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Organogenesis of corn plants (*Zea mays L.*) at various concentrations of auxin and cytokinin plant growth regulators in vitro

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ABSTRACT
This study aimed to determine the effect of auxin and cytokinin concentrations as plant growth regulators (PGR) on the organogenesis of corn plants in vitro. This research was conducted from June to September 2021 in the plant tissue culture laboratory of Cokroaminoto University, Palopo City, South Sulawesi, Indonesia. The research method was a completely randomized design (CRD) consisting of 15 experimental units, i.e., P0 (control), P1 (2 ml NAA (naphthaleneacetic acid) + 1 ml BAP (benzylaminopurine)), P2 (2 ml NAA + 1.5 ml BAP), P3 (2 ml NAA + 2 ml BAP), and P4 (2 ml NAA + 2.5 ml BAP). The results revealed that the administration of auxin and cytokinin plant growth regulators had a significant effect on the parameters of germination age and plantlet weight, but it had no significant effect on the parameters of plant height, number of roots, root length, and number of leaves. The effective concentration of auxin and cytokinin growth regulators on the organogenesis of corn plants was P2 (2 ml NAA + 1.5 ml BAP) on the parameters of 11 cm plant height, 7 root strands, 1 leaf, and 1 gram plantlet weight. This was due to the influence of the given concentration of PGR, which plays multiple important roles in plant development.

Contribution/Originality: The administration of the growth regulators naphthaleneacetic acid (NAA) and benzylaminopurine (BAP) had a significant effect on the parameters of germination age and plant weight, but it had no significant effect on the parameters of root length, number of leaves, plant weight, and number of roots.

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1. INTRODUCTION
Corn is the third largest staple food in the world after wheat and rice. Increasing the productivity of corn cultivation is highly dependent on the ability to provide and apply appropriate cultivation system techniques, including the use of high-quality seeds (Bheemanahalli, Vennam, Ramamoorthy, & Reddy, 2022), spacing (Zhang, Basso, Price, Putman, & Shuai, 2018), irrigation (Kaplan, Karaman, Kardes, & Kale, 2019; Orebo, Shanka, & Hadaro, 2021), pest and disease control (Bekeko et al., 2018), and fertilizers (Sajadinia, Ghazanfari, Naghavii, Naghavi, & Tahamipur, 2021). It is important to implement these techniques to support the growth and production of corn plants by meeting their nutritional needs. Several regions in Indonesia use corn as an alternative staple food to replace rice. Corn plants contain moisture, crude protein, crude fat, dietary fiber, carbohydrates (Langyan et al., 2022), and minerals (Ikram, Muhammad, & Arifa, 2010). In addition, the functions of corn plants include

antimicrobial activity (Abirami et al., 2021) and antioxidant capacity (Montero-Vargas, Ortíz-Islas, Ramírez-Sánchez, García-Lara, & Winkler, 2020).

Given the increasing human population, alternative technologies are needed to meet the increasing demand for corn. One of these technologies is the principle of tissue culture, which involves producing as many plant seeds as possible with a small amount of material. In this way, a single plant can produce a large number of new individuals in a relatively short time. The types of plant hormones widely used to stimulate in-vitro growth are auxins and cytokinins (Michniewicz et al., 2019; Wang et al., 2021; Zhao, Xiang, & Xue, 2013). To obtain the best results, the use of appropriate basic media is an important factor that must be considered in nurseries using tissue culture techniques.

Organogenesis is the process of forming organs, such as buds and roots, either directly or indirectly, through the formation of callus. Cell or tissue capacity, differentiation, and determination type are very important for explant organogenesis. Insufficient corn production may be caused by various factors, including drought, nutrient scarcity, and biotic stresses such as pests, weeds, and diseases; also, potassium (K) deficiency significantly reduces photosynthesis due to leaf chlorosis (Qi et al., 2019). A cell is defined as a competent cell if it can form competent cells in response to environmental signals (Tas & Mutlu, 2021) and hormones (Balzan, Johal, & Carraro, 2014; Ludwig, Zhang, & Hochholdinger, 2013). Developing research on corn includes efficient in-vitro split node explants derived from seeds (Mushke, Yarra, & Bulle, 2016), culture embryogenesis and direct regeneration (Hosseini, Ismaili, & Pour Mohammadi, 2014), and the influence of auxin homeostasis on the eighth internode length heterosis in corn (Balzan et al., 2014). The current study aimed to determine the effect of the concentration of the plant growth regulators (PGRs) auxin and cytokinin on the organogenesis of corn plants in vitro.

