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The effect of BA on inducing shoots of *Philodendron erubescent* 'Pink Princes' *in vitro*

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Abstract The study investigated the influence of plant growth regulator on the induction of direct shoots from node explants of *Philodendron erubescens* 'Pink Princes' *in vitro* condition. The result showed that the most of width, length, and height was observed in MS + BA 0.5 mg/l, measuring 3.40 cm × 3.22 cm × 1.96 cm and 1.74 g fresh weight. The highest number of shoots was consistently observed 60.44 shoots per nodal MDA content (1.91 nmol/g FW) and phenolic content (9.51 mg GAE/g FW) are lowest. The explant was cultured on MS medium performed highest chlorophyll content (chl A 213.96 μg/g FW, chl B 162.54 μg/ FW, total chlorophyll 304.64 μg/FW) and carotenoid content (2.61 μg/g FW) Moreover, they were the largest plant with green leaves when compared to another treatment.

Keywords: Shoot induction, Plant growth regulators

Introduction

Philodendron is the largest genus in the Araceae (Croat, 1997) The philodendron represents an entirely Neotropical genus , consisting of known to encompass 487 published species, with an estimated total of approximately 1500 species (Boyce and Croat, 2013), At present, the classification of Philodendron species involves their categorization into three distinct subgenera based on both floral and vegetative morphology as well as anatomical characteristics, Indigenous to regions of tropical and subtropical America, as well as the West Indies (Mayo *et al*.,1997). Philodendrons are widely recognized and valued for their aesthetically pleasing foliage as well as their capacity to thrive in indoor settings, thus making them a popular choice for interior landscaping. Within the genus Philodendron, two distinct growth types can be distinguished: the vining type, which held prominence in the foliage plant market from the 1950s to the early 1970s, and the self-heading type, which has gained popularity over the past

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four decades. This shift in popularity can be attributed to the introduction of numerous new hybrids with varying foliage colors that have been introduced to the market (Chen *et al*., 2012), Philodendron species hold a preeminent position in the foliage plant market, particularly in regions such as Thailand. *Philodendron erubescences* 'Pink Princess' known as 'Pink Princess' is a cultivated hybrid philodendron characterized by its relatively slow growth rate, variegated foliage displaying a striking interplay of colors, and an upright, vining growth habit. In its mature state, this cultivar can attain a height exceeding 4 feet and span a width of approximately 2 feet. The leaves, which are heart-shaped, feature a deep purplish-green hue complemented by vivid pink variegation, reaching dimensions of up to 8 inches in length and 5 inches in width. The pink variegation exhibits variability, manifesting as prominent patches, subtle streaks, or, on occasion, encompassing an entire leaf. Although houseplants of 'Pink Princess' infrequently produce typical arum-type inflorescences, these inflorescences would typically comprise a purple-red spathe enshrouding a white spadix. In meeting the demands of growers for potted philodendron plants, the development of rapid propagation techniques for elite cultivars becomes imperative. Traditional propagation of philodendron through stem cuttings is inherently slow, primarily due to the limited number of cuttings that can be obtained from each individual plant (Reffstrup and Boll, 1985) Thus making conventional methods of cutting propagation are not suitable for self-heading philodendron. In recent years, the propagation of hybrid self-heading philodendron varieties, which do not yield seeds that remain to prototype (Henny, 1988), In contrast, tissue culture presents a more attractive approach for Philodendron propagation, Furthermore, the application of tissue culture to tropical foliage plants has been proposed as an effective means of mitigating various systemic viral, fungal, and bacterial diseases that often afflict plants (Hartman,1974) This innovative approach has not only facilitated the mass production of several valuable hybrid varieties but has also effectively created a new product category. Unlike the majority of philodendron plants grown from seeds or traditional cuttings, those derived from Tissue culture exhibit a prevalence of basal shoots, contributing to the development of compact plants, even when cultivated in smaller containers. (Henley *et al.,* 2005) However, there have been investigations into the propagation of philodendron using a variety of tissue culture techniques, wherein plant parts were cultured on medium supplemented with plant growth regulator such as cultivation *Philodendron erubescens* cv. Red Emerald. They used shoot tip explants cultivated on MS medium supplement with IBA 2 mg/l +NAA 0.5mg/l and IBA 1 mg/l + NAA 0.5 mg/l resulted in the highest number of shoots 68.63 and 67.30 (Fahmy *et al.,* 1998), *Philodendron bipinnatifidum* Schott ex Endl used shoot tip explants

