JUATIKA
JURNAL AGRONOMI TANAMAN TROPIKA
VOL. 6 NO. 2 May 2024

DOI :https://doi.org/10.36378/juatika.v6i2.3670
eissn 2656-1727
pissn 2684-785X
Hal : 657 – 664

Micropropagation of Orchids (*Cattleya sp*) by adding Plantain Extract and Activated Charcoal to MS Media by in-vitro

Wendra Mayandres Pratama, Elfi Indrawanis, Tri Nopsagiarti*
Universitas Islam Kuantan Singingi
Jl. Gatot Subroto KM 7, Kebun Nenas, Teluk Kuantan, Sungai Jering Distric, Kuantan Singingi Regency, Riau 29511 Indonesia
*Email : trinopsagiarti@gmail.com

ABSTRACT

The *Cattleya sp* orchid, which belongs to the Orchidaceae family, is a popular ornamental plant that is widely enjoyed around the world. It is natural for orchids to be very difficult to cultivate, and in vitro techniques are one way to reproduce *Cattleya sp* orchids in controlled conditions. The purpose of the study was to investigate the growth of *Cattleya sp* orchid explants by introducing different levels of plantain extract and activated charcoal to MS media in a controlled environment. The research design employed a factorial Completely Randomized Design (CRD) involving 2 treatments: banana extract (with concentrations of 0 g/l, 25 g/l, 50 g/l, and 75 g/l) and activated charcoal (with concentrations of 0 g/l, 1 g/l, 2 g/l, and 3 g/l). The results indicated that the concentration of banana extract had a notable impact on the average number of leaves (5.00), the number of roots (7.92), and the length of the roots (1.31 cm). The ideal concentration of banana extract is 50 grams per liter of MS medium. A single treatment of activated charcoal had a significant effect on *Cattleya sp* orchid explants, with the best concentration of activated charcoal being 2 g/l MS media. This resulted in a leaf length of 1.28 cm, 7.44 roots, and a root length of 1.38 cm. In the meantime, the combination of plantain extract and activated charcoal did not significantly affect any of the parameters.

Keywords: Activated Charcoal, Banana Extract, *Cattleya sp*, Concentration, In-Vitro

1. INTRODUCTION

Cattleya sp orchids, classified within the Orchidaceae family, are esteemed as one of the most sought-after ornamental plants. These orchids exhibit a remarkable diversity in their forms, colors, and floral characteristics. Globally, the orchid family encompasses approximately 25.000 to 30.000 species, with *Cattleya sp* being a notable representative. The exquisite beauty of its blossoms has earned it the title of "queen of flowers." In Indonesia, *Cattleya sp* orchids are recognized for their significant economic value (Meilani, 2017).

The high market demand for orchids is attributed to their limited availability and the challenges associated with conventional cultivation methods. The scarcity of quality seeds further exacerbates the situation, making the cultivation of orchids particularly challenging due to their complex regeneration and seed production processes. To address these challenges, in vitro propagation techniques can be employed, facilitating the production of healthier seeds that are capable of optimal growth.

Karjadi (2016) posits that in vitro techniques represent the most effective method for plant propagation. This approach utilizes smaller plant materials, thereby minimizing damage to the parent plant. The in vitro environment is both aseptic and meticulously controlled, allowing for the rapid production of seedlings that are free from diseases. Additionally, this method requires relatively limited space to generate a substantial quantity of seedlings.

The efficacy of in vitro plant propagation is significantly influenced by the composition of the growth media employed. Typically, the media is supplemented with various additives, including vitamins, amino acids, growth regulators, or other organic substances, to optimize the growth of the explants. Among these organic additives, banana extract is noteworthy, as it is rich in carbohydrates and plant growth regulators, which can effectively promote the growth of explants.

The findings of the research conducted by Rahayu et al. (2021) indicated that the incorporation of 150 g/L of plantain extract into Vacin and Went (VW) media yielded the most effective concentration for promoting the growth of V. hookeriana Rchb. f. Orchid. Similarly, Fajri et al. (2020) reported that the optimal growth of lime explants was achieved with the addition of 50 g/L of plantain extract in MS media.

Building on these insights, the current study incorporated plantain extract into the micropropagation process of *Cattleya sp* orchids utilizing MS media. Additionally, activated charcoal was included in the in vitro culture media for the orchids. This substance possesses the capability to absorb toxic and inhibitory compounds released by the plantlets during their development. Furthermore, activated charcoal contributes to the stabilization of the media's pH, promotes root development, reduces light penetration into the plantlet media, mitigates callus formation, and encourages morphogenetic processes.

