JUATIKA

JURNAL AGRONOMI TANAMAN TROPIKA
VOL. 7 NO. 2 May 2025

DOI :https://doi.org/10.36378/juatika.v7i2.4264
eissn 2656-1727
pissn 2684-785X
pages : 364 – 371

RESEARCH ARTICLE Open Access

The Effect of Growth Regulatory Hormones with Various Concentrations on the Growth of Bentong Ginger Plants Through Tissue Culture

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Abstract

The ginger plant is known for its healing and warming properties because it contains various bioactive compounds that provide numerous therapeutic benefits. However, the quality of ginger production has declined due to disease infections. To address this issue, tissue culture techniques are being employed. The purpose of this study was to obtain high-quality ginger planting materials. This research was conducted at MARDI Malaysia, utilizing a completely randomized factorial design consisting of 2 factors. The first factor is hormones comprised of five levels, namely H 1 = GA 3, H 2 = IAA, H 3 = IBA, H 4= NAA and H 5 = BAP The second factor of hormone concentration, consists of five levels, K 1 = control, K 2 = 0.5 ppm, K 3 = 1 ppm K 4 = 1.5 ppm and K 5 = 2.5 ppm. The results of the study showed that the treatment of NAA concentration2.5 ppm had a significant effect on plant height, GA 3 treatment at a concentration of 0.5 ppm, IAA at a concentration of 2.5 ppm, IBA and BAP at a concentration of 1 ppm had a significant effect on the number of leaves, GA 3 treatment at a concentration of 0.5 ppm, IAA at a concentration of 2.5 ppm, IBA and BAP at a concentration of 1 ppm had a significant effect on the number of leaves,0.5 and 1ppm had a significant effect on root length, NAA treatment at concentrations of 0.5 and 1ppm had a significant effect on the number of roots, GA 3 treatment at concentrations of 0.5 ppm had a significant effect on wet weight. root. Hormone GA 3giveinfluenceWhichmoregood for the growth of ginger explants compared to auxin and cytokinin hormones.

Keywords: Concentration, Ginger, Hormone, Plant

1. Introduction

Ginger is widely grown in Asia, Europe, and the Middle East for its desirable properties as an herb or spice, and it is also used in traditional medicine. Bentong ginger (Zingiber officinale Roscoe) is known as the "King of Ginger" and is the most sought-after variety cultivated in the lowland areas. tall with clear water and fertile soil in the Bentong region (3.5222° LU, 101.9104° BT) Because taste and nutritional value are superior to other varieties. Its shape horn, colored yellowish, branched, and has a scaly structure with a spicy, lemon-like aroma (Mustafa and Chin 2023). Ginger rhizome contains various types of substance nutrients that are beneficial for the body, including energy, carbohydrates, fiber, protein, sodium, iron, potassium, and vitamins C. Besides, In addition, ginger rhizomes also contain magnesium, phosphorus, zinc, folate, vitamin B6, vitamin A, riboflavin, and niacin. At the moment, utility ginger is better for material food, beverages and cosmetics. Besides That, ginger also exported in fresh form and essential oils. The increasing demand for ginger is in line with the increasing development of industry traditional and other industries using ginger as the raw material and demand from abroad (Sari and Nasuha, 2021)

The results of Ahfani et al.'s research (2024) show that ginger has the potential to be a natural therapeutic agent in the treatment of various health conditions, including digestive problems, inflammation, cancer, and infections. Although ginger has extensive health benefits, further research in the field of toxicology is needed to ensure its long-term safety. With a deeper understanding of ginger phytochemicals, it is expected to continue to be developed as an effective and safe natural medicine. Ginger rhizomes can be used in traditional medicine and phytopharmaceuticals because the existence of gingerol,

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overcomes bone pain (the presence of active ingredients from the extract) In the study. Ginger is not only used as a spice and medicine but also as food, beverage and cosmetic ingredient. The active ingredients in ginger, especially essential oils, gingerol, shogal and zingerone, can be used as standardized herbal medicines and phytopharmaceuticals (Aryanti et al., 2015).

