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Application of NAA and BA to *Calotropis gigantea* (L.) W.T. Aiton in vitro

Muji Rahayu[1]*, Tyas Alivia Wulandari[1], Amalia Tetrani Sakya[1], Andriyana Setyawati[1], Sri Hartati[1,2], and Samanhudi[1,2]

[1]Study Program of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret
Jl. Ir. Sutami No. 36 A, Jebres Surakarta, Indonesia
[2]Research and Development Center for Biotechnology and Biodiversity, Universitas Sebelas Maret
Jl. Ir. Sutami No. 36 A, Jebres Surakarta, Indonesia
*Corresponding author: mujirahayu@staff.uns.ac.id

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Abstract

Crown flower (*Calotropis gigantea* (L.)) is a weed plant potentially to be used as raw material for textile fibers yet the utilization is not optimal. Efforts to optimize the utilization of *C. gigantea* are needed. This research aimed to determine the appropriate concentration of plant growth regulators, including NAA (Naphthalene Acetic Acid) and BA (Benzyl Adenine) to stimulate optimal growth of *C. gigantea* to be used as the basis for in vitro propagation of *C. gigantea*. The research was arranged in a completely randomized design (CRD). Explants were planted on Murashige and Skoog (MS) medium with various concentrations of NAA (0; 0.5; 1; 1.5; and 2 ppm) and BA (0; 1; 2; 3; and 4 ppm). Based on the research result, the addition of 3 ppm BA followed by increasing the concentration of NAA to 1.5 ppm could increase the number of *C. gigantea* shoots, and giving 1 ppm BA followed by adding 1.5 to 2 ppm NAA increased shoot height. The best plantlet response was found in media with 0 ppm NAA and 4 ppm BA, which could produce an optimal shoot height of 5.9 cm and a large number of shoots of 5.67 shoots. Increasing NAA concentration retarded root formation and reduced the root length and number of leaves, while the medium without NAA gave the earliest root emergence of 11 days after planting, the number of leaves of 20 strands, and the longest root of 10.9 cm. Applying a single substance BA did not accelerate all the variables observed.

INTRODUCTION

C. gigantea or crown flower is spread in Yunnan, China, India, Sri Lanka, Vietnam, Africa, and Indonesia. It can be utilized as a source of fiber raw materials in the textile industry (Jiang et al., 2012). Narayanasamy et al. (2020) state that *C. gigantea* are harvested to obtain the fibers from their stems for a wide range of applications. Also, fibers from *C. gigantea* are durable and can be useful for the creation of ropes, carpets, fishing nets, and sewing thread. Fiber of *C. gigantea* can be used as a textile material because fiber is environmental-friendly. They do not trigger allergies, are mild, and are hydrophobic so that they are not easy to get wet. In addition, *C. gigantea* is not only used as a textile fiber material but also has the potential to be developed as a medicinal raw ingredient. A study indicated that *C. gigantea* had activity of anti-inflammatory (Ahmad, 2020). Additionally, leaves of crown flower have been employed medicinally to treat injuries, measles, high body temperature, and respiratory ailments, such as coughs (Faradilla & Maysarah, 2019). *C. gigantea* fibers have cavities that function as media or trap the air to control heat flow. They can serve as renewable and eco-friendly materials for natural heat insulation (Karimah et al., 2021).

The fiber of the crown flower has thin walls and

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large holes, so it is relatively light like cotton wool, which has a length of 2–4 cm and a diameter of 18–37 µm (Cui et al., 2017). *C. gigantea* fiber can be environmentally friendly and become a new natural plant fiber in the textile industry. The study of Xiao et al. (2021) reported that *C. gigantea* fiber (CGF) derived from renewable plant resources had been employed to create a biodegradable foam-like oil sorbent known as PCF. The advantages of crown flower fiber over cotton fiber are that it is lighter, and the resulting surface is softer and smoother. High-quality textile fabrics can be produced with combination of crown flower fibers with cotton, rayon, and polyester fibers (Zhao et al., 2019). *C. gigantea* can grow wild in tropical and sub-tropical areas, for example, areas around the coast (Sakya et al. 2022). However, some people still do not use crown flower optimally, so an integrated effort is needed to use crown flowers.