2. MATERIALS AND METHODS

2.1. Research Time and Location

This research was conducted at the tissue culture laboratory of the Faculty of Agriculture, University of Cokroaminoto Palopo, Batupasi, Wara Subdistrict, Palopo City, South Sulawesi, Indonesia, from June to September 2022.

2.2. Research Design

This study used a completely randomized design (CRD) consisting of 5 treatments with 3 replications, creating a total of 15 experimental units. The treatments were: P0 (control), P1 (administration of MS medium + 2 ml NAA (naphthaleneacetic acid) + 1 ml BAP (benzylaminopurine)), P2 (administration of MS medium + 2 ml NAA + 1.5 ml BAP), P3 (administration of MS medium + 2 ml NAA + 2 ml BAP), and P4 (administration of MS medium + 2 ml NAA + 2.5 ml BAP).

2.3. Research Procedure

Room Sterilization. The room used as a laboratory must be clean and sterile from dirt and dust. The floor was cleaned using a vacuum cleaner and wiped with clean water, after which it was sterilized with formalin.

Sterilization of Bottles and Tools. For contamination control, it is very important to sterilize all tools and materials used to support the success of the in-vitro culture. The first step of the sterilization process was cleaning the bottles, Petri dishes, and tweezers with soap, after which the bottles and tools were put into the autoclave. The autoclave was closed and then turned on to sterilize the items. The autoclave process was at 121 °C for 15 minutes. Then the autoclave was left to stand for 35-45 minutes, after which the bottles were removed from the autoclave and stored on the storage rack.

Media Creation (MS). The medium used in this study was MS medium. First, the necessary tools and materials were prepared. Then each stock solution was placed in a beaker with a predetermined dose, and distilled water was added until it reached 1000 ml. Next, 30 grams of sugar and 7 grams of agar-agar were weighed, mixed with the broth in a chemical glass, and stirred until smooth. The storage media was then poured into the pan and heated. After the media was ready, it was transferred to the culture flask and covered with aluminum foil. Culture bottles covered with aluminum foil were autoclaved until the temperature reached 121°C. After the autoclave temperature stabilized, the media was stored in the storage rack.

Explant Sterilization. Sterilization of the explants was performed in a laminar air flow cabinet by immersing the explants in Ridomil. The explants were immersed in rifampicin and rinsed in sterile distilled water. They were sterilized back in chlorox and rinsed with sterile distilled water. Finally, the explants were soaked in 96% alcohol and dried using tissue or filter paper.

Explant Planting. The explants were planted in the laminar air flow cabinet. Before the bottles were planted, the bottle mouth was heated to avoid contamination. The bottle cap was carefully opened. To maintain the sterilization of the tool, tweezers were always heated before use. The plastic bottle cover was opened, the explants were taken with sterile tweezers, then the explants were planted on the media. After planting, the mouth of the bottle was heated again. Bottle caps were heated before use. The bottles were tightly closed using aluminum foil then labeled with the treatment and stored on a storage rack. Maintenance was performed every morning and evening by spraying the bottles with alcohol to protect the corn from contamination.

2.4. Observation Parameters

Observations were made after the plants had germinated, and subsequent observations were performed after the plants had grown (according to the observation parameters). Several parameters were observed and measured in this

study, including germination age (days), plant height (cm), number of roots (strands), root length (cm), number of leaves (strands), and plantlet weight (g).

2.5. Data Analysis

The data obtained were analyzed statistically using the analysis of variance (ANOVA). If the ANOVA showed a significant effect, then the data was further tested with the honest significant difference test ($\alpha = 0.01$ and $\alpha = 0.05$).

3. RESULTS AND DISCUSSION

3.1. Germination Age (Days)

Several concentrations of auxin and cytokinin growth regulators had a significant effect on the average germination age parameter in the in-vitro organogenesis of corn plants, as shown in Figure 1.

