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In vitro Growth of *Cattleya sp* Orchid from Leaf Explants with Growth Regulators

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Abstract

Using leaf explants, this research aimed at identifying the type of growth response that can be created and the optimal mix of media for the growth of Cattleya sp orchid. This was an experimental investigation employing the RAL method (completely randomized design) with a combination of 2.4-D (0;1; 2; 3 ppm) and Kinetin (0;0.3;0.6 ppm) repeated three times. According to the results of this study, the growth regulators 2.4-D and kinetin were unable to promote the development of all explants. Explants transferred to media containing polyvinylpyrrolidone (PVP) to prevent browning after 12 weeks of observation. Then the results of the study also found the emergence of shoots in the treatment P0 (control), P2 (2,4-D 0 + Kinetin 0.6 ppm), P8 (2,4-D 2 + Kinetin 0.6) and P11 (2.4- D 3 + K 0.6 ppm) in the 3rd week of observation, the appearance of callus at P5 (2.4-D 1 + Kinetin 0.6 ppm) in the 4th week of observation

Keywords: *Cattleya sp., Growth Regulators, Leaf Explants*

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INTRODUCTION

Orchid *Cattleya* sp is an orchid of exceptional beauty. The *Cattleya* sp orchid plant features huge, gorgeous flowers with vibrant colors and a pleasant fragrance (Nika et al., 2018); hence, this flower is known as The Queen of Orchids. The *Cattleya* orchid blossom is highly sought after by enthusiasts and collectors because to its popularity (Buyung, 2021). Orchids are a type of decorative plant whose natural population has diminished and is endangered with extinction. Since orchid seeds lack an endosperm as a food store, they require nutrients that promote seed development in order to germinate.

Because orchid seeds lack endosperm (food reserves), they cannot be propagated by normal seed culture; as a result, they can only germinate when grown on artificial media aseptically using in

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vitro seed culture. During two to three months, planted orchid seeds will germinate and create miniature plantlets. The germination of orchid seeds is characterized by the creation of the protocorm, followed by the emergence of the plumule and radicle. Many research findings suggested that genotype, explants, medium, incubation circumstances, inoculum density, and subculture time influenced orchid somatic embryogenesis. Propagation by tissue culture is a possible solution to this issue. One of the fundamental media utilized in tissue culture is MS, which is coupled with numerous Growth Regulatory Substances. Synthetic auxins such as NAA and 2,4-D are more effective since they are not degraded by IAA oxidase or other enzymes, allowing them to persist longer and be more stable, although BAP and kinetine are often employed in tissue culture research due to their low cost and resistance to degradation. Hormones 2.4-D and 1 ppm BAP may cause callus in explants of sipahutar pineapple shoots (Harahap et al., 2019). The optimal therapy for the development of dragon fruit explants is thus the injection of NAA hormone 0.4 ppm with Kinetine 4 ppm (Mahadi et al., 2013).

This study utilized orchid leaves as explants for the *Cattleya* orchid propagation by administering a growth regulators combination of 2.4-D + Kinetine . This was done since waiting for the orchids to develop seeds is a lengthy process.

RESEARCH METHODS

Tools and Materials

The tools used in this experiment were an autoclave, culture bottles, beaker glass, spatula, tweezers, petri dish, bunsen lamp, laminar air flow cabinet (LAFC), handsprayer, volume pipette, refrigerator, heater (stove), stir bar, heating pot, and culture rack. The materials used in this experiment were *Cattleya* sp. orchid leaf explants derived from *Cattleya* sp. orchid seeds. grown in vitro, 70% alcohol, 96% alcohol, and sterile distilled water. The combination media used were kinetoplastin (0, 0.3, and 0.6 ppm) and 2.4 dichlorophenoxyacetic acid (0, 1, 2, and 3 ppm).

Research Design

This was an experimental research employing the CRD (completely randomized design) approach, with 12 (twelve) treatments repeated three times.

Procedure

This research's implementation began with the sterilization of the instruments and continued with the production of 2.4-D combination medium containing BAP. *Cattleya* sp orchid seeds produce *Cattleya* sp orchid leaf explants. The explants are cultivated in vitro so that they may be planted directly. The explants were kept in an incubation environment between 23 and 25 degrees Celsius and maintained by daily alcohol spraying. 12 weeks of observations were performed. The proportion of living explants, the percentage of explants that swelled, the percentage of explants that produced callus, the percentage of explants that developed buds, and the period of shoot and callus formation were observed in this study. The obtained data are presented descriptively.

