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Research Article

The Effect of 2-iP (*2-isopentenyl adenine*) Concentration on the Growth of Ki Aksara Orchids (*Macodes petola* (Blume) Lindl.) *In Vitro*

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ABSTRACT

This study aims to determine the optimal concentration of 2-iP (*2-isopentenyl adenine*) for the in vitro shoot growth of Ki Aksara orchids (*Macodes petola* (Blume) Lindl.). The experiment was conducted at the Esha Flora Tissue Culture Laboratory, Kedung Waringin Sub-district, Tanah Sereal District, Bogor City, from December 2022 to March 2023. The method used a Completely Randomized Design (CRD) comprising six treatment levels, leading to a total of 30 experimental units. The treatment levels included P0 (Control), P1 (0.05 mg L^{-1}), P2 (0.10 mg L^{-1}), P3 (0.15 mg L^{-1}), P4 (0.20 mg L^{-1}), and P5 (0.25 mg L^{-1}). The observed parameters included the number of shoots, stem diameter, number of leaves, number of roots, plant height, and leaf colour. Subsequently, the collected data were analysed using Analysis of Variance (ANOVA), followed by a Least Significant Difference (LSD) test at a 95 % confidence level. The results showed that 2-iP concentration of 0.10 mg L^{-1} was the most effective in promoting the growth of apical shoots, stem diameter, root number, and plant height of Ki Aksara orchids. The two types of shoots observed were apical and lateral. However, the expected growth corresponded to apical shoots, which were predominantly found at 2-iP concentration of 0.10 mg L^{-1}.

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INTRODUCTION

Ornamental plants, such as orchids, are highly valued for their aesthetic appeal, particularly flowers and leaves. Orchids are part of the *Orchidaceae* family with around 5000 species globally, possessing significant economic value due to the striking colours and patterns (Herliana et al. 2019). However, the natural populations are threatened by habitat loss from urban and plantation development, as well as environmental degradation (Dewi et al. 2018). To protect endangered species, the Indonesian government, through Regulation No. 7 of 1999, has designated black, hartinah, sugarcane, star moon, and Ki Aksara orchids as protected. Ki Aksara orchids (*Macodes petola* (Blume) Lindl.) captivate attention due to the distinctive leaf patterns, resembling script or letters (Gunawan 2016). These species have been classified as Endangered (EN), facing a high risk of extinction unless protective measures are implemented within a certain timeframe (Rugayah et al. 2017).

One of the primary challenges in the cultivation of orchids is the limited availability of seedlings. An alternative method to produce a large number of seedlings in a short period is vegetative propagation through in vitro plant tissue culture. The in vitro method includes cultivating plant organs in sterile conditions in containers filled with growth media (Yuniardi 2020). The main objective of this method is to generate a high quantity of uniform seedlings in a shorter time compared to a conventional propagation strategy (Santoso et al. 2020). One of the key factors determining the success of in vitro propagation is the type and concentration of plant growth regulators, which must be selected according to the purpose and stage of cultivation.

Growth regulators are organic compounds not classified as nutrients but are capable of stimulating plant growth. These compounds are plant hormones deliberately added to accelerate the plant production process (Widiastoety 2014). The application of growth regulators can cause varying responses based on the type and concentration (Nurana et al. 2017). Among several plant hormones influencing cell division, cytokinin plays a central role. Cytokinin functions to stimulate cell division in meristematic tissues, promoting both cell division and enlargement, thereby facilitating growth (Rosniawaty et al. 2018). Administering high concentrations of cytokinin increases the frequency of shoot multiplication. This suggests that higher cytokinin concentration correlates with a greater number of shoots produced (Munggarani et al. 2018). A particular type of cytokinin used for promoting shoot elongation is 2-iP (*2-isopentenyl adenine*). Generally, 2-iP is often selected due to the relatively stable and extensive effect in stimulating shoot multiplication without inducing morphological abnormalities such as vitrification or excessive callus formation.

The application of 2-iP in the vitro method functions to stimulate plant growth and shows high activity in promoting cell division (Nurana et al. 2017). 2-iP-type cytokinin has been used in tissue culture to stimulate callus differentiation, induce axillary bud formation, and enhance cell division (Tongkok et al. 2018). According to Nurana et al. (2017), the application of 2-iP in combination with NAA to hybrid Dendrobium orchids at a concentration of 0.2 mL L^{-1} produced optimal growth of the plantlets, particularly in shoot development and production of leaves. Therefore, this study aims to determine the most effective concentration of 2-iP for promoting shoot elongation in Ki Aksara orchids (*Macodes petola*) under in vitro conditions.