The fulfillment of human needs for textiles and food must be carried out based on the principle of sustainable agriculture. This is essential because sustainable farming can protect the environment with concrete efforts to reduce erosion and maintain soil and water quality. Thus, it is necessary to emphasize that the use of natural resources must prioritize sustainable agriculture (Dubey et al., 2021). Tissue culture plays a role in the development of planting materials that have strong ecological characteristics. This potential is a solution to the problem of the seasonal plantation. Tissue culture can build a sustainable agricultural economy together with pest control and the addition of fertilization processes. The use of tissue culture in this research primarily focused on multiplication. Tissue culture offers several advantages that make it a valuable tool in pursuing these goals. It provides a reliable and efficient means of propagating plants with maintaining their genetic integrity and enables the propagation of cells or tissues with identical genetic characteristics.

Tissue culture or plant cultivation in vitro is a cultivation technique to efficiently produce substantial quantities of seeds in short time. According to Shnaishel (2019), in vitro culture is based on the fact that numerous plant cells possess the capacity to regenerate and form new plants (totipotent). Plant cultivation through tissue culture is carried out by providing nutrients and a sterile cultivation environment in a laboratory (Younas et al., 2020). Plant growth regulators are organic substrates naturally produced in plant tissue. They play a role to control or affect the growth or other physiological functions, the quality of the crops, and as stress protection (Desta & Amare, 2021). The application of growth regulators (auxin and cytokinin) can support the supply of nutrition. Giving of auxin and cytokinin can maintain and assist plant growth and development. An increase in cytokinin concentration followed by the addition of a low auxin can stimulate cell regeneration and division in tissue culture (Faisal et al., 2018).

NAA is a synthetic auxin known to influence various plant processes, including cell elongation, apical dominance, photoperiod responses, and geotropism (Ozkan et al., 2012). As an auxin, the treatment of 0.1 µM NAA can positively increase root length and form root hairs (Martins et al. 2018). Benzyl Adenin (BA) is one of plant growth regulators from cytokinin groups that play a role in cell division. This hormone can inhibit root induction and decrease apical dominance. However, the growth of axillary shoots can be promoted by this reduction in apical dominance (Mangena, 2020). Furthermore, the research of Taha et al. (2020) found that the addition of 1 mg/l NAA to the MS medium resulted in improved development and growth of the mulberry root system, leading to successful acclimatization.

The objective of the research was to obtain the appropriate NAA and BA concentrations to stimulate optimal growth of *C. gigantea* to be used as the basis for in vitro propagation of *C. gigantea*. This research was carried out due to the scarcity of the studies on *C. gigantea* in vitro culture. The types of growth regulators are diverse so that the effect on explant growth also varies. The growth regulators concentration also influences growth and differentiation of each type of explant. Thus, research related to growth regulator types and concentrations is necessary to support the development and supply of crown flower seeds.

MATERIALS AND METHODS

Plant material

The research was conducted over duration of four months, from August to December 2021. The research location was Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Indonesia. The initial stage was planting *C. gigantea* seeds in polybags in a greenhouse.

The seedlings were cultivated for a period of 1.5 months, or until the stems reached the appropriate stage for utilization. Plant maintenance was done by watering every day and controlling pests. The explant used was the second segment of the plant stem, which was 1.5 months old. Roots and leaves were cut off, and only stem segments were used as the explant.

Sterilization of the tools and explant

Sterilization of the tools was started by washing the tools thoroughly and sterilizing them using an autoclave. These tools include culture bottles, petridishes, and dissection tools (large tweezers, small tweezers, and scalpel knives). Several tools, such as petridishes and dissection equipment, were wrapped in opaque paper and sterilized using an autoclave before use. The pressure used in the autoclave was 1.5 psi with 30 minutes sterilization process, then 15 minutes for drying, and 1 hour and 15 minutes to let the pressure drops to 0 psi. This study used MS media, which was prepared by combining 50 mL of MS macronutrients, 50 mL of Fe-EDTA, 50 mL of vitamins, 10 mL of MS micronutrients, 30 grams of sugar, and growth regulators according to the treatments. After combining the mentioned ingredients, 1000 mL of distilled water was added to complete preparation of MS media. The materials used for making MS media were produced by the Merck factory. MS media is classified into media that has high salt content compared to other formulations (Uche et al., 2016).

