

The effectiveness of naphthaleneacetic acid and kinetin on the development of plantlet book cuttings of *Chrysanthemum* ornamental plants



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Abstract. The high demand for *chrysanthemum* flowers, coupled with the challenge of acquiring quality seeds in large quantities within a short timeframe, presents significant problems in this study. One potential solution is to propagate plants and utilize plant growth regulators to accelerate their development. Tissue culture techniques, which are essential in agriculture and biotechnology, are employed to produce new plants by isolating, developing, and duplicating specific plant cells or tissues in the laboratory. This study aims to assess the effects of two plant growth regulators Naphthalene Acetic Acid (NAA) and Kinetin on the growth and development of stem cuttings or nodes from ornamental *chrysanthemum* plants (*chrysanthemum*) in vitro using MS media. The research utilized a completely randomized design (CRD) factorial approach featuring two treatment factors: NAA and Kinetin. A total of nine treatment combinations were tested, with NAA concentrations of 0.5, 1.0, and 1.5 ml L⁻¹, and Kinetin concentrations of 1, 2, and 3 ml L⁻¹. Each treatment was repeated three times. Observations included measurements of plant height, leaf count, root count, and wet weight. The best results for each parameter were observed in treatment K₃N₂, which yielded a plant height of 30.73 mm and an average of 18.33 leaves. The optimal results for root count and wet weight were found in treatment K₃N₃, which produced an average of 13.83 roots and 3.57 grams, respectively. Overall, increasing the concentrations of NAA and Kinetin positively influenced all parameters evaluated.

Keywords: *Chrysanthemum* Ornamental Plants; Growth regulator; Kinetin; Naphthaleneacetic Acid

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflora* Tzelev syn.) is an ornamental flowering shrub commonly known as the *chrysanthemum* or golden flower. It originates from the plains of China. *chrysanthemum* plants from China and Japan were introduced to Europe, specifically France, in 1795. By 1808, Colvil from Chelsea had developed eight varieties of *chrysanthemum* in England. In the 17th century, *chrysanthemum* began to be introduced to Indonesia, and since 1940, they have been cultivated commercially in the country. Furthermore, *chrysanthemum* are the second most economically significant floricultural crop globally and are among the most important ornamental plants [1].

Due to the increased demand for decorative plants, companies who supply them might expect significant financial gains. *Chrysanthemums* are one of the most often used ornamental plants. The demand for *chrysanthemums* rises by 25% annually in Indonesia; even market demand has increased by 31.62%.

Chrysanthemum exports to foreign countries such as the Netherlands, Brunei, Singapore, Japan, and The United Arab Emirates (UAE) reached 1.44 million stalks. This high market demand means that *chrysanthemum* plants have bright prospects for development both now and in the future [2]. One challenge with traditional procurement methods for high-quality seeds is the difficulty in quickly acquiring large quantities. However, a key advantage of plant propagation through tissue culture techniques is the ability to rapidly produce substantial amounts of planting material [2].

Tissue culture, also known as controlled culture, is the process of growing plant parts—such as cells, tissues, or organs—in vitro (in a controlled environment) under aseptic conditions. This method utilizes artificial media that contains all the essential nutrients and growth regulators, along with carefully managed temperature and lighting conditions [3]. Various types of basic media are used in tissue culture techniques. Murashige and Skoog (MS) media contain sufficient inorganic nutrients



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to satisfy the needs of many types of plant cells in culture [4].

In tissue culture, two essential groups of hormones and plant growth regulators (PGRs) are cytokinins and auxins. The auxin group includes IBA (Indole-3-butyric acid), IAA (Indole-3-acetic acid), NAA (Naphthaleneacetic acid), and 2,4-D (2,4-Dichlorophenoxyacetic acid). The cytokinin group consists of Kinetin, Zeatin, and BA (Benzyladenine). Auxins primarily promote cell elongation, callus formation, and the development of adventitious roots, while they also inhibit the formation of axillary shoots [5].