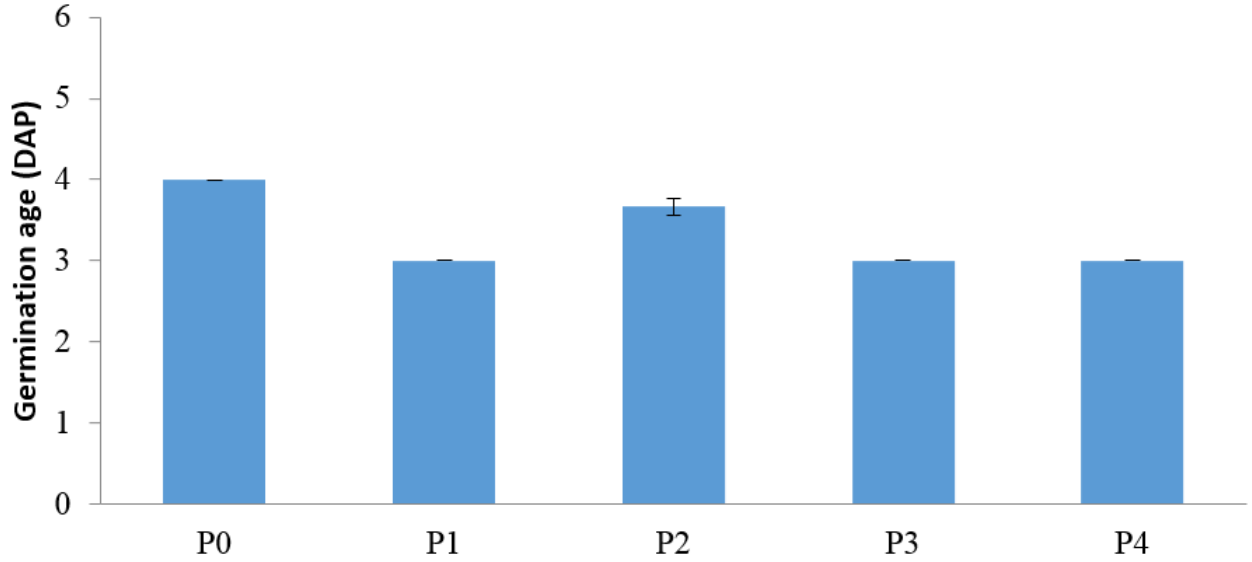

Figure 1. Diagram of the average germination age at various concentrations of auxin and cytokinin PGR on the organogenesis of corn plants in vitro (P0 (control), P1 (MS medium + 2 ml NAA + 1 ml BAP), P2 (MS medium + 2 ml NAA + 1.5 ml BAP), P3 (MS medium + 2 ml NAA + 2 ml BAP), P4 (MS medium + 2ml NAA + 2.5ml BAP).

Figure 1 shows the average germination age in corn plant organogenesis under the influence of various concentrations of auxin and cytokinin growth regulators. The lowest average germination age, or fastest sprouting age, was observed in treatments P1 (2 ml NAA + 1 ml BAP) and P3 (2 ml NAA + 2 ml BAP) at 2.7 days after planting (DAP). These were followed by P2 (2 ml NAA + 1.5 ml BAP) with an average of 3 DAP and P4 (2 ml NAA + 2.5 ml BAP) with an average of 3.3 DAP. Meanwhile, the slowest was P0 (control) with an average of 4.7 DAP. Treatment P1, therefore, produced the best germination age, and the difference with other treatments was significant. Plant germination can thus be affected by the addition of plant growth regulators.

3.2. Plant Height, Number of Roots, and Root Length

Table 1 shows that the average value for the plant height parameter in corn plants with the administration of auxin and cytokinin growth regulators from the highest to the lowest was P2 (2 ml NAA + 1.5 ml BAP) with 11 cm, P4 (2 ml NAA + 2.5 ml BAP) with 9.8 cm, P3 (2 ml NAA + 2 ml BAP) with 8.3 cm, P1 (2 ml NAA + 1 ml BAP) with 6.3 cm, and finally P0 (control) with an average value of 5.9 cm. The P2 treatment produced the best average plant height but the difference with the other treatments was not significant. Nevertheless, plant height gain can be affected by the addition of growth regulators.

Table 1 shows that several concentrations of auxin and cytokinin growth regulators had no significant effect on the average number of roots in the in-vitro organogenesis of corn plants. The average value for the number of roots parameter in corn plants with auxin and cytokinin growth regulators from the highest to the lowest was P2 (2 ml NAA + 1.5 ml BAP) with an average of 7 root strands, followed by P1 (2 ml NAA + 1 ml BAP) with 5.3 strands, P4 (2 ml NAA + 2.5 ml BAP) with 4.7 strands, P3 (2 ml NAA + 2 ml BAP) with 4.7 strands, while the lowest value was P0 (control) with an average value of 1.3 strands. Treatment P2 produced the best average root number, but the difference with the other treatments was not significant. Root growth did seem to be affected by the addition of growth regulators.