The explant preparation was started by taking the *C. gigantea* stems to the laboratory and washing them with sufficient detergent. The explants were then rinsed using running water and distilled water thoroughly. After that, the explants were soaked in a solution of bactericide (1 g) and fungicide (1 g), then added with distilled water up to 1000 ml for 1 hour and 45 minutes. The explants were rinsed again using distilled water before being brought to LAF. The first step during sterilization in LAF was washing explants in sterile distilled water twice for 1 minute. Next, explants were immersed in chlorin (100% bayclin) for 1 minute. Then the explants were immersed in 70% alcohol for 1 minute. Subsequently, the explants were rinsed twice using sterile distilled water, each time for a duration of 1 minute (Rahmawati dan Mila, 2019).

Explant initiation and maintenance

Sterilized explants were cut along approximately 2 cm. Then, explants were drained on sterile tissue. The explants were passed before the fire and then planted in culture bottles vertically. The explants were planted on MS media containing the combination of NAA and BA concentration, consisting of NAA (0 ppm (A1); 0.5 ppm (A2); 1 ppm (A3); 1.5 ppm (A4); 2 ppm (A5)) and BA (0 ppm (B1); 1 ppm (B2); 2 ppm (B3); 3 ppm (B4); 4 ppm (B5)). The culture bottles were then covered using plastic wrap to keep them tightly closed and to avoid contamination. They were stored in relative humidity in the growth room ranging from 70–75% (Singh, 2018). According to Mohit and Sirohi (2018), growth room is maintained in deep controlled environmental conditions with a temperature of around 22^{o}C. To prevent contamination in the culture bottles, explant maintenance was carried out by spraying the bottles with 70% alcohol every 2 days. Additionally, any contaminated explants were removed from the culture to ensure a clean and uncontaminated environment.

Data analysis

The experiment was arranged in a completely randomized design (CRD). The variables measured in this study were root emergence time, root length, number of shoots, shoot height, and number of leaves. The root emergence time was monitored every day after initiation. Root length, number of shoots, shoot height, and number of leaves were observed at the age of 10 weeks after planting. The data obtained were subjected to analysis of variance (ANOVA) at a 5% significance level test. If a significant difference was observed, the analysis was continued with the Duncan Multiple Range Test (DMRT) at a 5% significance level.

RESULTS AND DISCUSSION

Root emergence time

Root emergence time is one of the crucial stages in plantlet formation. The development of a large-scale production system is supported by the optimization of in vitro adventitious root formations (Tamyiz et al., 2022). The root growth in tissue culture is depending on growth regulators concentration (Agustina et al.,

2020). The data analysis revealed that the application of NAA had a significant impact on the root emergence time (Table 1). Factors that can affect the root and shoot formation process include light intensity, growth regulators, and the concentration of nutrients in the culture media (Faisal et al., 2018).

The administration of 0 ppm NAA was not significantly different from that of 0.5, 1, and 1.5 ppm NAA but significantly different from 2 ppm NAA. The increasing NAA concentration can retard root emergence time of crown flower. The root emergence time was 11–21 days after planting (DAP). The longest time taken to form roots was found in the 2 ppm NAA treatment, which was 21.13 DAP. This result is contradictive to auxin function in inducing roots. Sharma et al. (2021) stated that low concentration of auxin stimulated primary root growth in Arabidopsis and maize, while at elevated concentration, root growth was found to be inhibited. High concentrations of NAA can disrupt the delicate balance of hormonal signaling involved in root formation, leading to a delay or inhibition of root emergence in crown flower. This follows the opinion of Sessou et al. (2020), stating that increasing the concentration of NAA can inhibit root formation. In addition, according to Adugna et al. (2020), different genotypes of each explant also influence the root formation process. Treatment without NAA can accelerate root appearance, presumably because endogenous auxin present in explants has been able to stimulate root formation. The lower concentration of growth regulator in the form of auxin is suggested to induce rooting (Sugiyono et al., 2021).