The results of Telaumbanua's research, (2022) showed that the concentration of activated charcoal affected the increase in the number of leaves, number of shoots, number of roots, and height of Dendrobium sp orchid plantlets on VW (Vacin and Went) media, the best treatment was 2 g/L VW media. Furthermore, Nasution et al. (2021) stated that the addition of 2 g/L of activated charcoal to MS media resulted in the best shoot growth and leaf length for Dendrobium sp orchid explants. Meanwhile, research by Yusron et al (2020) showed that the administration of 2 g/L of activated charcoal in MS media combined with BAP had a significant effect on the growth of calamondin explants.

MS media is composed of both macro and micronutrients, including

myoinositol, niacin, pyridoxine HCl, thiamin HCl, glycine, and glucose. The Murashige and Skoog (MS) medium is extensively utilized in plant tissue culture due to its high concentrations of nitrate, potassium, and ammonium, which are essential for optimal plant growth.

2. MATERIAL AND METHODS

The research was conducted at the Tissue Culture Laboratory of the Riau Province Food Crops and Horticulture Seeds UPT, Jalan Kaharudin Nasution No. 133. Simpang Tiga Village, Bukit Raya District, Pekanbaru City. The design used was a factorial Completely Randomized Design (CRD) consisting of two factors, namely plantain extract and activated charcoal. The first factor of giving plantain extract consisted of 4 levels: plantain extract 0 g/l, 25 g/l, 50 g/l, and 75 g/l media. The second activated charcoal treatment consisted of 4 levels: activated charcoal 0 g/l, 1 g/l, 2 g/l, 3 g/l Media. There were 16 treatment combinations with 3 replications so 48 experimental units were obtained. Each experimental unit consisted of 1 culture bottle, each consisting of 2 explants.

The tools used in this study include a laminar air flow cabinet, autoclave, analytical balance, Erlenmeyer flasks, magnetic stirrer, graduated cylinders, beakers, Petri dishes, pipettes, glass stirrers, tweezers, scalpels, spirit lamps, hand sprayers, knives, pH meter, culture bottles, gas stoves, test tubes, volumetric flasks, scissors, plastic rubber, stationery, and washing equipment.

The materials employed in this research comprise extracts from Raja bananas, activated charcoal, and explants of *Cattleya orchids*, along with chemical media from Murashige-Skoog, sterile aquades, alcohol, agar powder, glucose, detergent, Tween, fungicides, and tissue paper. The observed parameters include the number of leaves, leaf length, root quantity, and root length. The observational data were analyzed statistically and presented in tables, with honest significant difference testing (HSD) at a 5% significance level using Microsoft Excel. The stages of the research are illustrated in the following diagram:

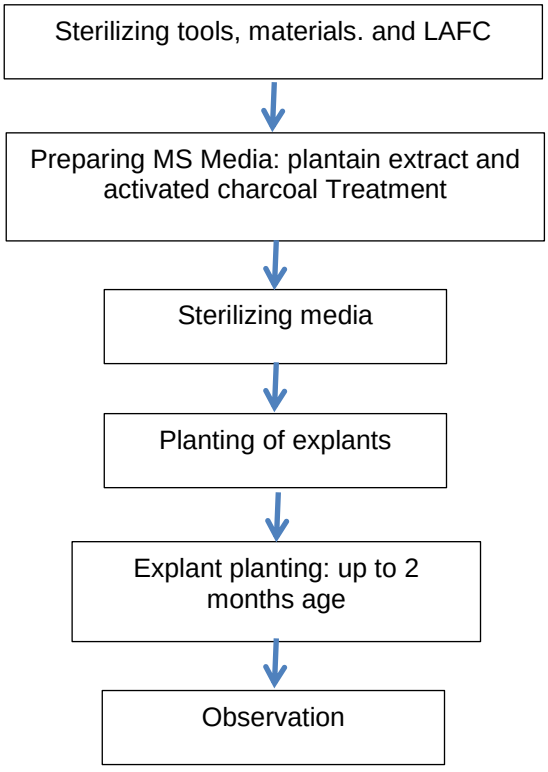

Figure 1. Research flow diagram

3. RESULT AND DISCUSSION

3.1 Number of leaves (strands)

The observation results on the number of leaves in *Cattleya sp* orchid explants indicate that the treatment with banana extract alone has a significant effect on leaf quantity. The best results were obtained at a concentration of 50 g/l in MS media, yielding an average of 5.00 leaves, with post hoc BNJ (5%) testing showing no significant differences among treatments. The application of activated charcoal alone and the interaction between banana extract and activated charcoal did not have a significant effect on the number of orchid leaves. For more detailed information, the data on the number of *Cattleya sp* orchid leaves can be found in Table 1.