cultured on MS medium supplement with BAP 1, IBA 0.5 and 1-2 NAA mg/l exhibited yielded more shoots than cytokinins alone, with the greatest number of axillary shoots 9.3 per explant obtained using both BAP 1mg/l and IBA 0.5mg/l (Alawaadh *et al*., 2020), In *Philodendron cannifolium*, they used shoot tips cultured on MS media supplemented with BA 0.5-10 mg/l or thidiazuron (TDZ) 0.05-0.1 mg/l exhibited shoot were growth on BA 1-3 mg/l (Han and Park, 2008), *Philodendron tuxtlanum* they used bud explants cultured on BM medium or MS medium with BA the result showed that MS medium supplemented with BA 20 mg/l was the best medium for shoot induction with the half-strength MS medium containing with BA 8 mg/l being most suitable for shoot development.(Jámborné Benczúr and Márta-Riffer, 1990), In the context of philodendron propagation (i.e. 'Imperial Green', 'Imperial Red' and 'Imperial Rainbow'), an investigation was conducted using three types of explants (leaf lamina, petiole, and stem nodal segment) cultured on MS medium supplement with 2,4-D , TDZ 0.5 mg/l, or a combination of both growth regulators. The results revealed that leaf lamina was relatively ineffective in inducing shoot formation, while petioles exhibited potential for generating adventitious shoots, with frequencies ranging from 2.8% to 11.1% across two of the tested cultivars. Notably, stem nodal segments displayed the highest responsiveness among the three explant types, with shoot formation occurring directly following TDZ treatment at frequencies ranging from 16.7% to 41.7%, depending on the cultivar. In terms of the efficacy of various cytokinins in promoting shoot proliferation within stem nodal segments, it was observed that treatments with 0.5 and 1 mg/l of Kn and BA resulted in higher shoot formation percentages compared to treatments with 0.5 and 1 mg/l TDZ in two out of the three cultivars. Additionally, BA were consistently produced more shoots across all three cultivars. (Chen *et al.*, 2012). Previous research indicated the significant involvement of hormones, particularly cytokinins, in the induction of new shoots for propagation across various Philodendron species. However, it is noteworthy that there is a limited body of research specifically addressing this aspect in the *Philodendron erubescent* 'Pink Princess' variety. The aims were to study the effect of plant growth regulator for inducing shoots of *Philodendron erubescent* in in vitro conditions.

Materials and methods

Explant source and culture media

Nodal explants (~5 mm) were cut from in vitro grown stock (*Philodendron erubescent* 'Pink Princes') preparation explants from plant in vitro condition by

cut leaf and roots all out after that cut nodal to size ~5 mm. Explant were cultured on MS medium (Murashiki and Skoog, 1962) combinations with plant growth hormone cytokinin BA at the concentration 0, 0.1, 0.5 and 1 mg/l. The MS medium supplemented with 3% sucrose pH of medium 5.5-5.7 before addition of 0.8% agar. Then was autoclaved at 121°C for 20 minutes.

Culture conditions

All the culture were arranged randomly on the shelf in growth room at 25 ± 2 °C under 16-hour photoperiod by white Light-Emitting Diode (LED) for 3 months.

Quantification of Chlorophyll a, Chlorophyll b and carotenoids content

Chlorophyll a (chl a), Chlorophyll b (chl b) and carotenoid content of explant were described by adaptive method from Lichhtenthaler (2001). Fresh explants were cleaned to remove medium and 0.1 g of fresh weigh was used to extracted chlorophyll by grinding in mortar with 80% acetone, 2 mL Volume, kept in darkness for 3 hr after that filter extract with filter paper No.93, 125m size. The extracts were dropped into elisa plate for measure of absorbance. The results were substituted for the chlorophyll a, b and carotenoid.