Ginger seeds are usually obtained from rhizome cuttings. Propagating ginger in this way requires a large number of seeds. Enormous, ranging between 2 to 3 tons/ha for old ginger plants to around 5 to 6 tons/ha for young ginger plants. The main problem in ginger cultivation is the high number of pest attacks and diseases such as Phyllosticta leaf spot, storage leaf rot, and bacterial wilt or rot. Rhizome is caused by bacteria living in the soil. Therefore, ginger producers currently use biotechnology equipment to produce pest and disease-free planting materials for high-quality and productive ginger production. Different parts of the plant, such as rhizome buds, shoot tips, leaf bases, and Inflorescences, can be selected as potential explants for micropropagation. Although methods for controlling bacterial wilt disease in ginger plants are not yet available, sources of resistance genes to this disease have been available. Improvement The resistance of ginger cultivars to bacterial wilt disease through conventional crossing cannot be carried out, due to low pollen fertility (<40%) and incompatibility between interspecies.

One of the efforts to eliminate disease attacks can be using disease-free seed sources. Large quantities of disease-free seeds can be obtained from propagation using tissue culture techniques (Mosie, 2019) and. According to Suharti et al. (2011), tissue culture of bacterial wilt disease Ralstonia solanacearum in ginger plants, can increase the resistance of ginger plants to wilt disease by 100%, and 4 other isolates have varying abilities in suppressing the disease. withered bacteria namely 66.5-83.3%. In addition to traditional propagation methods, ginger can be propagated vegetatively through tissue culture techniques. This approach offers several advantages, including shorter seedling times, large-scale seedling production, and disease control (Fika et al., 2025)

The use of hormones in ginger plant explant cultures to accelerate growth has been proven in several studies. Results Mawaddah et al.'s (2021) research shows that shoot growth and roots on ginger plant shoot explants are due to the addition of NAA (auxin) in vitro culture. The combination of NAA administration is able to accelerate growth optimally. According to (Almafri 2021), the Administration of Benzyl amino purine (BAP) at various levels of concentration on the micropropagation of ginger flowers has a significant effect on the parameters of the percentage of explants forming shoots, the number of shoots and the height of shoots at the ages of 2, 3, 4, 5, 6 MST. According to Ardiana and Advinda (2022), IAA is a natural auxin phytohormone and acts as a plant growth stimulant because it can regulate many critical physiological processes, including cell division and development, cell differentiation and protein synthesis. And in the research results of Abbas et al . (2011) explained that in the micropropagation of Hedychium spicatum (Zingiberaceae), the percentage of rooting (80%) was reported on MS media with l IAA, and in the results other that use IAA or IBA in the culture media affects root induction in plant shoots. According to, the type of cytokinin increases shoot multiplication red ginger in vitro. The best cytokinins were obtained at a concentration of 1 ppm which can increase the multiplication of ginger red in vitro. The interaction between the type and concentration of cytokinins only affects the length of shoots. According to (Almafri 2021), the administration of Benzyl amino purine (BAP) at various concentration levels to the micropropagation of flower ginger has a significant effect on the parameters of the percentage of explants forming shoots, the number of shoots and the height of shoots at the ages of 2, 3, 4, 5, 6 MST, IBA Also can help in the formation of shoots but IBA concentration more classified as in the formation of roots by providing high concentration levels. According to Saljuna et al. (2023), the provision of GA 3 (Gibberellin) increases the growth of ginger plants due to the role of GA 3 in accelerating cell division and elongation, which helps the internodes and stems to grow longer. In this study, the effect of growth regulators on the height of ginger plants was found to be significant. Stem elongation caused by the application of GA 3 may be due to increased mitotic activity, possibly through increased auxin production in the meristematic region. Auxin and gibberellin stimulate the development of vascular tissue and encourage cell division. In addition, it also promotes the growth of new roots (Arifin et al., 2022)

Previous research on administering Plant Growth Regulators (PGRs) to ginger plants has shown that auxin and cytokinin accelerate plant growth and propagation in vitro with smaller doses. So this study tested five different hormones with the addition of gibberellin and varying doses for optimal results. In addition, this study was conducted in Malaysia with a tropical climate of 1,400 meters above sea level.

Increasing global demand for ginger and challenges in cultivation. This study is to obtain the correct and optimal concentration of ZPT to obtain superior seeds in multiplying and producing ginger plants through tissue culture.

2. Material and Methods

2.1. Location and time of research

This research was conducted at the Malaysian Agricultural Research and Development Institute (MARDI), located in Serdang, Malaysia, with coordinates 2°58'52.3"N 101°41'55.0"E, at an altitude of 1,400 meters above sea level. The research took place in September 2024.

2.2. Materials and tools

The materials used are bentong ginger shoots, agar, distilled water, 70% ethanol, matches, bunsen burner, brown paper, paper label, shelves, tissue, aluminum foil, plastic wrap, auxin hormone (NAA, IBA, IAA), hormone gibberellin (GA 3), cytokinin hormone (BAP), sterile distilled water, spirits, 70% alcohol, 15% chlorox, Murashige and Skoog media (MS), NaOH 1 N, HCl 1 N.