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Table 1. Combination of the Hormones 2.4-D and Kinetine

Treatment Code	Information
P0	2.4-D 0 ppm + Kinetin 0 ppm
P1	2.4-D 0 ppm + Kinetin 0.3 ppm
P2	2.4-D 0 ppm + Kinetin 0.6 ppm
P3	2.4-D 1 ppm + Kinetin 0 ppm
P4	2.4-D 1 ppm + Kinetin 0.3 ppm
P5	2.4-D 1 ppm + Kinetin 0.6 ppm
P6	2.4-D 2 ppm + Kinetin 0 ppm
P7	2.4-D 2 ppm + Kinetin 0.3 ppm
P8	2.4-D 2 ppm + Kinetin 0.6 ppm
P9	2.4-D 3 ppm + Kinetin 0 ppm
P10	2.4-D 3 ppm + Kinetin 0.3 ppm
P11	2.4-D 3 ppm + Kinetin 0.6 ppm

Description : 2.4-D : 2.4 Dichloropenoxyacetic acid Hormone
Kinetin : Kinetin Hormone

RESULTS AND DISCUSSION

Percentage of Live Explants and Brown Explants

In this research, it was found that not all explants grew well. In Table 2, it can be seen that the explants that grew well were in the P0 (70%) and P2 (70%) treatments, while the rest experienced browning. The occurrence of browning in plants is due to injury to the explants releasing exudate secretions or phenolic compounds so that the plants experience browning. These phenolic compounds appear and accumulate usually due to the activation of the enzyme phlorophenol oxidase (Admojo & Indrianto, 2016). According to Hutami (2008), compounds in the form of proteins, amides, and polyamides can be added to the media so that they can react with phenol and restore the activity of the enzymes. In general, the polyamide that can be added is polyvinylpyrrolidone (PVP).

Table 2. The average percentage of live explants on *Cattleya* sp. Orchid Leaf Explants. with 2.4-D Combination Treatment and Kinetine Observation for 12th Weeks

Code	Treatment		Living Explants [%]	Brown Explants [%]	Explant Condition
	2.4-D [ppm]	Kinetin [ppm]			
P0	0	0	70	30	Growing shoots
P1	0	0,3	0	100	Brown Explants
P2	0	0,6	70	30	Growing shoots
P3	1	0	0	100	Brown Explants
P4	1	0,3	0	100	Brown Explants
P5	1	0,6	0	100	Brown Explants
P6	2	0	0	100	Brown Explants
P7	2	0,3	0	100	Brown Explants

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P8	2	0,6	0	100	Brown Explants
P9	3	0	0	100	Brown Explants
P10	3	0,3	0	100	Brown Explants
P11	3	0,6	0	100	Brown Explants

Description: The number 0 indicates the Explant is not growing

Explant Growth Response (Swelling of Explant, Growing Buds and Growing of Callus)

The results showed that using a combination of 24-D media with Kinetine can grow shoots. Melisa (2018) reported that the hormones 2.4-D and kinetine had no significant effect on the growth of PLB length and the orchid shoots formed, the Grammatophyllum scriptum orchid shoots were formed at a concentration of 4 mg/L 2.4-D + 2ml/L Kinetine . The results of observations on the 3rd MST of several treatments of shoot growth such as P0 (2.4-D 0 ppm + K 0 ppm), P2 (2.4-D 0 ppm + K 0,6 ppm), P8 (2.4-D 2 ppm + K 0,6 ppm) dan P11 (2.4-D 3 ppm + K 0,6 ppm).