The value of chlorophyll a, chlorophyll b and carotenoids were calculated as follows:- chlorophyll a ($\mu g\ ml^{-1}$) = 12.25(A663) - 2.79(A647), chlorophyll b ($\mu g\ ml^{-1}$) = 21.50(A647) - 5.10(A663) and carotenoids ($\mu g\ ml^{-1}$) = [1000(A470) - 1.82(chl a) – 85.02(chl b)]198.

The calculated values for chlorophyll a, chlorophyll b and carotenoid were calculated as follows:-Chlorophyll content ($\mu g\ g^{-1}$ fresh weight) = (chlorophyll value × 2 ml) initial $weight^{-1}$.

Quantification of Malondialdehyde content

Malondialdehyde content (MDA) of explants were described by adaptive method from Heath and Packer (1968). thiobarbituric acid reactive substances (TBARs), which are considered secondary byproducts arising from lipid peroxidation. samples of Explant, each weighing 0.2 g, underwent homogenization with 3 mL of 0.1% (w/v) trichloroacetic acid (TCA). Following centrifugation at 4°C and centrifuged 10,000 rpm for a duration of 20 minutes, 1 ml of the upper-layer solution was combined with 4 ml of 20% TCA containing 0.5% (w/v) thiobarbituric acid (TBA). The resulting mixture was subjected to heating in a boiling water bath for 30 minutes, followed by rapid cooling.

Subsequently, the contents were centrifuged at 10,000 rpm for 10 minutes, and the absorbance was recorded at 532 nm and 600 nm. The quantification of malondialdehyde (MDA) content was carried out using an extinction coefficient of 155 mm/cm, and the results were expressed as nmol/g FW (fresh weight).

Quantification of total phenolic

The quantification of total phenolic content followed the Folin-Ciocalteu method, following the procedures outlined by Ebrahimzadeh *et al.* (2009). In this process, 0.2 g of explant samples were ground in a mortar, and this resulting material was combined with 10 ml of 95% ethyl alcohol for a 72 hour shock period. Subsequently, the mixture was filtered using filter paper No. 93 with a 125micrometer size. Following filtration, 1 ml of the extract was drawn and added to 4.5 ml of distilled water, along with 0.5 ml of 2N Folin-Ciocalteu reagent. The resulting mixture was vortexed for 15 seconds and then enriched with 4 ml of 7.5% sodium carbonate. After another 15 second vortex, the mixture was allowed to incubate for 60 minutes at room temperature, in the absence of light. Following this incubation, the mixture was centrifuged at 6000 rpm for 5 minutes at 25°C. Finally, the absorbance was measured at 765 nm using a spectrophotometer. The quantification of total phenolic content was achieved by referring to a calibration curve based on gallic acid standards. The results were expressed as milligrams of gallic acid equivalent per gram of crude extract (mg GAE/g CE) obtained from the plant material.

Data recording and statistical analysis

The experiments design was factorial completely randomized design. Data were recorded plants size include width length and height, number of shoots per explant. The amount of chlorophyll and carotenoid contents including Malondialdehyde were recorded 4 times with 5 explants per treatment throughout the duration of the experiment. Recorded data were analyzed by the analysis of variance (ANOVA) using procedure computer program of IBM SPSS Statistics Version 29 statistical significances were tested using Duncan's multiple range tests (Gomez and Gomez, 1984) with a significance level of $p \leq 0.05$.

Results

Shoots induction from node and regeneration

Node from Philodendron Pink Princess cultivation on a MS medium (Murashige and Skoog, 1962) supplemented with BA 0.1 mg/l, BA 0.5 mg/l,

and BA 1 mg/l. Result was observed that in the 18th week of the experiment node of Philodendron Pink Princess cultivated on MS + BA 0.5 mg/l medium shown the greatest plant size in terms of width, length, and height when compared to other medium formulations. The average width was highest at 3.40 centimeters (Figure 1A), which showed statistically significant differences. The average length was highest at 3.22 centimeters (Figure 1B), and the average height was highest at 1.96 centimeters (Figure 1C), In parallel, there was also the highest average number of shoots per explant, with an average shoot count of 60.44 shoots per explant (Figure 1D).