The tools used are autoclaves, glassware, hot plates, stove, Laminar Air Flow Cabinet (which is a tool that is useful for working aseptically because BSC/LAF has a pattern of air flow settings and filters so that it becomes sterile and UV light applications several hours before use) filter paper, magnetic stirrer, pH indicator, tweezers, thermohygrometer, refrigerator, knife, scalpel, measuring pipette, scales analytic And tool other that supports research. The ideal temperature for tissue culture is 20°C to 25°C. Higher or lower temperatures can affect the growth or survival of the culture. The room's relative humidity is between 60% and 80% to avoid dehydration in tissue culture.

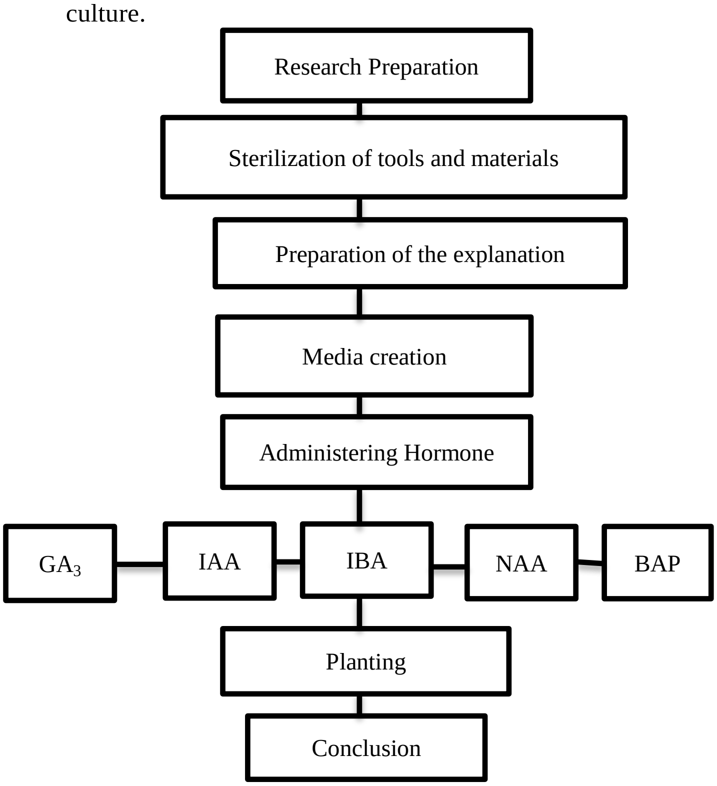

Figure 1. Research flow diagram

2.3. Research Implementation

This study used a completely randomized factorial design consisting of 2 factors, namely the first factor is growth hormone composed of five levels, namely H 1 = GA 3 , H 2 = IAA, H 3 = IBA, H 4 = NAA and H 5 = BAP The second factor is the concentration of hormones consisting of five levels, namely K 1 = control, K 2 = 0.5 ppm, K 3 = 1 ppm K 4 = 1.5 ppm and K 5 = 2.5 ppm. The combination of treatments is 25 treatments, with 2 repetitions and 50 experimental units are obtained.

2.4. Preparation and sterilization of media

The germinated ginger meristematic shoots were collected and cleaned under running tap water for one hour. Further explants were cleaned with a commercial laboratory detergent, namely Decon 5% (v/v), and the explants were rinsed thoroughly. Steps followed by immersion of the explants in 1% (v/v) fungicide for one hour, and after that, the submerged explants were washed under running tap water for five minutes. Next, the explants underwent surface sterilization, in which 10–20% Clorox with a few drops of Tween 20 was added to the flask containing the explants under sterile conditions. The explants were then rinsed with sterile distilled water several times to ensure thorough cleaning. Meristem sections were obtained by removing the outer layer of leaf sheaths from the explants with a sterile scalpel under aseptic conditions. The sterile meristems were then inoculated into Murashige and Skoog (mso) medium containing 3% sucrose and 3.0 mg/L. The pH of the medium was adjusted to 5.8 before autoclaving (15 min, 121℃). The cultured flasks were wrapped with parafilm to prevent contamination and incubated in a culture chamber under white, fluorescent light with a light intensity of 3000 lux and a photoperiod of 16 h at 25 ± 2°C.