Table 1. Average Number of *Cattleya sp* Orchid Explant Leaves Given Plantain Extract and Activated Charcoal (Sheets) on MS Media

Plantain extract (g/l MS media)	Activated Charcoal (g/l MS media)				Average Plantain extract
	0 g/l	1 g/l	2 g/l	3 g/l	
0 g/l	3.67	3.78	3.44	4.00	3.72a ± 0.23
25 g/l	4.44	4.33	4.44	4.56	4.44a ± 0.09
50 g/l	4.78	4.89	5.22	5.11	5.00a ± 0.20
75 g/l	4.78	4.78	4.89	4.44	4.72a ± 0.19
Activated Charcoal Average	4.42 ±0.52	4.44 ±0.50	4.50 ±0.77	4.53 ±0.45	
KK=	9.38%	BNJ plantain extract = 2.17			

Note: The numbers in the column followed by the same lowercase letter are not significantly different according to the further significant difference test (BNJ) at the 5% level.

The administration of 50 g/l of plantain extract in MS (A2) media was able to produce the largest number of leaves. When compared to the control (without the addition of plantain extract), the difference in the number of leaves was 1.28. These results indicate that the addition of plantain extract in MS planting media can stimulate the growth of *Cattleya sp*. orchid explant leaves.

The extract of Raja bananas contains a variety of minerals and vitamins. According to data from the Ministry of Health (2018), 100 grams of Raja bananas consists of: 65.8 g of water; 120 Kcal of energy; 1.2 g of protein; 0.2 g of fat; 31.8 g of carbohydrates; 5.3 g of fiber; 1.0 g of ash; 10 mg of calcium; 22 mg of phosphorus; 0.8 mg of iron; 35 mg of sodium; 582.2 mg of potassium; 0.31 mg of copper; 0.7 mg of zinc; 53 mcg of beta-carotene; 0.06 mg of thiamine; 0.14 mg of riboflavin; 1.2 mg of niacin; and 10 mg of vitamin C. Analyzing this composition, it is evident that a range of vitamins and minerals essential for plant growth is present. Thiamine (vitamin B1), for instance, serves as a coenzyme in carbohydrate metabolism and enhances the activity of hormones within plant tissues, which subsequently promote the division of new cells. According to Srilestari et al. (2020), vitamin B1 (thiamine) is required in culture media as a catalyst in metabolic processes, where thiamine can stimulate cell division.

The results of the study by Rahayu et al., (2021) showed that giving 100 g/l of plantain extract to VW (Vacin and Went) media was the best treatment to produce the largest number of leaves in Vanda hookeriana Rchb. f. orchids. There was a difference in the concentration of plantain extract given to produce the largest number of leaves, this was due to the different types of media and plants used, as stated by Lestari et al. (2015) that the success of orchid propagation by tissue culture was influenced by the

composition and combination of media. Tuhuteru (2012) stated that media is the main factor in plant propagation by tissue culture, media has an important influence on the growth and development of explants. Furthermore, Rahayu et al. (2021) stated that MS and VW media have almost the same substance content, but there is a slight difference in their composition.

3.2 Leaf Length (cm)

The observations on the shoot length parameters of *Cattleya sp* orchid explants indicate that the treatment with banana extract, both individually and in interaction, does not have a significant effect on shoot length. However, the application of activated charcoal alone shows a significant effect. The results of the honest significant difference test (HSD) at the 5% level can be seen in Table 2.

Table 2. Average Leaf Length of *Cattleya sp* Orchid Explants Given Plantain Extract and Activated Charcoal (cm) on MS Media.