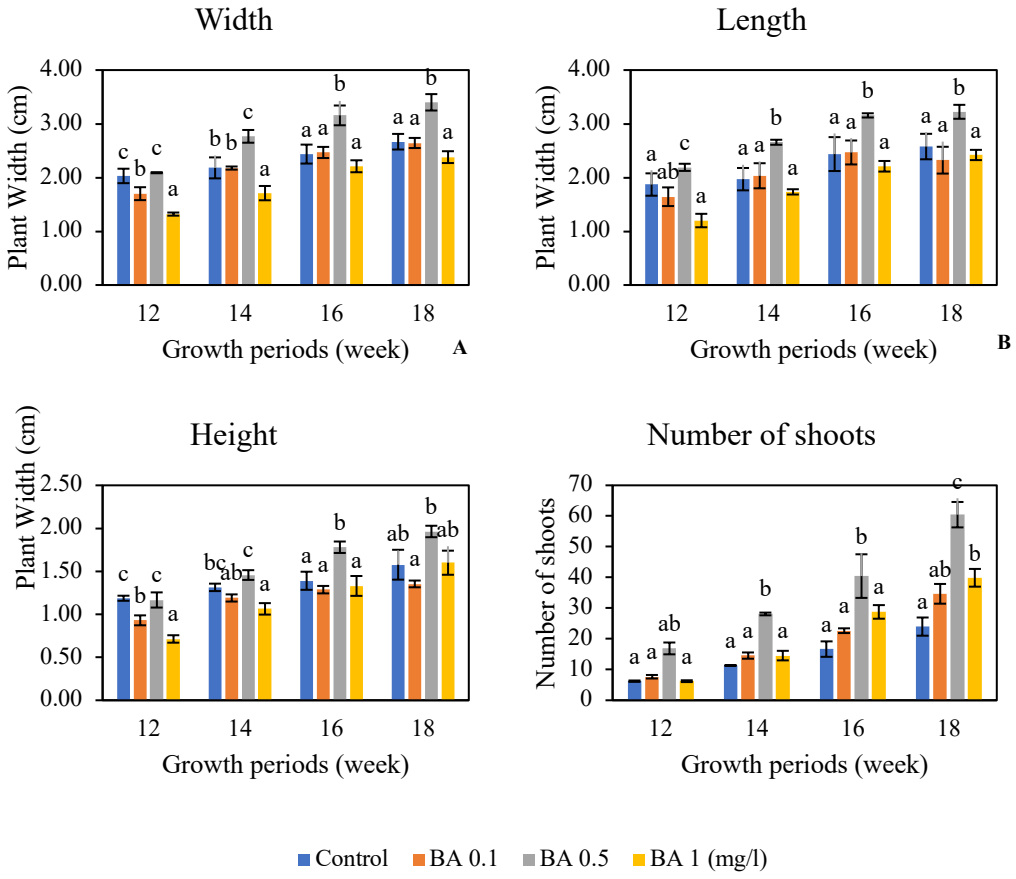

Figure 1. Effect of BA on plant size (A) width, (B) lenght, (C)height, and (D) number of shoots. The error bars represent standard error of the mean bars with the same letter are not significantly ($p > 0.05$) different