2.5. Explant Planting

The explants used are shoots from ginger rhizomes. The planting process begins by removing the shoots from the cotton media. Next, the shoots are placed in a petri dish and the ginger rhizome shoot explants are cut. The size of the leaf explants used is ± 1x1 cm. The leaf explants are then planted in the media according to the specified treatment. One bottle is planted with 5 ginger rhizome shoot explants. Explants planted in the medium are labeled with the planting date and the samples studied will be labeled.

2.6. Statistical Analysis

One-way ANOVA was used to analyze interactions and differences in treatment in data using. Using Measurable software Bundle for the Social Sciences (SPSS version 20.0), information Significance between means was tested using the Multiple Range Test. Duncan with $p \leq 0.05$. As many as 2 repeat times are used for each treatment in the analysis

3. Results and Discussion

3.1. Plant Height

A statistical analysis of variance was performed on the hormone administration and concentration as well as the interaction between these two treatments. This analysis revealed a significant effect on the height of ginger shoot explants, as illustrated in Table 1 and Figure 2.

Table 1. Average Height of Ginger Plant Explants with Various Types of Hormones and Concentrations at 8 MST.

Treatment	**Concentration (ppm)**					**Average**
	K_1	**K_2**	**K_3**	**K_4**	**K_5**	
			Cm			
H_1	8.0 ± 3.0 ^{c}	9.3 ± 3.5 ^{e}	9.0 ± 2.5 ^{e}	10.0 ± 3.5 ^{g}	9.0 ± 3.0 ^{e}	9.06 ^{d}
H_2	7.51 ± 1.58 ^{b}	7.0 ± 2.5 ^{a}	10.0 ± 3.8 ^{g}	9.0 ± 2.6 ^{e}	9.5 ± 2.5 ^{f}	8.6 ^{b}
H_3	8.0 ± 2.8 ^{c}	8.0 ± 3.1 ^{c}	8.2 ± 2.5 ^{c}	8.3 ± 3.0 ^{cd}	8.0 ± 1.8 ^{c}	8.1 ^{a}
H_4	7.5 ± 2.5 ^{b}	9.0 ± 3.0 ^{e}	8.0 ± 2.5 ^{c}	8.4 ± 1.5 ^{d}	**11.3** ± 4.1 $^{\mathbf{i}}$	8.84 ^{c}
H_5	8.0 ± 2.5 ^{c}	7.8 ± 2.2 ^{bc}	8.5 ± 2.4 ^{d}	9.8 ± 3.0 ^{g}	10.0 ± 3.1 ^{g}	8.82 ^{c}
Average	7.8a	8.22 ^{b}	8.74 ^{c}	9.1 ^{d}	9.56 ^{e}	8,684

Description: The same letters indicate results that are not significantly different at the 5% level based on Duncan's further test. Treatment H (type of hormone) and K (concentration).

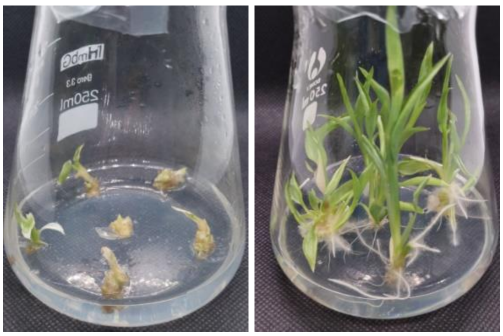

Figure 2. Plant height of Bentong ginger shoot culture with NAA treatment at 2 MST (left) and at 8 MST (right).

The combination of two treatments H 4 K 5 showed the highest average plant height at the age of 8 MST, 11.3 cm, the lowest average in the combination of H 2 K 2 treatments , namely 7 cm. growth of ginger shoot explant culture. Figure 1 shows the growth of plant height Ginger plant explants with the administration of NAA hormone can increase plant height growth, which plays a role not only as a nutrient supplement but also as a stimulant of morphogenesis growth in cells, tissues and organs. The addition of hormone to the media changes the permeability wall cells, which allows water enter the cell through diffusion (Rikardo et al., 2019) Ginger plant explants that are in direct active contact with MS media supplemented with hormones will receive sufficient nutrition so that they will stimulate cell division in ginger plant explants. This is following the opinion of Ngadiani and Jayanti (2021) that the auxin hormone (NAA) can play a role in stimulating cell division which results in the growth of new shoots in plants and induces root growth. In the research results of Mawaddah et al. (2021) the results of the study showed that the administration of plant growth regulators NAA 0.1, and NAA 15 mg/l.