The role of auxin in cell development indicates that auxin can enhance protein synthesis. This increased protein synthesis allows it to be utilized as a growth-promoting energy source [6]. Growth regulators, such as naphthalene, can be derived from fruit, making them safe for potential applications in food and safety analysis [7]. Auxin-binding protein formulas combined with air-stabilized lipid films can create portable sensors that quickly detect naphthalene acetic acid levels in fruits and vegetables.[8]

Plant root growth can be stimulated by a rooting hormone called auxin. This hormone is effective in enhancing root initiation, increasing both the quantity and quality of roots, promoting consistent rooting, and improving the success rate of root establishment. Due to its stability, NAA is commonly used as a hormone to promote root elongation [9].

Auxins and cytokinins are two essential hormones that play a crucial role in plant growth. Although they both regulate physiological functions in plants, their roles are distinct; they can enhance each other's effects at times but may also contradict one another. For healthy plant growth, a balance between auxin and cytokinin is necessary. Cytokinins promote shoot growth, while auxins tend to encourage root growth. Together, these two hormones regulate the overall growth of plants. For example, a higher concentration of auxin can lead to stronger roots, whereas increased cytokinin can result in more robust shoots. Additionally, this balance affects how plants respond to stressors such as drought or pest infestations [5].

The combination of BAP and NAA growth regulators is considered the most effective for promoting leaf and root production in *Atropa acuminata* Royal ex Lindl plants [9]. Additionally, the use of growth regulators can enhance the overall weight of the ginseng plant or cell biomass (*Panax vietnamensis*) [10].

Cytokinins play a role in promoting the growth of leaf buds and the multiplication of cells in a type of tissue known as explants. However, when applied to stimulate shoot growth in tissue culture or on mature plants, the results are often not optimal for adult plants [11]. Kinetin (6-furfuryl amino purine) is a type of cytokinin that regulates cell division and morphogenesis. In tissue growth, cytokinins and auxin interact to affect tissue differentiation in transgenic tobacco plants [12]. Auxins and cytokinins are known to either cooperate or conflict

in regulating important developmental processes, such as the establishment and maintenance of meristems [5].

This study aims to determine the appropriate concentrations of NAA and Kinetin, as well as their interaction, on *chrysanthemum* shoot growth in MS media.

METHODOLOGY

Materials

The research was conducted at the Network Culture Laboratory of the Main Seed Center for Horticulture, located in the Johor Building of the Food Crops and Horticulture Service in North Sumatra Province. The study took place from September 2022 to December 2022.

The materials used in this research included *chrysanthemum* plantlet cuttings and modified Murashige and Skoog (MS) media by adding growth regulators such as auxin (NAA) and cytokinin (Kinetin) at different concentrations. Sterilants used in the study comprised sterile distilled water, 96% alcohol, Dithane M-45, Clorox, Agrept 20 WP, HgCl₂, and detergent, as well as agar powder.

The equipment utilized included culture bottles, Erlenmeyer flasks, measuring cups, pipettes, a balance, a pH meter, an autoclave, a laminar air flow cabinet, an oven, a hot plate, a magnetic stirrer, glass stirrer rods, tweezers, scalpel knives, scissors, hand sprayers, funnels, aluminum foil, label paper, a camera, and stationery.

The study was designed as a factorial experiment using a completely randomized design (CRD) with two treatment elements: NAA and kinetin as growth regulators. There were three treatment combinations, and each was repeated three times.

Methods

Murashige and Skoog (MS) Media

Murashige and Skoog (MS) medium were prepared by adding a solution containing Fe EDTA, thiamine, 0.1 mg of pyridoxine, 0.5 mg of nicotinic acid, myo-inositol, sucrose, and agar. The acidity was adjusted to a pH of 5.8 using NaOH or HCl. The media was then sterilized by autoclaving at a temperature of 121°C to 126°C for 15 minutes [13]. Once sterilized, the media was stored on the incubation rack, making it ready for use.