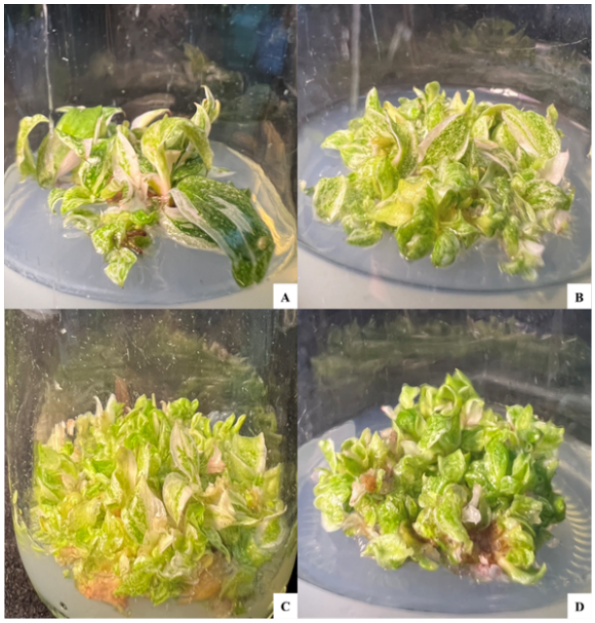

Figure 2. The characteristics of shoot regeneration of philodendron pink princess cultivated on MS medium supplemented with different concentrations of the growth regulator BA, (A) 0 , (B) 0.1 , (C) 0.5 and (D) 1 mg/l at the 18 week of cultivation

Chlorophyll a, Chlorophyll b, Total Chlorophyll and Carotenoids content

Node of philodendron pink princess cultivation on MS medium supplemented with varying concentrations of the growth regulator BA (0, 0.1, 0.5, 1 mg/l), it was observed that philodendron pink princess cultivation on MS medium without the addition of a growth regulator exhibited the highest average chlorophyll a (Chl a) content at 213.96 µg/g fresh weight (Figure 2A), the highest average chlorophyll b (Chl b) content (Figure 2B) at 162.54 µg/g fresh weight (Figure 2B), the highest average total chlorophyll (Total Chl) content at 304.64 µg/g fresh weight (Figure 2C), and the highest average carotenoid content at 2.61 µg/g fresh weight (Figure 2D) during the 14 week of the experiment. Subsequently, the Chl a, Chl b, Total Chl, and Carotenoid contents gradually decreased in the 16 and 18 weeks. In the 16 week, the average Chl a content was 182.42 µg/g fresh weight (Figure 3A), the average Chl b content was 92.57 µg/g fresh weight (Figure 3B), the average Total Chl content was 263.61 µg/g fresh weight (Figure 3C), and the average Carotenoid content was 2.18 µg/g fresh

weight (Figure 3D), representing the highest levels of Chl a, Chl b, Total Chl, and Carotenoid content observed.