The increasing concentration of NAA given, the increasing formation of shoots. Arif et al., (2017) added that if there is a balance between auxin, cytokinin and gibberellin, it will cause a balance in the formation of organs between roots, stems and leaves, thus providing the largest number of shoots. Plant hormones, or plant growth regulators, are organic compounds that are important in regulating plant growth, inhibition and movement.

3.2. Number of Leaves

The statistical analysis of the variance of hormone administration and concentration and the interaction of the two treatments showed a significant effect on the number of leaves of ginger shoot explants (Table 2, Figure 3).

Table 2. Average Number of Leaves of Ginger Plant Explants with Various Types of Hormones and Concentrations at 8 MST.

Types of hormones	**Concentration (ppm)**					**Average**
	K_1	**K_2**	**K_3**	**K_4**	**K_5**	
			Strands			
H_1	4.0 ± 3.0 ^{b}	**6.0** ± 2.7 $^{\mathbf{e}}$	4.0 ± 1.0 ^{b}	5.0 ± 1.5 ^{d}	4.0 ± 1.1 ^{b}	4.6 ^{c}
H_2	4.5 ± 1.9 ^{c}	4.0 ± 2.1 ^{a}	4.0 ± 1.2 ^{b}	4.0 ± 1.2 ^{b}	**6.0** ± 2.1 $^{\mathbf{e}}$	4.5 ^{b}
H_3	3.5 ± 2.0 ^{a}	5.0 ± 3.5 ^{d}	**6.0** ± 3.1 $^{\mathbf{e}}$	5.0 ± 2.1 ^{d}	4.0 ± 0.58 ^{b}	4.7 ^{d}
H_4	4.0 ± 1.8 ^{b}	4.0 ± 1.5 ^{b}	4.0 ± 0.8 ^{b}	5.0 ± 1.6 ^{d}	4.0 ± 0.1 ^{b}	4.2 ^{a}
H_5	4.5 ± 2.0 ^{c}	4.0 ± 0.5 ^{b}	**6.0** ± 2.9 $^{\mathbf{e}}$	5.0 ± 1.0 ^{d}	5.0 ± 1.5 ^{d}	4.9 ^{e}
Average	4.1 ^{a}	4.6 ^{b}	4.8 ^{c}	4.8 ^{c}	4.6 ^{b}	4.58

Description: The same letters indicate results that are not significantly different at the 5% level based on Duncan's further test. Treatment H (type of hormone) and K (concentration).

The study results showed a real interaction between the type of hormone and the concentration of the hormone applied. The interaction was shown in the parameter of the number of ginger plant leaves, hormones with the right concentration will have a good effect on the plant. Still, if excessive hormones are given, it can be an inhibitor for the plant; as seen in Table 2 above from the treatment of 5 hormones, only the NAA hormone did not have a significant effect; this is thought to occur because the dose given in the H4 (NAA) treatment is still insufficient or excessive. Although hormones are very important for normal plant growth and development, excess hormones can also cause problems. Both in natural conditions and due to excessive external applications, excess hormones can cause various negative effects on plants, according to Susanti (2011). Administration of hormones in appropriate concentrations can increase plant morphogenesis, but if growth regulators are given in excessive concentrations, they will inhibit plant morphogenesis growth.

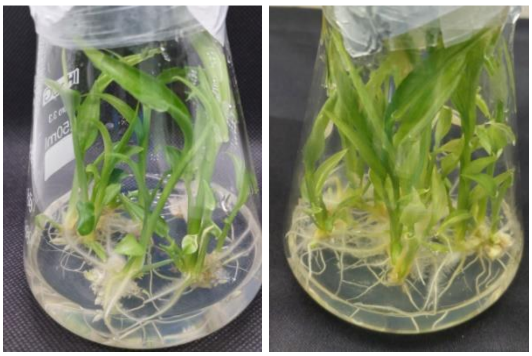

Figure 3. The number of leaves of Bentong ginger shoot culture at the age of 8 MST was lowest at H 3 K 1 (left) and the highest at H 3 K 1 (right).