Table 1 also shows that various concentrations of auxin and cytokinin growth regulators had no significant effect on the average root length parameter in the in-vitro organogenesis of corn plants. The average value of root length in corn plants with the administration of auxin and cytokinin growth regulators from the highest to the lowest was P4 (2 ml NAA + 2.5 ml BAP) with 1.9 cm, P3 (2 ml NAA + 2 ml BAP) with 1.8 cm, P1 (2 ml NAA + 1 ml BAP) with 1.7 cm, and P2 (2 ml NAA + 1.5 ml BAP) with 1 cm. The P4 treatment produced the best root length, but the difference with the other treatments was not significant. The increase in root length did appear to be affected by the addition of growth regulators.

Table 1. Plant height, number of roots, and root length at several concentrations of auxin and cytokinin PGR during the organogenesis of corn plants in vitro.

Treatments	Height	Number of roots (cm)	Root length (cm)
P0	5.9±2.88	1.3±0.5	0.5±0
P1	6.3±1.52	5.3±2.3	1.7±1.7
P2	11±4.85	7±3	1±0
P3	8.3±4.35	4.0±1	1.8±1.1
P4	9.8±5.86	4.7±2.5	1.9±1.1

3.3. Number of Leaves and Plantlet Weight

Table 2 shows the corn plants' average number of leaves with the administration of auxin and cytokinin growth regulators in the in-vitro organogenesis of corn plants. The average number of leaves from the highest to the lowest was P2 (2 ml NAA + 1.5 ml BAP) with 2 leaves, followed by P3 (2 ml NAA + 2 ml BAP) with 2.0 leaves, P1 (2 ml NAA + 1 ml BAP) with 2.0 leaves, and P4 (2 ml NAA + 2.5 ml BAP) with 2.0 leaves, while P0 (Control) had the lowest value of 1.7 leaves.

Table 2. Number of leaves and plantlet weight at several concentrations of auxin and cytokinin PGR during the organogenesis of corn plants in vitro.

Treatments	Number of leaves (Blades)	Plantlet weight (g)
P0	1.7±0.5	0.6±0.05
P1	2±0	0.9±0.09
P2	2±0.5	1±0.13
P3	2±0	1±0.21
P4	2±0	0.9±0.1

Table 2 shows the average plantlet weight of corn plants with the administration of auxin and cytokinin growth regulators in the in-vitro organogenesis of corn plants. The average value from the highest to the lowest was P2 (2 ml NAA + 1.5 ml BAP) with 1 gram, followed by P3 (2 ml NAA + 2 ml BAP) with 1.0 gram, and P1 (2 ml NAA + 1 ml BAP) and P4 (2 ml NAA + 2.5 ml BAP) with 0.9 grams, while P0 (control) had the lowest value of 1.7 grams. The P2 treatment produced the best root length, but the difference with the other treatments was not significant. The plantlet weight can be affected by the addition of growth regulators. Overall, the organogenesis of the tested corn plants is presented in Figure 2.

4. DISCUSSION

From the analysis of variance, the results showed a significant effect on the parameters of germination age and plantlet weight, while there was no significant effect on the parameters of plant height, number of roots, root length, or number of leaves. For the germination age parameter, P1 (2 ml NAA + 1 ml BAP) showed the best results with an average of 2.7 days (Figure 1). The addition of plant growth regulators auxin (NAA) and cytokinin (BAP) had the best results on the corn germination age. Cytokinin, in combination with auxin, plays an important role in cell division and the differentiation of tissues during the formation of shoots and root growth. Similarly, Rivas, Friero, Alarcón, and Salguero (2022) found that auxin was a plant hormone that could regulate many physiological processes, including growth, cell division and differentiation, and protein synthesis. Cytokinins are involved in cell division and promote the growth of shoots and stems.

For the plant height parameter, treatment P2 (2 ml NAA + 1.5 ml BAP) achieved the best results with an average value of 11 cm, while P0 (control) had an average value of 5.9 cm (Table 1). Therefore, the administration of the auxin growth regulator caused an increase in plant height. Davani, Nabipour, and Roshanfekr Dezfouli (2017) revealed that stem elongation occurs through the process of division, elongation, and expansion of new cells that occur in meristems or apical stems. This is said to increase plant size. For the number of roots parameter, treatment P2 (2 ml NAA + 1.5 ml BAP) obtained the best results with an average of 7 strands, while P0 (control) had an average of 1.3 strands.