MATERIALS AND METHODS

Materials

The materials used were one-year-old Ki Aksara orchids derived from multiplication, and practical Murashige and Skoog (MS) medium. Other materials included sugar, agar, 2-iP hormone, myo-inositol, glycine, peptone, plant pre-

servative mixture, sodium hypochlorite, distilled water, betadine, sterile water, sterile tissue, methylated spirits, pH meter, matches, aluminium foil, rubber bands, plastic culture containers, plastic wrap, label paper, latex gloves, and alcohol at concentrations of 70 % and 96 %.

Methods

Explant Preparation

The medium was prepared in a 1000 mL measuring cup containing 500 mL of distilled water. This solution was further supplemented with 4.43 g L^{-1} MS medium, 0.1 mg L^{-1} myo-inositol, 2 mL L^{-1} glycine, 0.20 g L^{-1} peptone, 30 g L^{-1} granulated sugar, and 0.2 mL L^{-1} PPM. The growth regulator 2-iP was added according to the treatment levels, comprising 0 mg L^{-1} (P0), 0.05 mg L^{-1} (P1), 0.10 mg L^{-1} (P2), 0.15 mg L^{-1} (P3), 0.20 mg L^{-1} (P4), and 0.25 mg L^{-1} (P5). After mixing all components, the pH of the solution was measured and adjusted to 5.8. When the desired pH was achieved, 6 g L^{-1} of agar was added. The prepared solution was transferred into a pan and brought to a boil. Subsequently, the medium was dispensed into culture bottles, each containing 15–20 mL, based on the treatment. The culture bottles were sterilized using an autoclave at 121 °C and 17.5 psi for 30 minutes. After sterilization, the medium was stored on a rack in the culture room for 1 week to monitor for possible contamination.

Explant Inoculation

The culture bottle containing 1-year-old Ki Aksara orchids was sprayed with 70 % alcohol before being brought into a Laminar Air Flow (LAF) cabinet. A sufficient amount of sterile water was poured into an empty culture bottle, followed by the addition of 6–8 drops of betadine. A similar procedure was applied to the petri dish by pouring in sterile water and adding 3–5 drops of betadine. This was followed by homogenising the solution to ensure coverage of the entire surface of the petri dish was coated. Subsequently, Ki Aksara orchids were removed from the culture bottle, and explant was cut using sterilised forceps with a scalpel. The explant used was taken from the top of the shoot. Each culture bottle was planted with 1 explant. After planting, the bottle was closed again using plastic and sealed with a rubber band to ensure it was airtight. The planted culture bottle was removed from LAF and covered using aluminium foil and plastic, sealed with a rubber band. Furthermore, the mouth of the bottle was sealed with plastic wrap to ensure a tight closure. Each bottle was labelled with the treatment code, planting date, and medium name.

Incubation and Maintenance

To ensure optimal growth of Ki Aksara orchids, temperature, humidity, and lighting conditions were regulated in the incubation room. The temperature was maintained at 24–26 °C, relative humidity was kept between 60–70 %, and lighting was set at 1000–3000 lux using TL lamps or white LEDs, with a photoperiod of 16 hours of light and 8 hours of darkness. Observations were conducted once a week to monitor explant growth and confirm the absence of contamination in the culture medium.

Study Parameters

The parameters of plantlet growth were recorded from 1 week after planting (WAP) to 12 WAP. In this study, the parameters assessed were the number of shoots, leaves, and roots, including stem diameter, plant height, and leaf colour.

Data Analysis

Data were analysed using analysis of variance (ANOVA) based on Completely Randomized Design (CRD) with a single factor to evaluate the effect of each treatment level. When ANOVA showed a significant effect of treatment, further analysis was conducted using Least Significant Difference (LSD) test at a 95 % confidence level to determine the differences among treatments.

RESULTS AND DISCUSSION

Results

The results were described based on several parameters including the number of shoots, leaves, and roots, as well as stem diameter, plant height and leaf colour. Specifically, Figure 1 shows the values obtained in line with the analysis conducted on Ki Aksara orchids at 12 WAP.