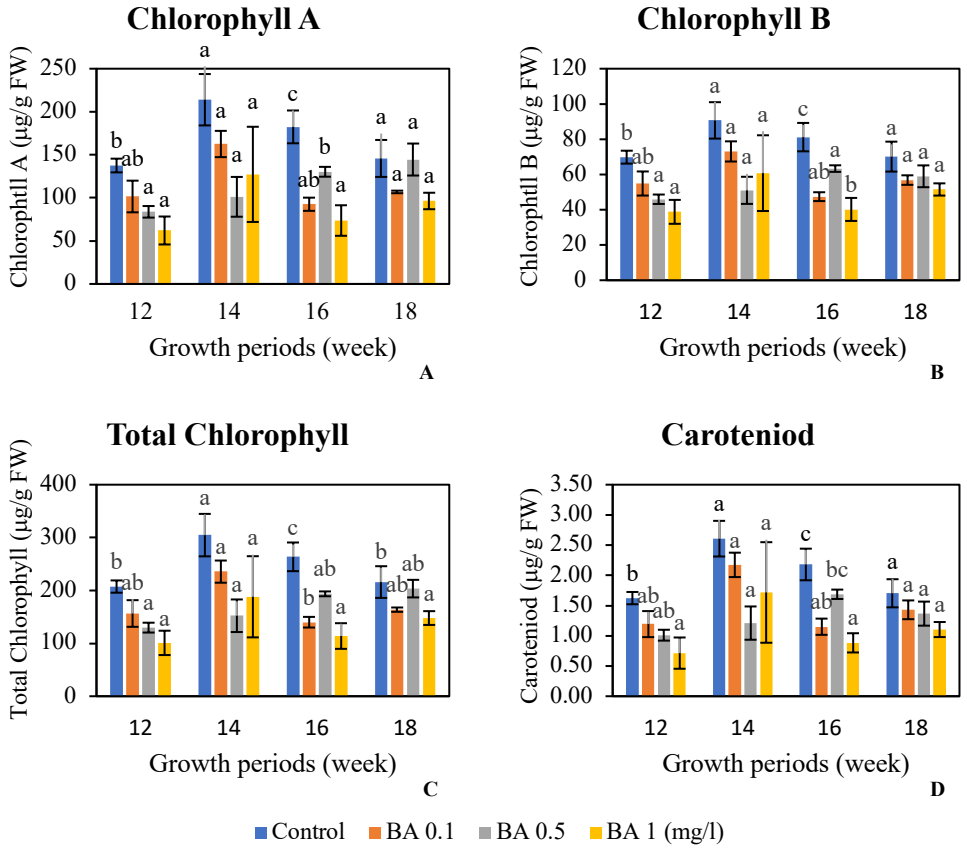

Figure 3. Effect of BA on (A) Chlorophyll a, (B) Chlorophyll b, (C) Total Chlorophyll and (D) Carotenoids content. The error bars represent standard error of the mean bars with the same letter are not significantly ($p > 0.05$) different

Malondialdehyde: MDA and total phenolic contents

Node of Philodendron Pink Princess cultivation on MS medium supplemented with varying concentrations of the growth regulator BA (0, 0.1, 0.5, 1 mg/l), cultivated on MS + BA 0.5 and 1 mg/l media exhibited the highest average levels of Malondialdehyde (MDA) (Figure 4A), measuring 20.75 and 18.57 nmol/g fresh weight, respectively. These levels were significantly higher when compared to philodendron pink princess cultivated on MS medium without the addition of a growth regulator and with other concentrations of BA. Subsequently, MDA levels gradually decreased in the 16th and 18th weeks.

Notably, in the 18th week, philodendron pink princess on MS + BA 0.5 mg/l medium demonstrated the lowest average MDA content at 1.91 nmol/g fresh weight. Phenolic content (Figure 4B) it was observed that philodendron pink princess cultivation on MS + BA 0.5 mg/l exhibited the lowest average total phenolic content, measuring 9.51 mg GAE/g FW, which was the lowest observed.

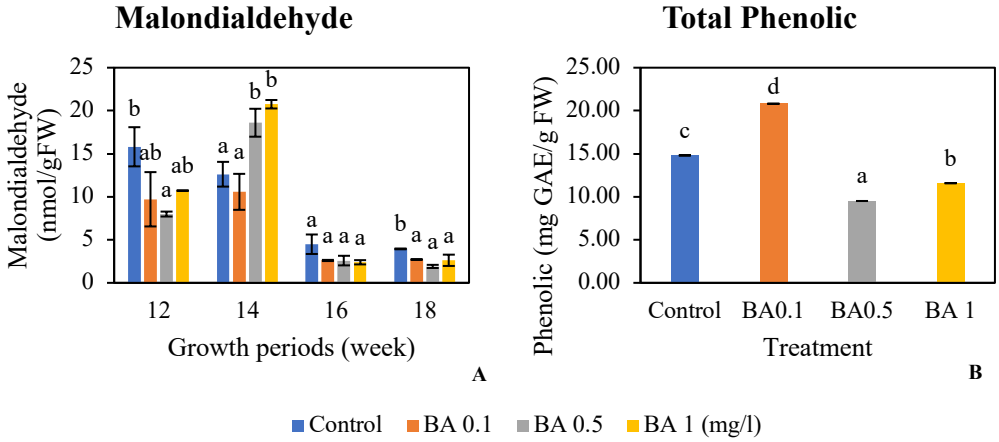

Figure 4. Effect of BA on (A) MDA, (B) Total Phenolic content. The error bars represent Standard error of the mean bars with the same letter are not significantly ($p > 0.05$) different