Plantain extract (g/l MS media)	Activated Charcoal (g/l MS media)				Average Plantain extract
	0 g/l	1 g/l	2 g/l	3 g/l	
0 g/l	1.04	1.08	1.23	1.17	1.13 ±0.07
25 g/l	1.18	1.09	1.21	1.13	1.15 ±0.04
50 g/l	1.09	1.14	1.36	1.22	1.20 ±0.10
75 g/l	1.07	1.10	1.33	1.11	1.15 ±0.10
Activated Charcoal Average	1.09 ±0.06 b	1.10 ±0.02 b	1.28±0.07 a	1.16 ±0.04 b	
KK= 6.09%	BNJ Activated Charcoal = 0.08				

Note: The numbers in the column followed by the same lowercase letter are not significantly different according to the further significant difference test (BNJ) at the 5% level.

Based on the data in Table 2. it is evident that the application of activated charcoal alone has a significant effect on the length of the shoots of *Cattleya sp.* orchid explants. The best shoot length, measuring 1.28 cm, was observed with the addition of 2 g/L of activated charcoal to the MS medium. This results in a shoot length difference of 0.17 cm compared to the control (without the addition of activated charcoal), indicating that activated charcoal can stimulate the growth of *Cattleya sp.* orchid shoots. The role of activated charcoal in in vitro culture media includes the absorption of toxic compounds or inhibitors secreted by the plantlet during growth. Additionally, activated charcoal helps stabilize the pH of the medium and reduces light penetration into it. By incorporating activated charcoal into the medium, optimal conditions for explant growth can be created, allowing the plants to thrive effectively.

The study results by Warisman and colleagues. The study (2024) indicated that using 3 g/l of activated charcoal in VW media resulted in the highest leaf length of 3.66 cm in Dendrobium welirang orchid explants. In this study, the best leaf length was achieved by using 2 g/l of activated charcoal in MS media, resulting in a leaf length of only 1.28 cm. Increasing the concentration to 3 g/l of media resulted in a decrease in leaf length, suggesting that the ideal concentration to promote the growth of *Cattleya sp.* is 2 g/l of MS media. orchid tissues. In Ismaini's (2021) study, it is mentioned that the incorporation of activated carbon into in vitro culture media serves to absorb toxic compounds generated by plants.

3.3 Number of Roots

The observation results regarding the number of root explants of *Cattleya orchids* indicate that the treatment of Plantain extract and activated charcoal applied individually has a significant

effect on the number of root explants of *Cattleya sp.* However, the interaction between the two does not have a significant effect. The application of 50 g/l of Plantain extract in the MS medium resulted in the highest number of roots. This concentration is optimal for stimulating the growth of root explants. The results of the BNJ (5%) further test show a significant difference compared to other treatments.

Table 3. Average Number of Roots of *Cattleya sp.* Orchid Explants Given Plantain Extract and Activated Charcoal on MS Media

Plantain extract (g/l MS media)	Activated Charcoal (g/l MS media)				Average Plantain extract
	0 g/l	1 g/l	2 g/l	3 g/l	
0 g/l	5.67	5.89	7.00	6.44	6.25 ±0.79 c
25 g/l	6.89	7.11	6.67	7.00	6.92 ±0.19 b
50 g/l	7.33	7.67	8.89	7.78	7.92 ±0.67 a
75 g/l	7.22	6.78	7.22	6.67	6.97 ±0.28 b
Activated Charcoal Average	6.7±0.7 6 b	6.86±0.74 b	7.44±0. 9 a	6.97±0.5 8 b	
KK= 7.78%			BNJ plantain extract/ activated charcoal = 0.61		

Note: The numbers in the column followed by the same lowercase letter are not significantly different according to the further significant difference test (BNJ) at the 5% level.

The research conducted by Fajri et al. (2020) identifies that the optimal treatment of banana extract is 50 g/L in MS medium to promote the growth of lime explants. This study shares a similar concentration with the findings of the present research. Conversely, the study by Rahayu et al. (2021) indicates that to achieve the highest number of roots in Vanda hookeriana Rchb. f. orchid explants, a concentration of 150 g/L in VW medium is required. It can be observed from these two studies that the amount of banana extract added when using MS medium is lower compared to using VW medium, due to the differing nutrient compositions of MS and VW, particularly in nitrogen content. According to Istiqomah (2021), both MS and VW media essentially contain similar substances, with the difference lying in their composition, which imparts distinct characteristics that influence the cultured explants differently.