Based on Table 2, it can be seen that the average number of leaves of ginger plant explants was highest in the treatments H 1 K 2 , H 2 K 5 , H 3 K 3 and H 5 K 3, with an average of 6 leaves per explant plant, this treatment is significantly different from other treatments. This shows that the hormone given at the right concentration can stimulate plant growth leading to leaf formation. Leaves are where the photosynthesis process takes place, producing carbohydrates as a source of plant energy. The more leaves that appear on the explant, the better the explant growth. Many leaves will produce a lot of photosynthate, so plant growth will improve. This follows the opinion of Khalil et al., (2021). Plant growth regulators affect plant physiology and thus create positive and negative impacts. Endogenous hormones in some plants can be used as natural ZPTs to stimulate growth and development in other plants. Karyanti et al . (2021) cytokinins (BAP, IAA and IBA) in this study were able to form shoots and leaves faster; the number of shoots and leaves was greater after being combined with several concentrations. Cytokinins with high concentrations reduce shoot length. Cytokinins do not function to stimulate shoot height but play a greater role in cell division.

3.3. Root Length

The statistical analysis of the variance of hormone administration and concentration and the interaction of the two treatments showed a significant effect on the root length of ginger shoot explants (Table 3, Figure 4). Based on Table 3, the longest root length treatments were the H 1 K 2 and H 1 K 3 ppm treatments with an average root length of 7 cm. While the lowest average root length was in H 4 K 4 and H 4 K 5. Root length observations were carried out at the end of the observation at 8 MST. The study results showed that the H 1 K 2 and H 1 K 3 treatments given affect the growth of root length. These results indicate that both treatments individually cause changes. The concentration of GA 3 greatly influences root length growth. The coefficient of determination of GA 3 concentration on root length at a concentration of GA 3 0.5 ppm and GA 3. These results show that the relationship between GA 3 concentration and plant growth is very strong in roots, leaves and stems.

Table 3. Average Root Length of Ginger Plant Explants with Various Types of Hormones and Concentrations at 8 MST.

Types of hormones	Concentration (ppm)					Average
	K_1	K_2	K_3	K_4	K_5	
			cm			
H_1	5.5 ± 1.4^{f}	**7.0** $\pm 3.1^{\mathbf{fg}}$	**7.0** $\pm 1.58^{\mathbf{fg}}$	6.5 ± 2.4^{f}	4 ± 1.9^{d}	6^{d}
H_2	5.5 ± 1.0^{f}	3.0 ± 1.2^{c}	3.5 ± 0.5^{cd}	2.5 ± 0.5^{bc}	2.5 ± 0.3^{bc}	1.7^{b}
H_3	5.5 ± 2.1^{f}	3.0 ± 0.3^{c}	3.0 ± 1.2^{c}	3.0 ± 0.9^{c}	2.0 ± 0.5^{ab}	1.65^{b}
H_4	4.5 ± 1.7^{de}	2.0 ± 1.0^{ab}	2.5 ± 0.4^{ab}	1.5 ± 0.2^{a}	1.5 ± 0.1^{a}	1.2^{a}
H_5	6.5 ± 3.0^{i}	6.0 ± 2.1^{h}	6.0 ± 2.0^{h}	5.0 ± 1.8^{e}	4.0 ± 1.0^{d}	2.75^{c}
Average	5.5^{d}	4.2^{c}	4.4^{c}	3.7^{b}	2.8^{a}	4.12

Description: The same letters indicate results that are not significantly different at the 5% level based on Duncan's further test. Treatment H (type of hormone) and K (concentration).

According to Asra et al ., (2020) who said that Gibberellin (GA 3) is one of the growth regulators that plays a physiological role in plants by stimulating plant growth, stimulating seed germination and dormant bud growth, flowering, transporting food and mineral elements to young sprouts in food reserves. According to (Chien et al., 2023) GA 3 treatment forces germination is not very effective but has a very good effect on increasing ginger growth results.

Based on the root length data obtained the highest in the H1K2 and H1K3 treatments with an average of 7 cm. It is suspected that gibberellin (GA3) in MS media can support root elongation in bentong ginger shoots and concentration, sufficient to increase root length so that additional gibberellin hormone concentration is no longer needed.

Figure 4. The length of the shoot culture roots of bentong ginger plants at the age of 8 WAP was highest in the H 1 K 2 treatment (left) and lowest in the H 1 K 3 treatment (right).

According to Saljuna et el (2023). Plant growth is accelerated by GA3, which also accelerates cell division and elongation, thereby helping plant growth. Gibberellin hormone occurs in young leaves (main site), shoot tips, root tips and immature seeds (embryos) and gibberellin precursors, the results of the study by Saljuna et el., (2024) showed that the administration of GA3 at 100 and 150 ppm recorded the highest plant height, leaf length, root length, clump length, and clump area so that it stimulates growth significantly increased by 36.4%, in this study using a lower concentration than before.