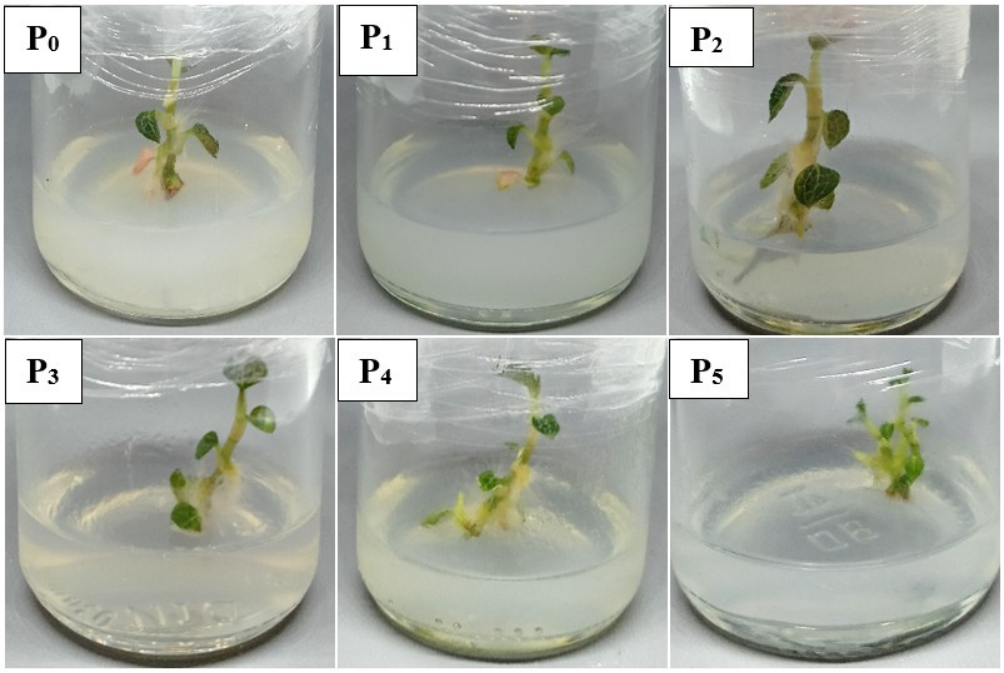

Figure 1. The growth of Ki Aksara orchids was observed at 12 weeks of age following the administration of 2-iP.

Number of Shoots

The observation results of the effect of 2-iP concentration on the number of shoots of Ki Aksara orchids at 12 WAP are shown in Figure 2.

Based on the results in Table 1, LSD concentration of 2-iP showed that the treatment had significant effect on the number of buds at 1 to 12 WAP. At 12 WAP, the highest average number of shoots (7.20) was found in the 0.25 mg L^{-1} treatment, consisting of lateral shoots.

Stem Diameter

LSD test results on the effect of 2-iP concentration on the stem diameter of Ki Aksara orchids are presented in Table 2. Based on the 12 WAP observation, the 2-iP treatment at 0.10 mg L^{-1} was significantly different from 0.05 mg L^{-1}, 0.15 mg L^{-1}, 0.20 mg L^{-1}, and 0.25 mg L^{-1}, but not significantly different from 0 mg L^{-1}. The highest average stem diameter of 0.29 cm was found in the 0.10 mg L^{-1}, while the lowest at 0.21 cm was observed in the 0.20 mg L^{-1} treatment.

Number of Leaves

LSD results on the concentration of 2-iP on the number of leaves are shown in Table 3. The 2-iP treatment showed significant differences in number of