Characteristics of Philodendron pink princess

In the context of the cultivation experiment involving Philodendron Pink Princess on MS medium supplemented with varying concentrations of the growth regulator BA (0, 0.1, 0.5, 1 mg/l), notable distinctions in plant characteristics were observed. philodendron pink princess grown on MS medium without the inclusion of a growth regulator exhibited larger stem and leaf sizes compared to those cultivated on MS medium with BA supplementation. However, this group showed a reduced number of shoots. Conversely, philodendron pink princess cultivated on MS + BA 0.1 mg/l and MS + BA 0.5 mg/l (Figure 5B, C) medium show that smaller stem and leaf sizes but exhibited a higher number of shoots in comparison to the plants on MS medium without a growth regulator (Figure 5A). Particularly noteworthy is the observation that Philodendron Pink Princess on MS + BA 1 mg/l (Figure 5D) exhibited characteristics akin to those cultivated on MS medium without a growth regulator, showcasing an equivalent average

number of shoots. However, they also show a distinctive feature of compact callus formation concurrently.

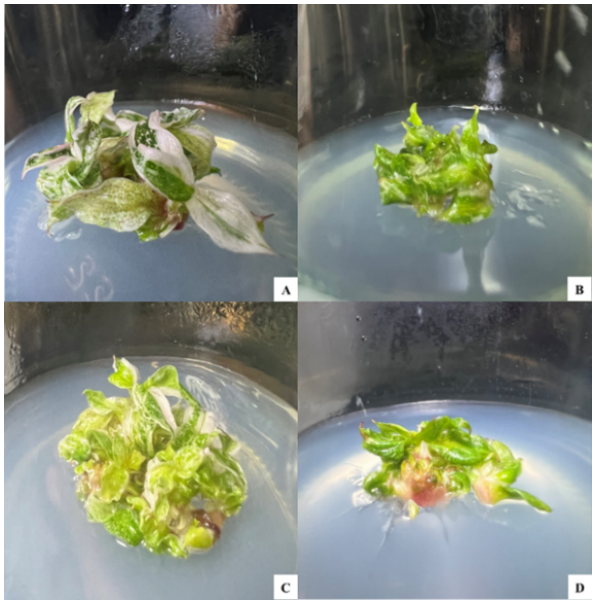

Figure 5. The characteristics of Philodendron Pink Princess Plant cultivated on MS medium supplemented with different concentrations of the growth regulator BA, (A)0, (B) 0.1, (C)0.5 and (D)1 at the 18 weeks of cultivation

Discussion

In this study, the induction of shoots in Philodendron Pink Princess was investigated Node Explant were cultivated on MS medium combine with cytokinin, which demonstrated superior efficacy in promoting the emergence of new shoots compared to other plant parts, studied by Chen *et al*. (2012). Notably, Node explants were cultivated on MS+BA 0.5 medium for 18 weeks exhibited the largest sizes (i.e., width, length and height) due to the shoots emergence that higher that other treatment of BA concentration. However, Cytokinin, recognized as plant growth regulators (PGRs), are pivotal plant-specific chemical messengers or hormones that play a central role in governing the plant cell cycle and numerous developmental processes, as highlighted by Lennarz and Daniel Lane (2003). Cytokinin serve multiple critical functions in plant development and morphogenesis, particularly in regulating cell division and stimulating the growth and proliferation of buds and shoots, both in intact plants and in tissue culture, as emphasized by Srivastava (2002); Mehbub *et al*. (2022). And Fahmy

et al. (1998) demonstrated that cytokinin are involved in stimulating plant cell division. Several studies have explored the use of cytokinin, such as BAP, in the induction of shoots in Philodendron species. For instance, a study on *Philodendron erubescens* cv. Red Emerald revealed that cytokinin, particularly BAP at concentrations of 2.5 and 5.0 mg/l, resulted in the highest average numbers of shoots and leaves per culture. Additionally, research on *Philodendron bipinnatifidum* Schott ex Endl. showed that the addition of 1 mg/l BAP significantly enhanced shoot multiplication compared to other cytokinin. Furthermore, Chen *et al*. (2012) investigated the impact of cytokinin, specifically BA and Kn, in three philodendron cultivars ('Imperial Green,' 'Imperial Red,' and 'Imperial Rainbow'). Their findings underscored that both the type and concentration of cytokinin (i,e, BA, Kn) exerted significant effects on shoot regeneration in Philodendron. In 'Imperial Green' and 'Imperial Red,' BA and Kn treatments exhibited higher shoot formation percentages. Regarding the number of shoots produced per explant, BA outperformed Kn in all three cultivars. Notably, there was no discernible difference between the 0.5 mg/l and 1 mg/l BA treatments in two of the three cultivars. It is worth noting that the specific amount of cytokinin required to induce shoot emergence can vary among different plant species and cultivars.