Sterilization of Media Equipment

The bottles and iron tools are thoroughly washed with detergent and then soaked in a mixture of Clorox and water for 3 hours. After soaking, they should be rinsed with running water and allowed to drain. The bottles are then baked in the oven at 150°C for 4 hours. The iron tools must be wrapped tightly before being placed in the oven.

Laminar Air Flow Cabinet (LAFC)

LAFC is disinfected using 96% alcohol by wiping the inner surface of the laminar flow hood with a cotton or tissue that has been sprayed with 96% alcohol.

Additionally, 96% alcohol is sprayed around the LAFC and then subjected to UV light for 60 minutes.

Preparation and Planting of Plantlets

The plantlets used in this experiment are sterile and derived from *chrysanthemum* cuttings previously grown in vitro culture. The plantlets were obtained from the UPT for Horticultural Parent Seeds of the Food Crops and Horticulture Service of North Sumatra Province. Each plantlet is cut from nodes one through four, measuring 1.5 to 2 cm in length and having 2 to 3 leaves. These plantlets are cultivated in media treated with NAA and Kinetin. Each culture bottle contains one *chrysanthemum* plantlet and is sealed with a plastic cap. The bottles are then placed in a culture/incubation room for observation over a period of eight weeks. After this period, the culture bottles are transferred to a growth room that features 24-hour lighting provided by an 80-watt incandescent lamp. The growth room maintains a temperature of 18 to 21°C and humidity levels of 60 to 80%. The plantlets are kept in this environment for a duration of three months.

Parameters

Observations were conducted by measuring the height of the plantlets and counting the number of roots and leaves, as well as recording their wet weight. These observations took place two weeks after planting and continued every two weeks until the plants reached eight weeks of age. The total number of roots was counted at the end of the study.

Data Analysis

The first factor, NAA, included three levels: N₁ (0.5 ml L⁻¹ water), N₂ (1 ml L⁻¹ water), and N₃ (1.5 ml L⁻¹ water). The second factor, kinetin, also had three levels: K₁ (1 ml L⁻¹ water), K₂ (2 ml L⁻¹ water), and K₃ (3 ml L⁻¹ water). Observations recorded in the study included plantlet height, number of leaves, number of roots, and wet weight

RESULTS AND DISCUSSION

Plantlet Height Analysis (mm)

The addition of kinetin to the growing medium stimulates cell division (cytokinesis), leading to an increase in the number of cells. Divided cells will absorb more water, which causes cell enlargement and elongation, resulting in increased culture height [15,16].

In contrast, auxin, particularly at high concentrations or when interacting with other hormones, can inhibit plant growth. This inhibition often occurs through apical dominance, increased ethylene production, or an imbalance with other growth hormones. However, in this study, a balanced concentration of cytokinin (kinetin) and auxin (NAA) was found to enhance plant growth by stimulating cell elongation, maintaining apical dominance, collaborating with gibberellin, promoting cell division in the meristem, and increasing the differentiation of vascular tissue.

Table 1. Plantlet height when given NAA and kinetin

Treatment Kinetin (ml L ⁻¹ water)	NAA (ml L ⁻¹ water)			Average
	N ₁ (0.5mL)	N ₂ (1mL)	N ₃ (1.5mL)	
K ₁ (1 mL)	20.33	23.17	26.33	23.28b
K ₂ (2 mL)	23.00	23.83	26.33	24.39b
K ₃ (3 mL)	28.00	30.73	28.50	29.08a
Average	23.78	25.91	27.06	