Number of shoots

The data analysis indicated that NAA and BA interaction had a significant impact on the number of shoots formed in the *C. gigantea* (Table 2). The application of high and low concentrations of BA with an increase in NAA concentration could reduce the number of shoots. In contrast, the application of low concentrations of NAA (0 and 0.5 ppm) followed by raising the concentration of BA resulted in an increase in the number of shoots formed. Recommendations that can be taken for the propagation of *C. gigantea* seeds are 0 ppm NAA

Table 1. Effects of NAA on root emergence time (DAP) in *C. gigantea* explants

NAA treatment (ppm)	Root emergence time (DAP)
0	11.13 a
0.5	18.80 ab
1	14.47 ab
1.5	16.13 ab
2	21.13 c

Remarks: Means sharing the same letters within the same column exhibit no significant difference at a 5% level of DMRT.

Table 2. Interaction effects of NAA and BA on the number of shoots in *C. gigantea* explants aged 10 WAP

BA (ppm)	NAA (ppm)					NAA Average
	0	0.5	1	1.5	2	
0	2.00 ad	2.67 af	1.33 ab	1.67 ac	0.67 a	1.68
1	3.00 af	4.67 cf	0.33 a	4.00 bf	1.00 ab	2.60
2	2.33 ae	5.00 df	0.67 a	0.67 a	1.00 ab	1.93
3	3.33 af	0.67 a	2.00 ad	3.33 af	1.33 ab	2.13
4	5.67 f	5.33 ef	1.00 ab	0.33 a	1.33 ab	2.73
BA average	3.26	3.67	1.06	2.00	1.06	+

Remarks: Means sharing the same letters within the same column exhibit no significant difference at a 5% level of DMRT; (+): present an interaction.

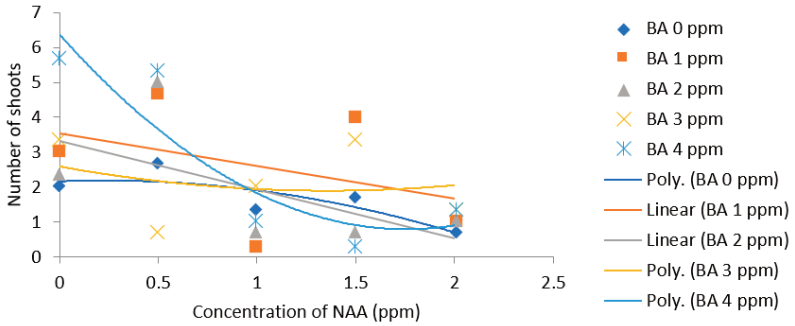
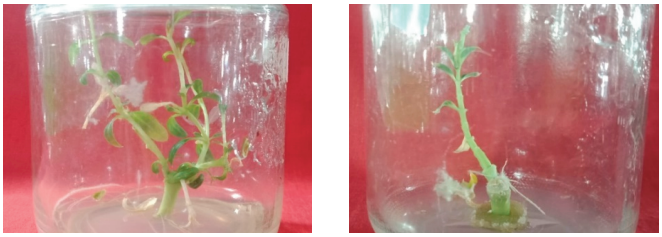


Figure 1. Effects of giving NAA and BA on the number of shoots in *C. gigantea*

Remarks: Y (BA 0 ppm) = -0.1186x2 + 0.3454x + 1.936 R^2 = 0.6907
Y (BA 1 ppm) = -0.467x + 4.001 R^2 = 0.1545
Y (BA 2 ppm) = -0.699x + 4.031 R^2 = 0.3586
Y (BA 3 ppm) = 0.0943x2 – 0.6997x + 3.194 R^2 = 0.0536
Y (BA 4 ppm) = 0.4529x2 – 4.0851x + 10.006 R^2 = 0.8265



(a) (b)

Figure 2. Number of shoots as affected by the addition of (a) 0 ppm NAA + 4 ppm BA, (b) 2 ppm NAA + 1 ppm BA

and 4 ppm BA.

Treatments of 0 ppm NAA and 4 ppm BA are recommended because the results revealed that this concentration was able to produce a large number of shoots and optimal shoot height, and it could not produce callus on crown flower explants. This result proved the experiment of Karimpour et al. (2020), reporting that the giving of BA affected the number of regenerated shoots. Furthermore, Pramita et al. (2018) stated that shoot multiplication rate was higher in medium with addition of growth regulators compared to the medium without growth regulators. This proves that growth regulators can produce higher shoot multiplication. The addition of BA concentration led to an increase in the number of regenerated shoots. The value of R^2 (Figure 1) shows how much NAA and BA affects the number of *C. gigantea* shoots. The R^2 value of 0.8265 or 82.65% indicates that NAA and BA affect the number of shoots by 82.65%.