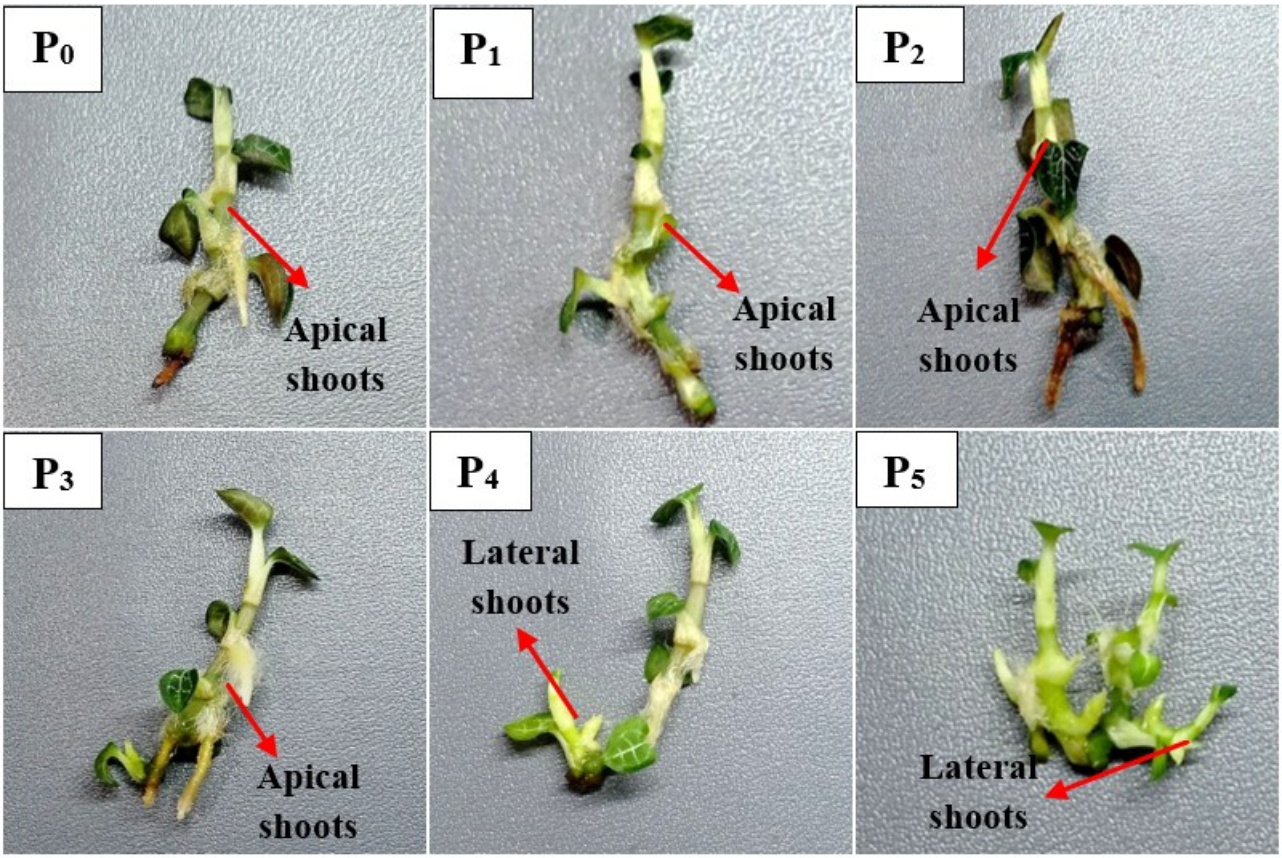

Figure 1. Number of Shoots of Ki Aksara Orchid Plants at 12 Weeks of Age Following the Application of 2-iP Concentration.

Table 1. The Effect of 2-iP Concentration on the Number of Shoots of Ki Aksara Orchid Plants.

	Number of Shoots				
Concentration	Week 1	Week 3	Week 6	Week 9	Week 12
0 mg L^{-1}	0.00 c	1.00 c	2.20 b	3.20 b	5.00 b
0.05 mg L^{-1}	0.80 ab	1.60 ab	2.80 b	3.80 b	4.80 b
0.10 mg L^{-1}	1.00 a	1.20 bc	2.60 b	3.80 b	5.20 b
0.15 mg L^{-1}	1.00 a	1.80 a	2.60 b	3.60 b	5.00 b
0.20 mg L^{-1}	1.00 a	1.80 a	2.40 b	3.60 b	5.20 b
0.25 mg L^{-1}	0.60 b	1.40 abc	3.60 a	5.00 a	7.20 a
LSD 5 %	0.37	0.58	0.67	0.69	0.96

Notes: Numbers followed by the same letter in the same column are not significantly different according to the 95 % LSD test.

leaves at 1 to 12 WAP. Observation at 12 WAP showed that 0.25 mg L^{-1} treatment was significantly different from 0.05 mg L^{-1}, 0.15 mg L^{-1}, and 0.20 mg L^{-1}, but there was no substantial variation between 0 mg L^{-1} and 0.10 mg L^{-1}. The highest average number of leaves (7.60) was found in the 0.25 mg L^{-1} treatment, while the lowest (5.80) was observed in the 0.20 mg L^{-1} treatment. The results showed that the use of 2-iP in the multiplication of Ki Aksara orchids could stimulate the formation of leaves. This was because the highest number of leaves that grew on lateral shoots.

Number of Roots

As shown in Table 4, the LSD results showed that 2-iP treatment significantly different affected the number of roots from 1 to 12 WAP. In comparison, a concentration of 0.10 mg L^{-1} was significantly different from 0.05 mg L^{-1} 12 WAP. However, there was no significant difference from the treatment of 0 mg L^{-1}, 0.15 mg L^{-1}, 0.20 mg L^{-1}, and 0.25 mg L^{-1}. The highest average for the

Table 2. The Effect of 2-iP Concentration on Stem Diameter of Ki Aksara Orchid Plants.