The administration of activated charcoal alone had a significant effect on the number of roots of *Cattleya orchid* explants. The best treatment was given 2 g/l of MS media, which was 7.44. The results of the study by Warisman et al. (2024) showed the same results, where the administration of 2 g/l of activated charcoal in VW media resulted in some roots of Dendrobium welirang orchids of 6.75. Meanwhile, the best number of leaves and leaf length were at a concentration of 3 g/L of VW media.

3.4 Root Length (cm)

The findings from the observations regarding the root lengths of Cattleya sp orchid explants indicate that the application of plantain extract and activated charcoal, when administered separately, significantly influenced root development. The outcomes of the subsequent honest significant difference (HSD) test at the 5% significance level are presented in Table 4.

The application of 50 g/l of Plantain extract in MS medium is the best treatment for stimulating the root length growth of *Cattleya sp.* orchid explants. Subsequent tests showed significant differences compared to the control treatment (0 g/l) and the 25 g/l treatment. This concentration represents the optimal amount for all growth parameters of the explants. Plantain extract is capable of aiding cell division, leading to faster cell growth (Fajri et al., 2020), and it provides a favorable growth and development response for orchid plant explants

(Rahayu et al., 2021). The auxin content in Plantain extract plays a vital role in root growth, thus its presence in certain quantities will yield optimal explant growth.

Table 4. Average Root Length of *Cattleya sp* Orchid Explants Given Plantain Extract and Activated Charcoal (cm) on MS Media.

Plantain extract (g/l MS media)	Activated Charcoal (g/l MS media)				Average Plantain extract
	0 g/l	1 g/l	2 g/l	3 g/l	
0 g/l	1.02	1.09	1.29	1.16	1.14±0.11 b
25 g/l	1.09	1.09	1.30	1.11	1.15±0.10 b
50 g/l	1.12	1.21	1.54	1.37	1.31±0.18 a
75 g/l	1.16	1.23	1.40	1.26	1.26±0.11 a
Activated Charcoal Average	1.10±0.06c	1.16±0.05b	1.38±0.17a	1.22±0.11b	
KK= 5.82%		BNJ A= 0.08		BNJ B= 0.08	

Note: The numbers in the column followed by the same lowercase letter are not significantly different according to the further significant difference test (BNJ) at the 5% level.

The extract of plantain extract also contains several vitamins, one of which is thiamine. According to Srilestari et al. (2020), the thiamine present in plantain extract can stimulate the growth of root meristems, resulting in longer and more numerous root cells. The application of plantain extract extract at higher concentrations (75 g/l) or lower concentrations (25 g/l) in the media results in lower growth of *Cattleya sp.* orchid explants. In comparison to previous studies, there is a consistent finding that a concentration of 50 g/l of plantain extract extract is optimal for enhancing the growth of explants propagated in vitro (Fajri et al., 2020; Delviandra et al., 2021).

The administration of activated charcoal alone had a significant effect on the root length of *Cattleya sp.* orchid explants. The best treatment was at a concentration of 2 g/l MS media, the results of further tests were significantly different from other treatments. Compared with the research of Nisyawati et al. (2013) and Warisman et al. (2024), the concentration of 2 g/l media is the optimum amount to stimulate explant growth.

4. CONCLUSION

The findings of the research indicate that:

1. The application of 50 g/l of plantain extract in MS media (A2) yields optimal results for the growth of *Cattleya sp.* orchid explants, resulting in an average of 5.00 leaves, 7.92 roots, and a root length of 1.31 cm.
2. The most effective concentration of activated charcoal is determined to be 2 g/l in MS media, which corresponds to an average leaf length of 1.28 cm, a root count of 7.44. and a root length of 1.38 cm.
3. The combination of plantain extract and activated charcoal does not exhibit a significant impact on any of the measured parameters.

REFERENCES

Delviandra, D., Nopsagiarti, T., & Haitami, D. A. (2021). Uji Berbagai Ekstrak Pisang Sebagai Suplemen Terhadap Pertumbuhan Eksplan Tanaman Pisang Roti Pada Media Ms. *Jurnal Green Swarnadwipa,* 10 (1):109-116.

Fajri, K., Nopsagiarti, T., & Okalia, D. (2020). Respon Pertumbuhan Eksplan Jeruk Nipis *(Citrus Aurantifolia L)* Dengan Pemberian Berbagai Konsentrasi Ekstrak Pisang Raja Dan Arang Aktif Pada Media Ms. *Jurnal Green Swarnadwipa,*9(2): -231.