Studies involving the application of the plant hormone cytokinin in tissue culture did not yield significant improvements in chlorophyll and carotenoid synthesis in Philodendron Pink Princess, as evidenced by experiments conducted on parts cultured on MS medium without the addition of hormones because plants cultured on MS medium are mostly grown as single plants, which may allow the plants to receive nutrients and have more space to grow than plants grown in groups of shoots at the same time, plants raised on MS will grow into mature plants more quickly. That may affect the rate of photosynthesis in the leaves of plants that are larger. However, it is important to note that cytokinin plays a multifaceted role in regulating the development and activity of chloroplasts, as supported by research findings. Cytokinin has been reported to exert a promoting influence on chloroplast ultrastructure and chlorophyll synthesis, particularly during the transition from etioplasts to chloroplasts. Additionally, cytokinin has been found to play a protective role for the photosynthetic apparatus, particularly under conditions of high light stress (Cortleven and Schmülling, 2015). Furthermore, cytokinins are known to block or slow down the aging process in plants by preventing the loss of chlorophyll, thereby maintaining the green coloration of leaves. This effect is achieved through the inhibitory action of phytohormones on the degradation of the green pigment (Sosnowski *et al*. 2023). In another study involving Rhododendron 'Kazimierz Odnowiciel' in In vitro cultures, it was observed that micro shoots

developed on a medium containing the ZEA+2iP treatment exhibited the highest contents of chlorophyll and carotenoids (Nowakowska *et al.,* 2021), study by Akram and Aftab (2015) found that MS+BA 0.22 μM affected the amount of chlorophyll in *Tectona grandis* L. The highest amount of chlorophyll was found to be 1.86 Chl a, 1.30 Chl b mg/g. FW. These findings collectively underscore the intricate role of cytokinins in influencing chlorophyll and carotenoid synthesis in various plant species.

The measurement MDA content is a widely used parameter as a measure of lipid peroxidation in plant tissue that increases under oxidative stress (Morales and Munné-Bosch, 2019; Tulkova and Kabashnikova, 2023), When plants confront diverse forms stress or undergo senescence, they experience oxidative stress, resulting in lipid peroxidation. This process leads to the degradation of cell membrane systems and the breakdown of proteins. Consequently, it impairs crucial plant processes such as photosynthesis and respiration and can ultimately lead to cell death, particularly in severe cases (Janků *et al.,* 2019) As the level of damage inflicted upon the plant cell increases, there is a elevation in MDA content. The results showed that the effects of MDA increased at week 14 due to sub-culture, which may be a significant contributor to the increase in MDA dosage. Phenolic compounds, characterized by their redox properties, are essential constituents of plants and are accountable for their antioxidant activity (Soobrattee *et al*., 2005) there have been documented to play various roles in plants in response to stress, encompassing functions such as free radicals free radicals to acquiring resistance to both biotic and abiotic stress conditions (Boo *et al.,* 2011) which tends to be consistent with the amount of MDA a measure of lipid peroxidation marker of stress in plant

In conclusion, the study showed that the most effective results were observed when the node explants of Philodendron erubescens 'Pink Princess' were cultivated on an MS medium supplemented with 0.5 mg/l of BA. This treatment resulted in the largest plant size and the greatest number of shoots. Moreover, the treatment exhibits a best amount of MDA and total phenolic content in comparison to alternative approaches. The MS Medium thereafter exhibits the highest levels of chlorophyll and carotenoid concentrations.

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