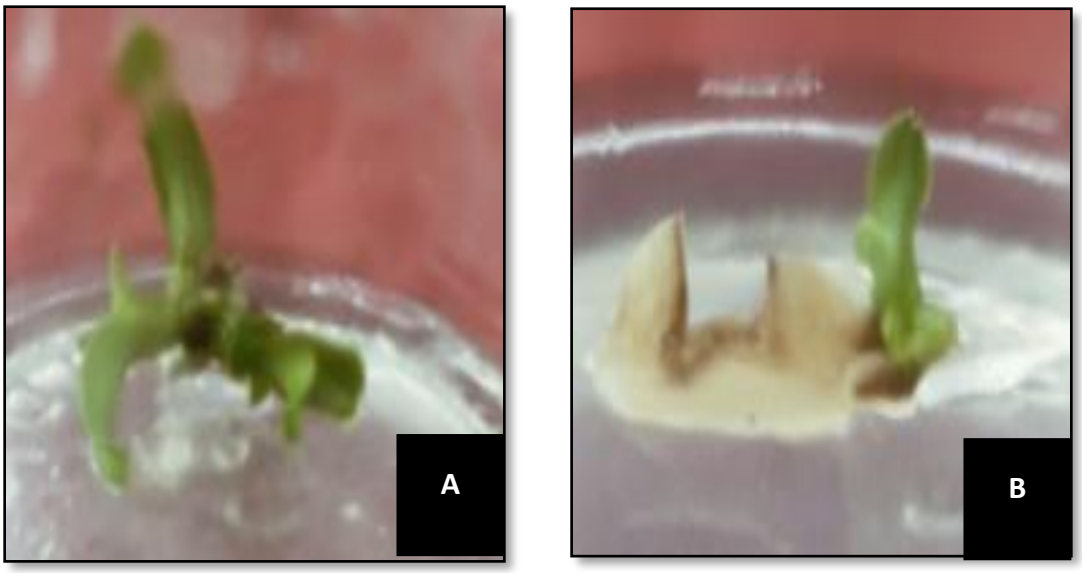

Figure 1. Growth Response of *Cattleya* sp. Orchid leaf Explants on a) P0 (2.4-D 0 ppm + Kinetin 0 ppm) and P2 (2.4-D 0 ppm + Kinetin 0.6 ppm)

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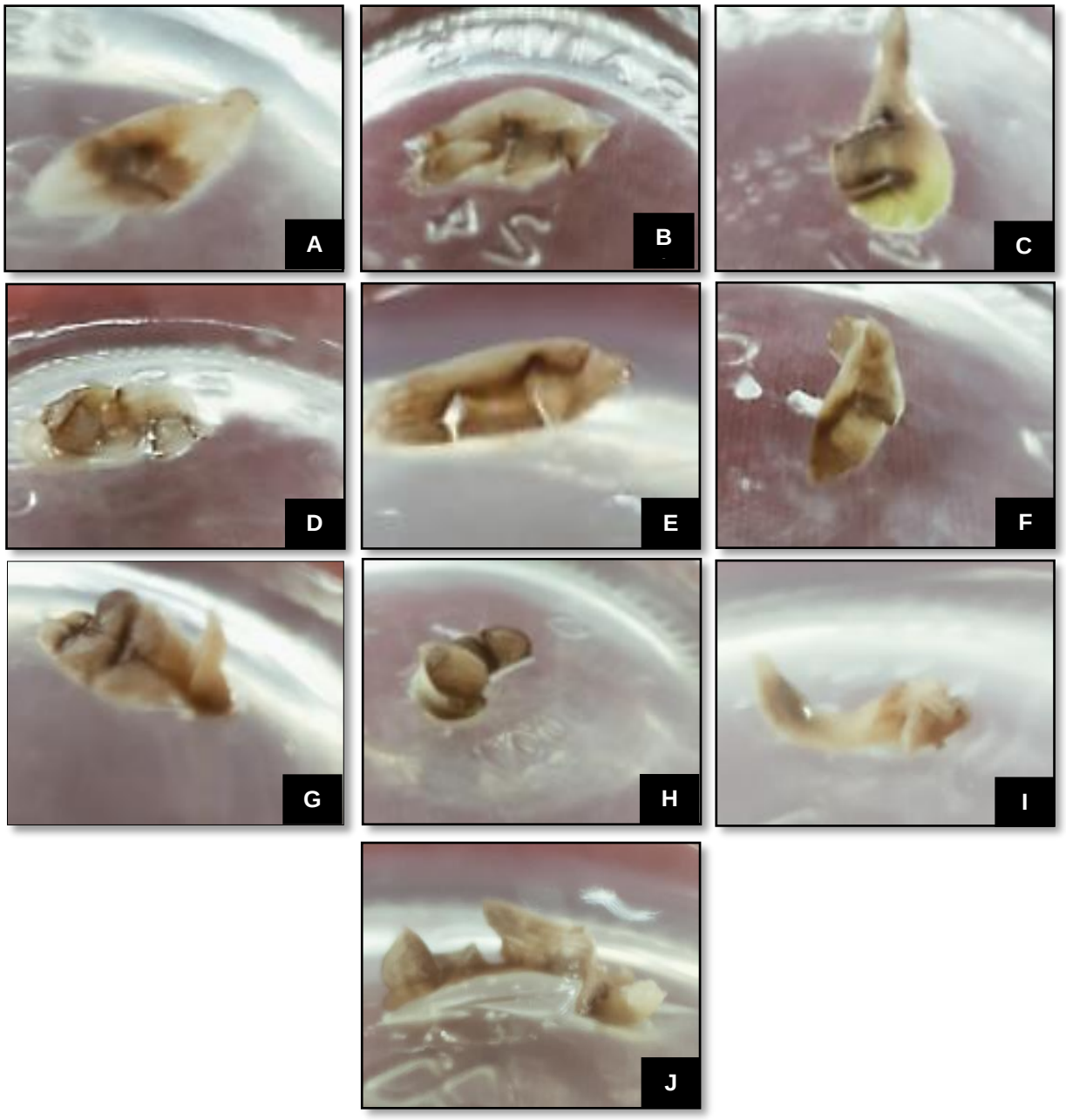