Ismaini, L. 2021. Pengaruh Komposisi Media Pada Perbanyakan Medinilla Beamanii Secara *In Vitro*. *Prosiding Semnas Biologi Ke-9 Tahun 2021. Fmipa Universitas Negeri Semarang:* 10–16. Https://Proceeding.Unnes.Ac.Id/Index.Php/Semnasbiologi/Article/View/751.

Istiqomah, A.M., Setiari, N., Nurchayati, Y. 2020. Pengaruh Media Ms Dan Vw Terhadap Pertumbuhan *Planlet* Anggrek Bulan (*Phalaenopsis Amabilis L* Blume) Setelah *Transplanting.* Prosiding Seminar Nasionalpendidikan Biologi Dan Saintek Ke-5. Universitas Muhammadiyah Surakarta: 476-480.Https://Publikasiilmiah.Ums.Ac.Id/Handle/11617/12298.

Karjadi, A. K. (2016). *Kultur Jaringan Dan Mikropropagasi Tanaman Kentang (Solanum tuberosum L)* (Vol. 2016. Issue 008).

Lestari, N, D. Ni, W, D. (2015). Perbanyakan Anggrek Hitam (*Coeloegyne pandurata*) dengan Media Organik dan *Vacin Went* Secara *in Vitro*. *Jurnal Virgin. 1*(1), 30-39. https://www.jurnal.undhirabali.ac.id/index.php/virgin/article/view/49.

Meilani, S. N. (2017). Efektifitas penambahan media organik ekstrak ubi jalar (*Ipomoea batatas* L) Pada pertumbuhan subkultur Anggrek Cattleya sp. *Florea*, *4*(1), 5–11.

Nasution, L.Z., Manurung, E.D., Muainah Hasibuan1. dan Hardayani, M.A. 2021. Pengaruh Arang Aktif (Charcoal) pada Media MS untuk Meningkatkan Pertumbuhan Anggrek pada kultur In vitro. Prosiding Seminar Nasional dalam Rangka Dies Natalis ke-45 UNS Tahun 2021. Membangun Sinergi antar Perguruan Tinggi dan Industri Pertanian dalam Rangka Implementasi Merdeka Belajar Kampus Merdeka, Vol 5(1): 1372-1738.

Nisyawati, & Kariyana, K. (2013). Effect Of Ascorbic Acid, Activated Charcoal And Light Duration On Shoot Regeneration Of Banana Cultivar Barangan (*Musa Acuminata* L .) *In vitro* Culture. *In vitro*, *15:* 13–17. https://www.cabdirect.org/cabdirect/abstract/20133290250

Rahayu, S., Utami, E. S. W., & Indraloka, A. B. (2021). Pengaruh Ekstrak Yeast dan Pisang Raja Terhadap Pertumbuhan Tunas Embrio Vanda hookeriana Rchb.f. *Al-Kauniyah: Jurnal Biologi*, *14*(1), 138–151. https://doi.org/10.15408/kauniyah.v14i1.16713.

Srilestari, R., Dan Suwardi. 2021. Induksi Akar Pisang Abaka Secara *In Vitro* Dengan Menggunakan Macam Media Dan Thiamin. *Agrivet*, Vol 26: 1 – 7

Telaumbanua, S. M. (2022). Pengaruh Konsentrasi Air Kelapa Dan Dosis Arang Aktif Terhadap Pertumbuhan Planlet Anggrek *Dendrobium Sp* Dengan Media Vw Secara In Vitro. *Jurnal Sapta Agrica*, 1(1): 26-33.

Tuhuteru. S., Hehanussa. L, Raharjo, S.H.T. 2012. Pertumbuhan Dan Perkembangan Anggrek *Dendrobium Anosmum* pada Media Kultur In Vitro Dengan Beberapa Konsentrasi Air Kelapa. *Jurnal Agrologia*. 1(1), 1-12.

Warisman, A.N.P., Rahayu2 , P., Mulyaningrum. E.R.Pengaruh Penambahan Variasi Konsentrasi Karbon Aktif Pada Media Kultur *In Vitro* Untuk Pertumbuhan Anggrek *Dendrobium Welirang. BIOEDUSAINS: Jurnal Pendidikan Biologi dan Sains,* 7 (1): 309-321. DOI:10.31539/bioedusains.v7i1.9870