	Stem Diameter				
Concentration	Week 1	Week 3	Week 6	Week 9	Week 12
0 mg L^{-1}	0.10 a	0.15 ab	0.22 ab	0.24 a	0.28 ab
0.05 mg L^{-1}	0.10 a	0.14 ab	0.20 b	0.20 b	0.24 bc
0.10 mg L^{-1}	0.11 a	0.21 a	0.23 a	0.24 a	0.29 a
0.15 mg L^{-1}	0.10 a	0.13 b	0.21 ab	0.22 ab	0.22 c
0.20 mg L^{-1}	0.10 a	0.16 ab	0.21 ab	0.21 b	0.21 c
0.25 mg L^{-1}	0.10 a	0.16 ab	0.20 b	0.22 ab	0.22 c
LSD 5 %	0.00	0.07	0.00	0.00	0.04

Notes: Numbers followed by the same letter in the same column are not significantly different according to the 95 % LSD test.

Table 3. The Effect of 2-iP Concentration on the Number of Leaves of Ki Aksara Orchid Plants.

	Number of Roots				
Concentration	Week 1	Week 3	Week 6	Week 9	Week 12
0 mg L^{-1}	0.00 a	0.00 b	1.40 b	2.60 ab	4.00 ab
0.05 mg L^{-1}	0.00 a	0.20 ab	1.40 b	2.20 b	3.40 b
0.10 mg L^{-1}	0.20 a	0.60 a	2.20 a	3.00 a	4.40 a
0.15 mg L^{-1}	0.20 a	0.40 ab	1.80 ab	2.80 ab	3.80 ab
0.20 mg L^{-1}	0.00 a	0.40 ab	1.60 ab	2.60 ab	3.60 ab
0.25 mg L^{-1}	0.00 a	0.00 b	1.80 ab	2.60 ab	3.60 ab
LSD 5 %	0.33	0.55	0.65	0.71	0.98

Notes: Numbers followed by the same letter in the same column are not significantly different according to the 95 % LSD test.

number of roots at 4.40 was found in the 0.10 mg L^{-1}, while the lowest at 3.40 was observed in 0.05 mg L^{-1} treatment. This suggested that at high concentrations, 2-iP was more effective for cell division, shoots, and leaf development compared to roots growth.

Table 4. The Effect of 2-iP Concentration on the Number of Roots of Ki Aksara Orchid Plants.

	Number of Leaves				
Concentration	Week 1	Week 3	Week 6	Week 9	Week 12
0 mg L^{-1}	3.00 a	3.00 c	4.40 ab	5.60 abc	7.20 ab
0.05 mg L^{-1}	3.00 a	3.20 bc	4.40 ab	4.80 c	6.80 bc
0.10 mg L^{-1}	3.20 a	3.80 a	4.80 ab	6.00 ab	7.40 ab
0.15 mg L^{-1}	3.00 a	3.60 ab	4.80 ab	5.80 ab	6.40 cd
0.20 mg L^{-1}	3.00 a	3.20 bc	4.20 b	5.40 bc	5.80 d
0.25 mg L^{-1}	3.00 a	3.80 a	5.20 a	6.40 a	7.60 a
LSD 5 %	0.23	0.55	0.82	0.80	0.75

Plant Height

As presented in Table 5, LSD results showed that the treatment of 2-iP was significantly different on the height of Ki Aksara orchids at 1 to 12 WAP. At 12 WAP, the 2-iP treatments at 0 mg L^{-1}, 0.10 mg L^{-1}, and 0.15 mg L^{-1} were significantly different from 0.25 mg L^{-1}, but not significantly different from 0.05 mg L^{-1} and 0.20 mg L^{-1}. The highest average height of 3.50 cm was found in 0.10 mg L^{-1} treatment, while the lowest at 2.90 was observed in 0.25 mg L^{-1}.

Colour of Leaves

Leaf colour was observed at 12 WAP visually using the Munsell Colour Chart Ver 1.0.0 application by KSGc. Based on the observation results in Table 6, there were two categories: dark green and light green.

Table 5. The Effect of 2-iP Concentration on Ki Aksara Orchid Plant Height.

	Plant Height				
Concentration	Week 1	Week 3	Week 6	Week 9	Week 12
0 mg L^{-1}	1.45 a	1.67 ab	1.85 ab	2.24 a	3.45 a
0.05 mg L^{-1}	1.44 ab	1.52 b	1.75 b	2.03 a	3.33 ab
0.10 mg L^{-1}	1.69 a	1.85 a	2.13 a	2.32 a	3.50 a
0.15 mg L^{-1}	1.50 ab	1.67 ab	1.77 b	2.22 a	3.46 a
0.20 mg L^{-1}	1.53 ab	1.72 ab	2.04 ab	2.18 a	3.28 ab
0.25 mg L^{-1}	1.39 b	1.64 ab	1.86 ab	2.27 a	2.90 b
LSD 5 %	0.27	0.30	0.30	0.36	0.43

Notes: Numbers followed by the same letter in the same column are not significantly different according to the 95 % LSD test.