Figure 2. *Browning* Explants on *Cattleya* Orchid Leaf Explants on the 12th MST. a) P1 (2.4-D 0 ppm + Kinetin 0.3 ppm); b) P3 (2.4-D 1 ppm + Kinetin 0 ppm); c) P4 (2.4-D 1 ppm + Kinetin 0.3 ppm); d) P5 (2.4-D 1 ppm + Kinetin 0.6 ppm); e) P6 (2.4-D 2 ppm + Kinetin 0 ppm); f) P7 (2.4-D 2 ppm + Kinetin 0.3 ppm); g) P8 (2.4-D 2 ppm + Kinetin 0.6 ppm); h) P9 (2.4-D 3 ppm + Kinetin 0 ppm); i) P10 (2.4-D 3 ppm + Kinetin 0.3 ppm); and j) P11 (2.4-D 3 ppm + Kinetin 0.6 ppm).

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Table 3. Growth Response of Explants at 3rd MST and 7th MST on *Cattleya* sp Orchid Leaf Explants with 2.4-D and Kinetine Combination Treatment

Code	Treatment		3 Weeks After Planting			7 Weeks After Planting		
	2.4-D [ppm]	Kinetin [ppm]	Swelled [%]	Shoots [%]	Callus [%]	Swelled [%]	Shoots [%]	Callus [%]
P0	0	0	0	35	0	0	35	0
P1	0	0,3	0	0	0	0	0	0
P2	0	0,6	0	70	0	0	70	0
P3	1	0	0	0	0	0	0	0
P4	1	0,3	0	0	0	0	0	0
P5	1	0,6	35	0	0	0	0	35
P6	2	0	0	0	0	0	0	0
P7	2	0,3	0	0	0	0	0	0
P8	2	0,6	0	35	0	0	0	0
P9	3	0	35	35	0	35	0	0
P10	3	0,3	0	0	0	0	0	0
P11	3	0,6	0	35	0	0	35	0

Time Appears Buds and callus

In table 4 it can be seen that not all explants can grow callus or buds. Some have grown shoots or callus but can't last long, as can be seen in table 2 showing the percentage of explant life that survived to the 12th weeks after planting, namely at P0 (2.4-D 0 ppm + K 0 ppm) around 70% and P2 (2.4 -D 0 ppm + K 0.6 ppm) around 70%, while the rest experienced browning.

Table 4. Callus and budding time on explants of *Cattleya* sp. with 2.4-D Combination Treatment and Observation BAP for 12th Weeks after Planting.

Code	Treatment		Repetition			
	2.4-D (ppm)	Kinetin (ppm)	U1	U2	U3	Explant Condition
P0	0	0	0	0	3	It appears buds
P1	0	0,3	0	0	0	-
P2	0	0,6	3	3	0	It appears buds
P3	1	0	0	0	0	-
P4	1	0,3	0	0	0	-
P5	1	0,6	4	0	0	Explant swelling
P6	2	0	0	0	0	-
P7	2	0,3	0	0	0	-
P8	2	0,6	3	0	0	It appears buds
P9	3	0	0	0	0	-
P10	3	0,3	0	0	0	-
P11	3	0,6	0	3	0	It appears buds

Figure Description,
1,2,3,... : There was a change in the observations in the weeks after growth.
U1,U2,U3 : Repeats

In Table 4 above, it can be seen that the explants have different responses, which are due to differences in the endogenous hormones contained in these explants (Sriskandarajah et al., 2006), despite the addition of auxin and cytokinins at the same concentration. In table 3, it can be seen that from all treatments, the emergence of shoots dominated, as in treatment P0 (control), P2 (2.4-D 0 ppm + Kinetine 0.6 ppm), P8 (2.4-D 2 ppm + Kinetine 0.6 ppm), and treatment P11 (2.4-D 3 ppm + Kinetine 0.6 ppm), which grew at the 3rd week after planting. Whereas those who experienced callus growth were only in treatment P5 (2.4-D 1 + Kinetine 0.6 ppm), which appeared in the 4th week after planting. After being observed until the 12th week, many explants experienced browning. Ideally, after the explants show signs of browning, they are transferred to new media with the addition of polyamide, namely polyvinylpyrrolidone (PVP). However, due to the time limitations of the researchers, who only observed for 12 weeks, the action to move the explants and add polyvinylpyrrolidone (PVP) could not be carried out.

