanaman Budi Daya.* Jakarta: Bumi Aksara