Table 6. The Effect of 2-iP Concentration on Colour of Leaves of Ki Aksara Orchid Plants.

Treatment	Munsell Colour	Figure
P_3 (2-iP 0.15 mg L^{-1})	Munsell Value: 7.5 GY 1/4	
P_0 (2-iP 0 mg L^{-1})	Munsell Value: 7.5 GY 2/4	
P_5 (2-iP 0.25 mg L^{-1})	Munsell Value: 7.5 GY 4/8	

Discussion

Cytokinin plays an essential role in plant development, including regulating the formation and proliferation of shoots as well as promoting cell division and elongation (Ashraf et al. 2014). A study conducted by Kadapatti and Murthy (2022) showed that 2-iP at an optimal concentration significantly enhanced the direct regeneration of adventitious shoots from explants without callus formation. This led to a high number of shoots, uniform growth, and good regeneration efficiency, suggesting that higher concentrations required more time for shoot formation. Murashige and Skoog (MS) medium contains high levels of micronutrients that support plant growth (Lawrie et al. 2021). MS medium provides complete micronutrients, supporting the physiological activity of 2-iP through synergy with auxin in regulating apical dominance and inducing organogenesis. Generally, the mechanism of cytokinin in cell division in shoots occurs through cooperation with auxin, stimulating cell division and influencing cell differentiation pathways. Cytokinin enters plant's shoot system through the roots by signalling axillary buds to initiate growth (Wiraatmaja 2017).

2-iP cytokinin shows a distinct molecular mechanism by binding to histidine kinase receptors located in the endoplasmic membrane, thereby activating a two-component phosphorylation system. The phosphate is transferred through AHP proteins to type-A response regulators (RRs), which stimulate the expression of genes regulating cell division and differentiation (Cárdenas-Aquino et al. 2023). This pathway is essential, allowing a more targeted hormonal response and minimizing side effects such as unwanted callus formation. Based on the analysis conducted, observations on stem diameter showed that lower concentrations led to optimal growth. The phenomenon was due to the role of 2-iP in increasing stem diameter, which is related to its function in stimulating cell division. According to Nurana (2017), 2-iP functioned to stimulate growth with high activity in promoting cell division in tissue culture. This showed that lower concentrations supported optimal growth due to the role of 2-iP, increasing the stem diameter through the ability to stimulate cell division.

Observations on number of leaves showed that the use of 2-iP in the propagation of *Macodes petola* orchids could stimulate the formation of leaves. This was because the highest number of leaves occurred from lateral shoots, indicating that 2-iP played two interrelated roles, namely cell division and chloroplast formation. The ongoing cell division process enlarges leaf surface area and causes chloroplasts to form and develop. According to Dewanto et al. (2019), formation of leaves in explants can be triggered by the availability of nitrogen (N) and potassium (K), acting as macronutrients in the culture medium. In in vitro propagation, photosynthesis is facilitated by artificial light. However, the activity is generally very low because the propagated plantlet is typically not yet autotrophic, showing the need for assistance by the presence of sugar (glucose) as an energy source. Similar results were also reported by Nurana et al. (2017), who applied 2 ppm 2-iP to *Dendrobium* Hybrid orchids, producing the highest number of leaves compared to other cytokinin growth regulators. The application of 2-iP as a plant growth regulator could influence the number and length of leaves.

The change in colour of leaves observed during the study was presumed to be associated with the physiological activity of the plant, which was influenced by the concentration of the applied hormone. Leaves showing a dark green colour indicated a high chlorophyll content. Similarly, Astuti et al. (2016) stated that the intensity of green coloration was directly proportional to chlorophyll accumulation. The high nitrogen content in MS medium also supported chlorophyll formation through the enhancement of endogenous cytokinin (Joni et al. 2014). The presence of 2-iP hormone can stimulate cell division and chloroplast formation, particularly in lateral leaves. The dark green colour indicates a high chlorophyll level in accordance with the hormonal mechanism and nitrogen presence in MS medium.

Auxin is generally produced in the shoots, stems, and roots (Nurana et al. 2017). In this study, explants used were shoot tips of *Macodes petola* orchids, showing the occurrence of root development in the area. The results showed that higher concentrations of auxin produced fewer roots. The auxin hormone present in the shoot was produced and transported from the apical meristem to the basal stem, roots, and young tissues. Root formation often occurs after the cultured explants form shoots, which stimulate development (Yatim 2016).