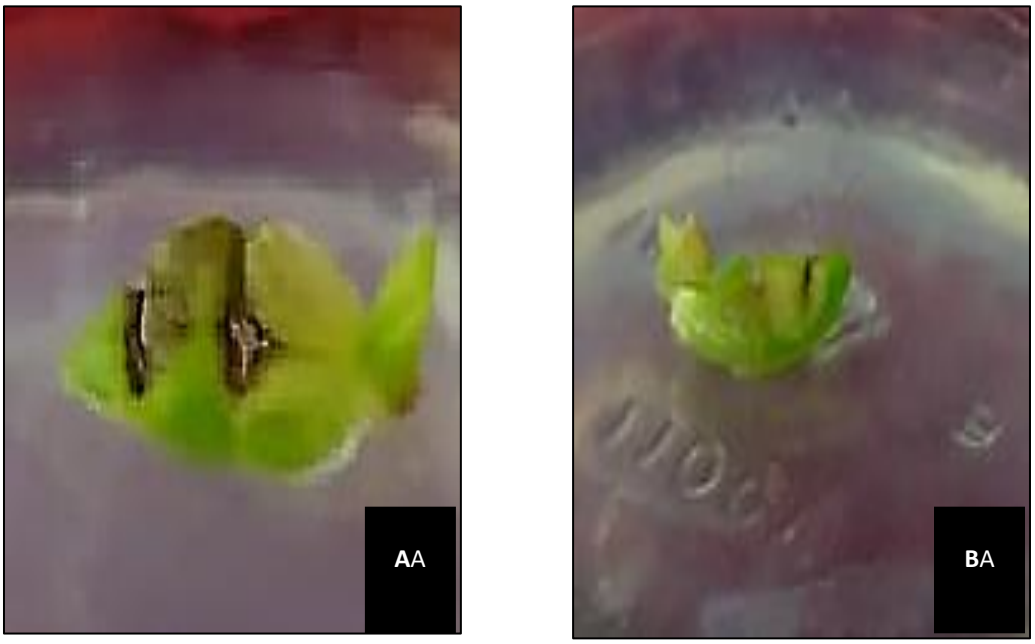

Figure 3. Growth of shoots in the 3rd week after planting at a) P8 (2.4-D 2 ppm + Kinetine 0.6 ppm) and b) P11 (2.4-D 3 ppm + Kinetine 0.6 ppm)

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Figure 4. Explant swelling at weeks 4th after planting at P5 (2.4-D 1 ppm + Kinetin 0.6 ppm)

In this study, it was found that even though 2.4-D was added at a dose of 0; 1; 2; 3 ppm combined with kinetine at a dose of 0; 0.3; 0.6, it did not cause callus. This is contrary to what Hariyadi et al., (2023) obtained by using a combination of growth regulatory substances 2.4-D at doses (0, 1, 2, and 3 ppm) combined with BAP at doses (0, 0.3, and 0.6) to produce research results as follows: Combination treatment of 2.4-D with BAP was able to produce a response in the form of discoloration, explant swelling, and callus formation in several treatments, namely P3 (2.4-D 1 ppm + BAP 0 ppm), P5 (2.4-D 1 ppm + BAP 0.6 ppm), P7 (2.4-D 2 ppm + BAP 0.3 ppm), and P8 (2.4-D 2 ppm + BAP 0.6 ppm). The best concentration to stimulate callus growth of *Cattleya* orchid leaf explants was treatment P5 (2.4-D 1 ppm + BAP 0.6 ppm) with a percentage of 35% callus formation characterized by green callus color, compact callus texture, and moderate callus growth (+ +).

CONCLUSION

Giving growth regulators 2.4-D and kinetine did not allow all explants to grow. After 12 weeks of observation, the explants were alive and growing well in treatment P0 (control) at around 70% and P2 (2.4-D 0 ppm + kinetine 0.6 ppm) at around 70%, while the rest experienced browning. This happened due to the limited time for the study, which was only around 12 weeks. The explants should have been transferred to media that had been given polyvinylpyrrolidone (PVP) to prevent browning. Then the results of the study also found the emergence of shoots in the treatments P0 (control), P2 (2,4-D 0 + Kinetine 0.6 ppm), P8 (2,4-D 2 + Kinetine 0.6 ppm), and P11 (2.4-D 3 + K 0.6 ppm) in the 3rd week of observation, and the appearance of callus at P5 (2.4-D 1 + Kinetine 0.6 ppm) in the 4th week of observation.

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