3.4. Number of Roots

The statistical analysis of the variance of hormone administration and concentration and the interaction of the two treatments showed a significant effect on the number of ginger plant shoot roots (Table 4, Figure 5).

Table 4. Average Number of Roots of Ginger Plant Explants with Various Types of Hormones and Concentrations at 8 MST.

Treatment	Concentration (ppm)					Average
	K_1	K_2	K_3	K_4	K_5	
			strands			
H_1	3.5 ± 0.5 ^{cd}	3.0 ± 1.0 ^{c}	3.0 ± 0.3 ^{c}	2.0 ± 0.1 ^{b}	1.0 ± 01 ^{a}	2.5 ^{a}
H_2	3.0 ± 1.2 ^{c}	5.0 ± 1.5 ^{e}	4.0 ± 1.0 ^{cde}	4.0 ± 1.2 ^{cde}	1.0 ± 0.2 ^{a}	3.2 ^{b}
H_3	4.5 ± 0.5 ^{de}	6.0 ± 1.2 ^{f}	6.0 ± 2.5 ^{f}	5.0 ± 0.58 ^{e}	4.0 ± 1.2 ^{cde}	5.1 ^{d}
H_4	6.0 ± 1.5 ^{f}	**9.0** ± 4.1 $^{\mathbf{h}}$	**9.0** ± 3.5 $^{\mathbf{h}}$	8.0 ± 2.5 ^{g}	4.0 ± 1.5 ^{cde}	7.2 ^{e}
H_5	6.5 ± 2.5 ^{fg}	6.0 ± 1.0 ^{f}	5.0 ± 1.7 ^{e}	5.0 ± 1.5 ^{e}	1.0 ± 0.1 ^{a}	4.7 ^{c}
Average	4.7 ^{b}	5.8 ^{d}	5.4 ^{c}	4.8 ^{b}	2 ^{a}	4.54

Description: The same letters indicate results that are not significantly different at the 5% level based on Duncan's further test. Treatment H (type of hormone) and K (concentration).

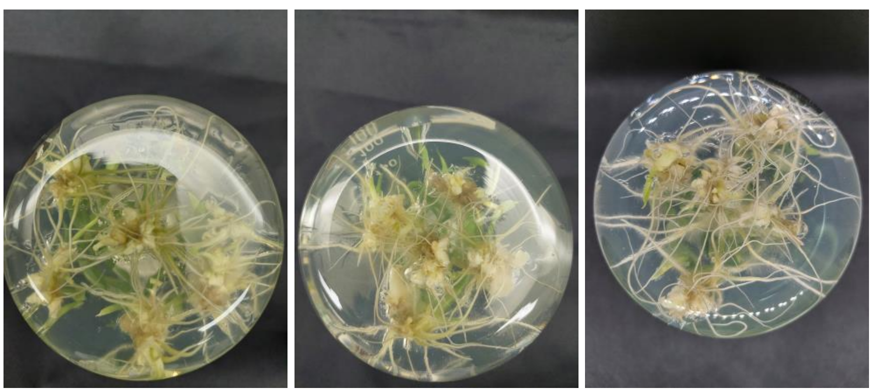

Figure 5. Number of roots of ginger shoot culture in H4K2 treatment at 8 MST (left) H4K3 treatment (middle) H4K4 treatment (right).

In Figure 5, the roots of the ginger plant shoots grow well and quickly. This result is because the administration of NAA can stimulate root formation. NAA is the right choice to stimulate roots because NAA can stimulate the growth of new shoots and roots auxin is found in the tips of young shoots and in the meristem tissue at the tip of the root; this hormone functions as a regulator of cell enlargement and triggers cell extension in the area behind the tip meristem and helps the growth process. The addition of Auxin (NAA) has an effect on the tissue development process, which will directly affect the overall growth rate and development. In addition, the amount of nutrients plants absorb is generally always directly proportional to the plant's growth rate and dry weight (Rikardo et al., 2019). The research results from Zahid et al. (2021) stated that NAA produces a sufficient number of roots per plant and plants rooted in this culture are successfully acclimatized in ex vitro conditions. After proper acclimatization, the 'Bentong' ginger plant was raised in vitro and successfully grew 100%.