An increase in explant height indicates that growth and development are influenced by the availability of nutrients in the medium (Karyanti 2017). The nutrient content in MS medium is generally sufficient to influence the growth of explants. According to Isda and Fatonah (2014), the use of MS medium with the addition of the appropriate growth regulator can enhance explant growth. Growth regulators are non-nutrient organic compounds that,

when applied in low concentrations, can promote explant growth. However, when administered in high concentrations, there is a tendency to inhibit plant growth and development (Nurhanis et al. 2019).

The application of 2-iP at varying treatments can lead to changes in leaf colour. This is presumably due to the different physiological responses of the plant during development. Improper use of growth regulators can cause leaves to turn light green or even pale (Indriani et al. 2014). Chlorophyll levels are influenced by leaf pigments, where greener colour indicates higher chlorophyll content (Astuti et al. 2016). MS is an in vitro culture medium containing high nitrogen levels, which is capable of enhancing endogenous cytokinin (Joni et al. 2014). This endogenous cytokinin assists the explants in forming chlorophyll in leaves. The application of cytokinin at an optimum concentration can increase nitrogen content, which will be used as a building block for plant organs including roots, stems, and leaves.

CONCLUSIONS

In conclusion, the administration of 2-iP in in vitro culture medium significantly affects the growth of Ki Aksara orchids. The concentration of 0.10 mg L^{-1} is the most effective treatment in increasing the number of apical shoots, leaves, and roots, including stem diameter and plant height. The dark green colour of leaves with a Munsell value code of 7.5 GY 1/4 produced under this treatment, indicates a high chlorophyll content and optimal physiological condition.

AUTHOR CONTRIBUTION

A.W. collected and analysed data, and wrote the manuscript. E.S. and Y designed and supervised the entire process.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ashraf, M.F. et al., 2014. Effect of cytokinin types, concentrations and their interactions on in vitro shoot regeneration of chlorophytum borivilianum sant. & fernandez. *Electronic Journal of Biotechnology*, 17(6), pp.275–279. doi: 10.1016/j.ejbt.2014.08.004.

Astuti, A.F., Hardjoko, D. & Rahayu, M. 2016. Kombinasi serat batang aren dan pasir merapi pada hidroponik substrat kailan. *Agrosains: Jurnal Penelitian Agronomi*, 18(2), pp.50-56. doi: 10.20961/agsjpa.v18i2.18692.

Cárdenas-Aquino, M.D.R. et. al., 2023. The Cytokinins BAP and 2-iP modulate different molecular mechanisms on shoot proliferation and Root Development in Lemongrass (*Cymbopogon citratus*). *Plants*, 12(20), 3637. doi: 10.3390/plants12203637.

Dewanto, H.A., Saraswati, D. & Hadjoeningtijas, O.D., 2019. Pertumbuhan Kultur Tunas Aksilar Kentang (*Solanum tuberosum* L.) dengan Penambahan Super Fosfat dan KNO3 Pada Media AB Mix Secara In Vitro. *Agritech*, 20(2), pp.71–81. doi: 10.30595/agritech.v20i2.3991.

Dewi, E.R.S., Ary, S.N. & Maria, U., 2018. Menuju Desa Konservasi Anggrek di Desa Ngesrep Balong, Limbangan Kendal. *Journal of Dedicators Community*, 2(2), pp.100-107. doi: 10.34001/jdc.v2i2.702.

Gunawan, G., 2016. *Pengaruh Zat Pengatur Tumbuh Tunas (BAP, IBA, GA3, Myoinositol Dan Mineral) dan Umur Kultur Terhadap Pertumbuhan Tanaman Macodes Petola Secara In Vitro*. Universitas Islam Negeri Sunan Gunung Djati Bandung.

Herliana, O., Harjoso, T. & Rokhminarsi, E., 2019. Pemberdayaan Mantan Buruh Migran Melalui Introduksi Budidaya Anggrek *Dendrobium* sp dengan Berbagai Jenis Media Tanam dan Aplikasi Pupuk Hayati Mikoriza di Kabupaten Banyumas. *Jurnal Panrita Abdi*, 3(1), pp.9-17. doi: 10.20956/pa.v3i1.3696.

Indriani, B.S., Suwarsi, E. & Pukan, K.K., 2014. *Efektivitas Substitusi Sitokinin dengan Air Kelapa pada Medium Multiplikasi Tunas Krisan (Chrysanthemum indicum L.) Secara In Vitro*. Universitas Negeri Semarang.