The results show that the administration of auxin and cytokinin growth regulators stimulates plant growth and development. The addition of auxins or cytokinins can increase the concentration of growth regulators from the body itself in the cells, which can be a triggering factor for the process of tissue growth and development. The more explant leaves, the better the explant photosynthetic process (Wei, Wang, Jiang, & Dong, 2018). This can indicate the growth and development of explants or planted seeds. The study's findings on the number of roots parameter are also in accordance with the study of Yan, Xu, Li, Wang, and Han (2021), which stated that auxin plays a very important role in stimulating the formation of roots, or cell division. The use of auxin is known to accelerate the process of root formation (Li et al., 2018). The root length observations showed that P4 (2 ml NAA + 2.5 ml BAP) gave the best results with an average value of 1.9 cm.

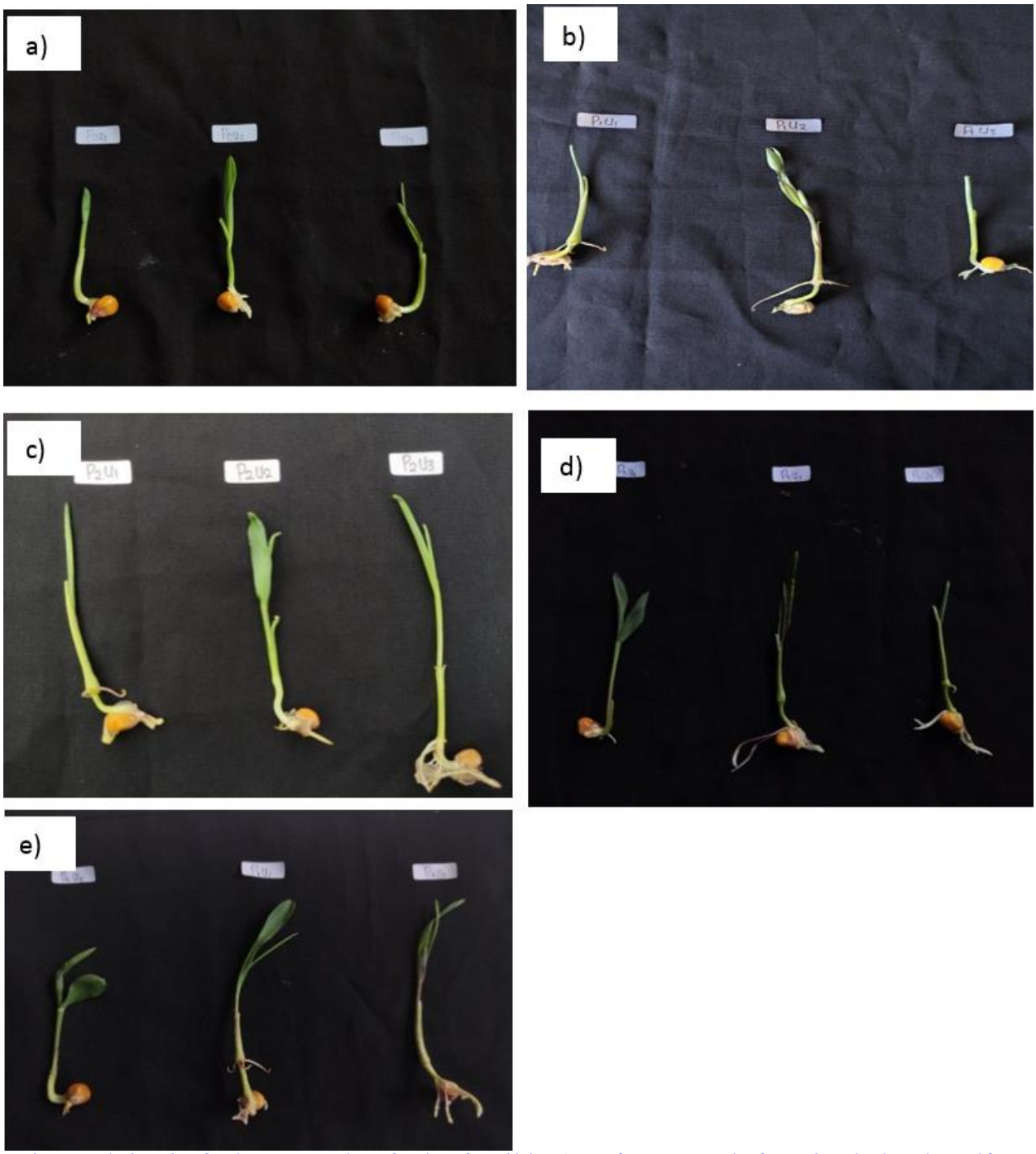

Figure 2. Final results of various concentrations of auxin and cytokinin PGR on the organogenesis of corn plants in vitro. a) P0: without treatment (control), b) P1: 2 ml NAA + 1 ml BAP, c) P2: 2 ml NAA + 1.5 ml BAP, d) P3: 2 ml NAA + 2 ml BAP, e) P4: 2 ml NAA + 2.5 ml BAP.

Meanwhile, P0 (control) had the lowest average root length with 0.5 cm. This result provides evidence for the effect of auxin and cytokinin administration as growth regulators on root formation and elongation. This is in line with Boivin, Farde, and Frugier (2016), who reported that auxin is necessary for root formation as it stimulates cell division. The use of auxin is known to accelerate the root formation process. Growth regulators in concentrations high enough for explants have different effects on the growth and development of each plantlet's roots, stems, and leaves. The effectiveness of exogenous auxin and cytokinin growth regulators depends on the concentration of endogenous hormones in plant tissues (Jones & Ljung, 2011; Schaller, Bishopp, & Kieber, 2015).

Regarding the number and length of roots and their role as a determinant of the direction of tissue development, the administration of auxins and cytokinins must take the concentration and ratio in the media into account. When auxin is higher than cytokinin, it causes differentiation leading to root growth. The findings showed that the treatment with MS medium + 2 ml NAA + 2.5 ml BAP resulted in a better number of roots and root length, while none of the treatments had a significant effect on root length. The observations on the number of leaves showed that for the average number of leaves parameter, the best result of administering the auxin growth regulator was with treatment P2 (2 ml NAA + 1.5 ml BAP), which resulted in 2 blades. Meanwhile, P0 (control) obtained the lowest value with an average of 1.7 blades. The effect of exogenous growth regulators in in-vitro media, whether they are