The addition of cytokinin increased the number if shoots and leaves. BA with higher concentration combined with NAA could produce more shoots. Talitha et al. (2022) recommended the higher concentration of cytokinin because it produced a large number of shoots. However, if the concentration is too high, it can inhibit the induction of shoots and leaves. According to Torres-Silva et al. (2018), the use of growth regulators from auxin and cytokinin groups can increase the number of shoots. The number of shoots can also have some variation because it is influenced by the type of explant and genotype. The number of shoots produced by an explant can be an indicator of competition for the formation of seeds in a short period and large quantities.

Based on Figure 2, the number of shoots produced in the treatments of NAA 0 ppm and BA 4 ppm wa higher than that in NAA 2 ppm and BA 1 ppm. According to Pramita et al. (2018), media added with growth regulators can produce higher shoot multiplication than media without growth regulators. Cytokinin is also known to induce shoot formation in vitro culture.

Shoot height

The application of a single treatment of NAA and a single treatment of BA did not demonstrate a significant impact on shoot height. Still, interaction between the two growth regulators significantly affected shoot height (Table 3). The analysis results indicated that NAA and BA interaction had a notable impact on shoot height in crown flower. According to Naaz et al. (2019), a critical balance between auxins and cytokinin can affect the meristematic activity of axillary buds. Based on the test results of several auxins with BA, NAA showed a better effect on shoot morphogenesis. Application of BA with concentrations of 1 and 4 ppm followed by an increase in NAA concentration increased shoot height. Application of NAA with a concentration of 0 and 2 ppm followed by an increase in BA concentration also increased shoot height (Table 3).

Recommendations that can be taken for the propagation of *C. gigantea* seeds are 0 ppm NAA and 4 ppm BA. This concentrations are recommended because the results demonstrated that the specific concentration used was capable of generating a large number of shoots and optimal shoot height. According to Mangena (2020), benzyladenine is a widely recognized cytokinin that functions as a plant hormone, either naturally occurring or synthetic, which stimulates cell division and frequently promotes the formation of adventitious shoot buds. BA is commonly used in tissue culture to stimulate shoot elongation and overall growth. This result is in line with Grossman et al. (2012), stating that BA seems to encourage plant shoot growth. The value of R2 (Figure 3) shows how much NAA and BA affects the shoot height of *C. gigantea*. The R^2 value of 0.8552 or 85.52% indicates that NAA and BA affect shoot height by 85.52%.

Table 3. NAA and BA interaction effects on the shoot height (cm) in *C. gigantea* explants

BA (ppm)	NAA (ppm)					NAA Average
	0	0.5	1	1.5	2	
0	3.33 ad	6.10 cd	4.20 ad	3.50 ad	1.00 ab	3.63
1	5.26 ad	4.83 ad	0.33 a	4.16 ad	6.67 d	4.25
2	4.00 ad	7.06 d	2.16 ad	2.10 ad	2.00 ad	3.46
3	5.36 ad	2.06 ad	5.23 ad	6.67 d	1.50 ac	4.16
4	5.90 bd	3.56 ad	3.00 ad	0.53 a	3.23 ad	3.24
BA average	4.77	4.72	2.98	3.39	2.88	+

Remarks: Means sharing the same letters within the same column exhibit no significant difference at a 5% level of DMRT; (+): present an interaction.