Isda, M.N. & Fatonah, S., 2014. Induksi Akar Pada Eksplan Tunas Anggrek *Grammatophylum scriptum* var. Citrinum Secara In Vitro Pada Media MS dengan Penambahan NAA dan BAP. *Al-Kauniyah Jurnal Biologi*, 7(2), pp.53–57. doi: 10.15408/kauniyah.v7i2.2715.

Joni, Y., Efendi, D. & Roostika, I., 2014. Morfogenesis Eksplan Keping Biji dari Tiga Klon Manggis (*Garcinia mangostana* L.) pada Tiga Jenis Media Dasar. *Horticultura*, 24(2), pp.94–101. doi: 10.21082/jhort.v24n2.2014.p94-101.

Kadapatti, S.S. & Murthy, H.N., 2022. Micropropagation of Andrographis producta through axillary and adventitious shoot regeneration. *Journal of Genetic Engineering and Biotechnology*, 20(1), 152. doi: 10.1186/s43141-022-00438-w.

Karyanti, K., 2017. Pengaruh Beberapa Jenis Sitokinin Pada Multiplikasi Tunas Anggrek Vanda Douglas Secara In Vitro. *Jurnal Bioteknologi & Biosains Indonesia (JBBI)*, 4(1), pp.36-43. doi: 10.29122/jbbi.v4i1.2200.

Lawrie, M. D. et al., 2021. In Vitro Germination and Flowering of Dendrobium capra J.J. Smith, An Endemic Orchid of Java. *HAYATI Journal of Biosciences*, 28(2), pp.172–172. doi: 10.4308/hjb.28.2.172.

Munggarani, M. et al., 2018. Multiplikasi Tunas Meriklon Kentang Pada Berbagai Jenis dan Konsentrasi Sitokinin. *Agrologia*, 7(2), pp.80-89. doi: 10.30598/a.v7i2.766

Nurana, A.R., Wijana, G. & Dwiyani, R., 2017. Pengaruh 2-iP dan NAA terhadap Pertumbuhan Plantlet Anggrek *Dendrobium Hibrida* pada Tahap Subkultur. *Agrotrop*, 7(2), pp.139–146. doi: 10.24843/AJoAS.2017.v07.i02.p05.

Nurhanis, S.E., Wulandari, R.S. & Suryantini, R., 2019. Korelasi Konsentrasi IAA dan BAP Terhadap Pertumbuhan Kultur Jaringan Sengon (*Paraserianthes falcataria*). *Jurnal Hutan Lestari*, 7(2), pp.857-867. doi: 10.26418/jhl.v7i2.34552.

Rosniawaty, S., Anjarsari, I.R D. & Sudirja. R., 2018. Aplikasi Sitokinin untuk Meningkatkan Pertumbuhan Tanaman Teh di Dataran Rendah. *Jurnal of Industrial and Beverage Crops*, 5(1), pp.31-38. doi: 10.21082/jtidp.v5n1.2018.p31-38.

Rugayah, K.S.Y. et al., 2017. *Tumbuhan langka Indonesia: 50 Jenis Tumbuhan Terancam Punah*. BRIN.

Santoso, I.B. et al., 2020. Teknologi Kultur Invitro Anggrek untuk Meningkatkan Keragaman Tanaman Di Agrowisata Serang. *Prosiding Seminar Nasional LPPM Unsoed*, 9, pp.294-303.

Tongkok, P., Prasart, K. & Kaewsuralikhit, C., 2018. Effect of n6-(2-*isopentenyl adenin*) (2iP) on The Grwoth of Tropical Seagrass Enhalus Acroides After Germination. *Journal of Fisheries and Environment*, 42(1), pp.16–23.

Widiastoety, D., 2014. Pengaruh Auksin dan Sitokinin Terhadap Pertumbuhan Planlet Anggrek Mokara. *Jurnal Hortilultura*, 24(3), pp.230-238. doi:10.21082/JHORT.V24N3.2014.P230-238

Wiraatmaja, I.W., 2017. *Bahan Ajar Zat pengatur tumbuh Giberelin dan Sitokinin*. Universitas Udayana.

Yatim, H., 2016. Multiplikasi Pisang Raja Bulu (*Musa paradisiaca* L. AAB GROUP) pada Beberapa Konsentrasi Benzyl Aminopurine (BAP) Secara In Vitro. *Jurnal Agroekoteknologi*, 4(3), pp.1989-1995. doi: 10.32734/ja.v4i3.2075.

Yuniardi, F., 2020. Aplikasi Dimmer Switch pada Rak Kultur Sebagai Pengatur Kebutuhan Intesitas Cahaya Optimum Bagi TanamanIn Vitro. *Indonesian Journal of Laboratory*, 1(4), pp.8–13. doi: 10.22146/ijl.v1i4.52991