the same or different from endogenous growth regulators (in plant tissues), is determined by their content (Hussain et al., 2021). In general, the effect of exogenous BAP in the growth medium on plant growth, i.e., the number of leaves, is determined by the content of BAP growth regulators or other endogenous cytokinin groups and auxin groups, including NAA and indole-3-acetic acid (IAA). The observations revealed that the administration of MS medium + 2 ml NAA + 1.5 ml BAP resulted in a significant effect on leaf length compared to other treatments and the control. Meanwhile, none of the treatments had a significant effect on leaf width.

The increase in leaf length growth was due to the acceleration of cell division and the encouragement of the differentiation process (Table 2). Cell division requires high energy, which is obtained from auxins, cytokinins, and other nutrients. Energy in the form of ATP, which results from the respiration process, is used to synthesize essential compounds, such as proteins, carbohydrates, fats, and others. These compounds are needed for the process of cell division that occurs in apical meristems and intercalary meristems. BAP plays an important role in the direct formation of somatic embryogenesis (Lan, Hong, Huang, Chang, & Lin, 2009). The observation results of plantlet weight (grams) showed the highest average plantlet weight for P2 (2 ml NAA + 1.5 ml BAP) with the highest value of 1 gram (Table 2). Meanwhile, the lowest result was achieved by P0 (control) with an average value of 0.6 grams. This was because the administration of growth regulators stimulated growth and development in the plantlets. The formed plantlets are influenced by the presence of auxins, both endogenous and exogenous. Cytokinins also affect the proliferation of shoots, so that callus can develop, and the total fresh weight produced increases. In the practice of tissue culture, cell differentiation is determined by the balance between auxins and cytokinins. Stem elongation occurs due to the process of division, elongation, and enlargement of new cells that occurs in the apical meristem and stem segments, which causes the plant to increase in height. The administration of BAP in the culture media will affect tissue growth. BAP growth regulators have the properties of being more easily absorbed by plant cells, not breaking down easily, and functioning to promote morphogenetic activity (Ling, Tan, & Hussein, 2013).

5. CONCLUSION

This study concluded that the administration of auxin and cytokinin growth regulators had a significant effect on the parameters of germination age and plantlet weight, but it had no significant effect on the parameters of plant height, number of roots, root length, and number of leaves. This was due to the influence of the given growth regulator concentrations, which play an important role in plant development.

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