Based on the root number data in Table 4, the largest number of roots was produced in the H4K2 treatment, with an average of 9 roots per explant. This result shows that a higher auxin concentration than cytokinin can cause tissue morphogenesis towards root formation. The growth of the number of roots is better when compared to several other treatment combinations. This proves that the cells in the roots have sufficient or almost sufficient auxin for normal root growth. This follows the opinion of Mawaddah et al., (2021) who said that the growth regulator auxin has a broad effect on growth such as stimulating and accelerating root growth, as well as increasing root quantity and quality. The 7.5μm NAA treatment was more effective in inducing more roots per plant than IAA and IBA. In the study by Mawaddah et al. (2021), Observations on the percentage of root formation and root length showed that the administration of NAA and kinetin had an effect on root formation with a percentage reaching 33.33% -100%, in this study using a combination of two hormones.

3.5. Root Wet Weight

The statistical analysis of the variance of hormone administration and concentration and the interaction between the two treatments showed a significant effect on the number of ginger plant shoot roots (Table 5).

Table 5. Fresh Weight of Ginger Plant Explant Roots with Various Types of Hormones and Concentrations at 8 MST.

Treatment	Concentration (ppm)					Average
	K1	K2	K3	K4	K5	
			g			
H1	0.076 ± 0.00 ^{b}	**0.325** ± 0.08 ^{e}	0.107 ± 0.02 ^{c}	0.84 ± 0.01 ^{g}	0.066 ± 0.01 ^{b}	0.401 ^{e}
H2	0.083 ± 0.01 ^{bc}	0.037 ± 0.01 ^{a}	0.037 ± 0.01 ^{a}	0.045 ± 0.01 ^{ab}	0.048 ± 0.01 ^{ab}	0.025 ^{c}
H3	0.084 ± 0.03 ^{bc}	0.037 ± 0.01 ^{a}	0.041 ± 0.01 ^{ab}	0.037 ± 0.01 ^{cd}	0.040 ± 0.00 ^{ab}	0.023 ^{b}
H4	0.085 ± 0.01 ^{bc}	0.130 ± 0.05 ^{cd}	0.147 ± 0.03 ^{d}	0.142 ± 0.02 ^{cd}	0.136 ± 0.01 ^{cd}	0.064 ^{d}
H5	0.078 ± 0.02 ^{b}	0.031 ± 0.01 ^{a}	0.041 ± 0.01 ^{ab}	0.037 ± 0.01 ^{a}	0.032 ± 0.00 ^{a}	0.021 ^{a}
Average	0.081 ^{b}	0.112 ^{c}	0.074 ^{a}	0.220 ^{e}	0.1832 ^{d}	0.134

Description: The same letters indicate results that are not significantly different at the 5% level based on Duncan's further test. Treatment H (type of hormone) and K (concentration).

Based on the data presented in Table 5, it is evident that the average wet weight of ginger plant roots in the H1K2 treatment significantly differs from that of the other treatments, with a total root weight of 0.325 grams. This finding suggests that the GA3 hormone, at a concentration of 0.5 ppm, is adequate, and there is no need to increase the concentration in ginger shoot culture. When administered at the appropriate concentration, the hormone can facilitate cell division, thereby increasing the number of roots and, consequently, the wet weight of ginger plant roots. This aligns with the assertion made by Wahyuningsih (2020) that gibberellin is a plant growth regulator (ZPT) proven to enhance plant growth and yield. Gibberellin is also a chemical substance produced in developing tissues, such as leaves, branch primordia, root tips, and seeds. According to Murni et al. (2024), the increase in wet weight is attributed to the rise in cell numbers and the influx of water from the media into the cells, resulting in cell enlargement. Water enters the cells due to increased permeability, which is accompanied by a decrease in cell wall pressure influenced by auxin, leading to an increase in cell volume. Callus cell growth occurs due to enhanced cell division driven by hormonal influence.

4. Conclusion

The results indicated that NAA treatment at a concentration of 2.5 ppm significantly affected plant height. Additionally, NAA treatment at a concentration of 0.5 ppm had a notable impact on the number of leaves, the number of roots, and the wet weight of the roots. Furthermore, GA3 treatment at a concentration of 1 ppm significantly influenced root length. IAA at a concentration of 2.5 ppm, along with IBA and BAP at a concentration of 1 ppm, also significantly affected the number of leaves. Overall, the GA3 hormone demonstrated a more pronounced effect on ginger explants' growth than the auxin and cytokinin hormones.

Acknowledgment

Thanks to the Dean of the Faculty of Agriculture, Muhammadiyah University of North Sumatra and the Deputy Chairperson of the Board of Directors of MARDI (Malaysian Agricultural Research and Development Institute) who have provided the opportunity to conduct research in the PJT Biotechnology and Nanotechnology laboratory.

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