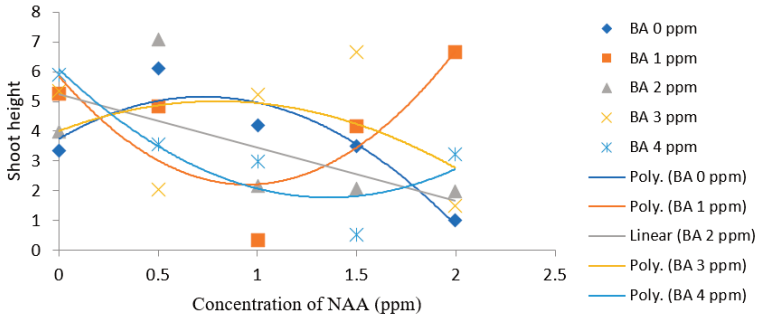


Figure 3. Effects of giving NAA and BA on *C. gigantea* shoots height

Remarks: Y (BA 0 ppm) = -0.6671x2 + 3.2769x + 1.134 R^2 = 0.8552
Y (BA 1 ppm) = 1.015x2 – 5.875x + 10.71 R^2 = 0.6590
Y (BA 2 ppm) = -0.896x + 6.152 R^2 = 0.4243
Y (BA 3 ppm) = -0.3907x2 + 2.0333x + 2.362 R^2 = 0.1524
Y (BA 4 ppm) = 0.5836x2 – 4.3384x + 9.84 R^2 = 0.8075

Root length

The data analysis revealed that applying NAA on MS media had a notable impact on root length. Treatment of 0 ppm NAA exhibited a significant difference from the treatments of 0.5; 1; 1.5; and 2 ppm NAA, but the 0.5 ppm NAA treatment did not show a significant difference from 1; 1.5; and 2 ppm NAA. MS medium with 0 ppm NAA resulted in the longest roots, which was 10.93 cm long (Table 4). NAA 0.5; 1; 1.5; and 2 ppm treatments resulted in shorter roots ranging from 1–3 cm. This described that increasing NAA concentration decreased root length in crown flower explants. This is following the research of Adugna et al. (2020), reporting that increasing NAA concentration from 1.0 to 2.0 mg/L can reduce the average number of roots per shoot and the average root length from 1.63 ± 1.03 to 0.27 ± 0.45 and 0. 84 ± 0.54 to 0.14 ± 0.24 cm.

Treatment of 0 ppm NAA can produce the longest roots because the explants can develop using their endogenous hormones so that they can still form roots even though the media is not added with NAA. According to Gallei et al. (2020), the addition of auxin inhibited root elongation and growth. Several hormone signals, including auxins, were proved to operate downstream of ethylene, playing a role in inhibiting root elongation. The response of plantlets depends on the current auxin concentration in the cell. A combination of NAA and BA, according to Kaviani et al. (2019), was found to be effective in inducing root length in Aglaonema thistles. NAA is plant growth regulator supporting the initiation process of roots and stems at specific concentrations.

Number of roots

The number of roots formed on explants has an essential role in supporting nutrients absorption process for plant growth. According to Latifah et al. (2017), an increasing number of roots can help expand the range roots in the absorption of nutrients so that it becomes more optimal. Juras et al. (2019) stated that the combination of different treatments from BA and NAA gave different effects on root

Table 4. Effects of NAA on the number of roots and root length in *C. gigantea* explants aged 10 WAP

NAA treatment(ppm)	Number of roots (strands)	Root length (cm)
0	0.0 a	10.93 b
0.5	3.0 a	3.07 a
1	6.3 a	2.48 a
1.5	2.0 a	1.59 a
2	8.9 a	2.32 a

Remarks: Means sharing the same letters within the same column exhibit no significant difference at a 5% level of DMRT.

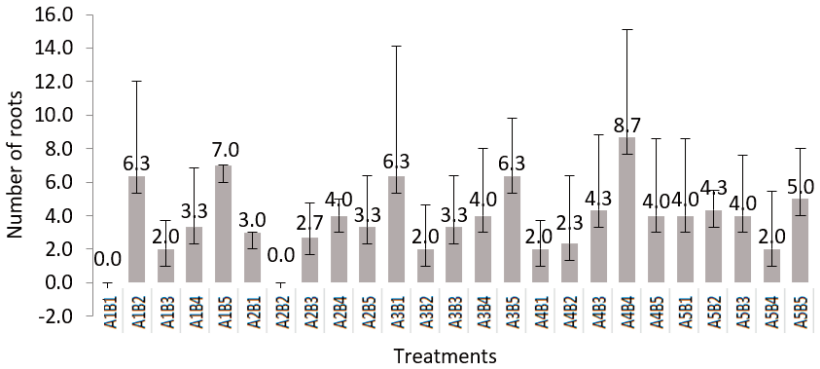


Figure 4. Effects of addition of NAA and BA on the number of roots in *C. gigantea* at 10 WAP

Remarks: A1 = NAA 0 ppm; A2 = NAA 0,5 ppm; A3 = NAA 1 ppm; A4 = NAA 1,5 ppm; A5 = NAA 2 ppm; B1 = BA 0 ppm; B2 = BA 1 ppm; B3 = BA 2 ppm; B4 = BA 3 ppm; B5 = BA 4 ppm; I= error bar.

Table 5. Effects of NAA on the number of leaves in *C. gigantea* explants aged 10 WAP

NAA treatment (ppm)	Number of Leaves (strands)
0	20.13 b
0.5	17.27 b
1	6.67 a
1.5	9.60 a
2	8.93 a

Remarks: Means sharing the same letters within the same column exhibit no significant difference at a 5% level of DMRT.

development in cattleya orchids (Cattleya Xanthina). However, some treatments did not produce root.

The analysis result showed that NAA and BA application had no significant effect on the number of roots in crown flower (Figure 4). The treatment of NAA did not significantly affect the number of roots (Table 4). The number of roots in *C. gigantea* roots in various treatments ranged from 0–8.7 roots. The average number of roots in all treatments was 3.8 roots. Most of the roots are produced on NAA 1.5 ppm + BA 3 ppm as 8.7 roots. According to Sri Lestari and Suwardi (2020), explants that sprout will form roots. However, based on Figure 4, several treatments did not produce roots, which were NAA 0 ppm + BA 0 ppm and NAA 0.5 ppm + BA 1 ppm, although both treatments increased the number of shoots. According to Akbar et al. (2017), several explants did not form roots probably because of the lack of concentration of NAA given and the formation of ethylene due to auxin hormone in plant cells that inhibits root formation. According to Chen et al. (2019), in general, the number of roots increases as the NAA concentration is increased.

Srilestari and Suwardi (2020) stated that the formation of roots was closely associated to endogenous auxins and cytokinin levels in plant tissues through cell elongation and enlargement. In addition to endogenous auxins and cytokinin effects, root formation process is also affected by the intensity of light in the culture room. According to Hlophe et al. (2020), root formation process in explants is influenced by content of endogenous and exogenous auxin in plants, and the application of BA at low concentrations is known to have a low inhibitory effect on root formation.

Number of leaves

Analysis of data revealed that NAA application had a notable impact on crown flower leaves number (Table 5). Treatment of 0 ppm NAA did not show a significant difference from 0.5 ppm NAA but substantially different from the treatments of 1; 1.5; and 2 ppm NAA. More leaves were produced on MS medium with 0 ppm NAA (20.13) and on MS medium with 0.5 ppm NAA (17.27 leaves). Meanwhile, the treatments of 1; 1.5; and 2 ppm NAA produced between 6–9 leaves. This indicated that increasing the concentration of NAA decreased the number of leaves formed on *C. gigantea* explants. This result follows the results of Kalve et al. 2020) research, reporting that low concentrations of auxin, particularly NAA, resulted in an increase in leaf blade size of up to 37%, while the higher concentration inhibited leaf growth. In addition, the addition of NAA concentrations of 1; 1.5; and 2 ppm inhibited leaf formation or resulted in a lower number of leaves (Table 5).

Jing and Strader (2019) state that in formation of lateral roots, there are some roles of plant hormones that have been revealed by physiological and genetic studies. Lateral root initiation and development are affected by the strong roles of auxin and cytokinin. The formation of shoots and leaves in plants generally requires cytokinin, while auxin can be inhibitor if the concentration is too high. Haida et al. (2020) stated that auxin and cytokinin combination could have an impact on both the number and weight of fresh leaves formed in explants. Based on research conducted by Monfort et al. (2018), plantlets cultured in vitro and supplemented with growth regulators produced leaves in nearly every treatments.

CONCLUSIONS

Based on research result, the best response was found in media with 0 ppm NAA and 4 ppm BA combination, which produced a large number of shoots of 5.67 and an optimal shoot height of 5.9 cm. Increasing NAA concentration decreased root formation and reduced root length and the number of leaves, while the medium without NAA resulted in the earliest root emergence of 11 DAP, the number of leaves of 20 strands, and the longest root of 10.9 cm. Applying a single substance BA did not stimulate all the variables observed.

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