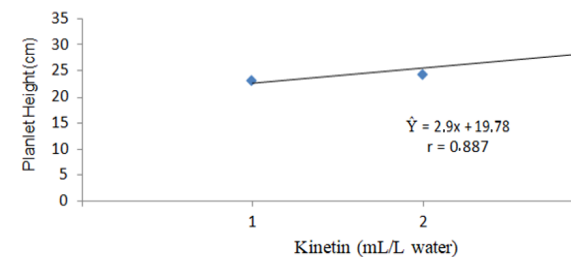


Figure 1. Relationship between Kinetin and *Chrysanthemum* Plantlet Height

Increasing auxin concentrations up to 1.5 ml L⁻¹ positively interacted with kinetin, resulting in an increase in plant growth rates up to 30.73 mm at the K₃N₂ treatment level. These findings contradict previous studies that indicated an increase in auxin and cytokinin concentrations up to 10 mg L⁻¹ reduced plant height from 4.5 mm to 0.2 mm [16].

Figure 1 illustrates the positive relationship between the height of *chrysanthemum* shoots and the administration of kinetin. The linear equation representing this relationship is $\hat{Y} = 19.78 + 2.9x$, with an R-value of 0.887. This indicates that as the concentration of kinetin increases, the height of *chrysanthemum* shoots also increases, which aligns with findings from previous research [17]. Other studies have shown that increasing the concentration of kinetin to 3 ml L⁻¹, without auxin, results in the highest average plant shoot height [12]. It is noted that auxin does not inhibit plant height growth. Cytokinin plays a physiological role in stimulating morphogenesis, cell division, shoot formation, and apical dormancy. In this study, a combination of kinetin at 3 ml L⁻¹ and NAA at 1 ml L⁻¹ emerged as the most effective mix of auxin and cytokinin for promoting shoot height, thereby adhering to the principle of balance among plant growth regulators.

Number of Leaves (pieces)

Cytokinins play a vital role in enhancing leaf growth by stimulating cell division, accelerating the differentiation of leaf tissues, increasing photosynthesis, delaying aging, and improving nutrient distribution. However, high levels of auxin can inhibit leaf growth, especially when it interacts with other hormones like cytokinins and ethylene. The mechanisms that lead to leaf growth inhibition include apical dominance, increased

Table 2. Number of Leaves with NAA and Kinetin

Treatment Kinetin (ml L ⁻¹ water)	NAA (ml L ⁻¹ water)			Average
	N1 (0.5mL)	N2 (1mL)	N3 (1.5mL)	
K ₁ (1 mL)	14.17	12.67	15.33	14.06b
K ₂ (2 mL)	13.50	14.83	16.83	15.06ab
K ₃ (3 mL)	15.00	18.33	15.67	16.33a
Average	14.22	15.27	15.94	

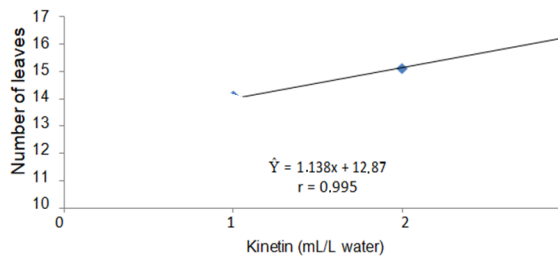


Figure 2. Relationship between Kinetin and Number of Leaves

production of ethylene, and the suppression of leaf cell division and differentiation. When auxin is balanced, it contributes positively to healthy leaf development by promoting cell division and elongation. This hormone works in conjunction with cytokinins to enhance nutrient transport, delay leaf aging, and activate leaf growth genes.

Table 2 demonstrates that applying kinetin and NAA at concentrations of up to 1.5 ml L⁻¹ can result in the production of up to 18.33 leaf strands in the K₃N₂ treatment. Previous research also indicated that varying concentrations of kinetin significantly affect the height of *Eucalyptus pellita* F. Muell microcuttings at the age of 7 days and the number of leaves at 28 days [18]. The interaction between cytokinins and auxin is crucial for determining the optimal pattern of leaf growth.

Figure 2 demonstrates that administering kinetin can increase the number of leaves. From the figure, a positive linear equation is derived: $\hat{Y} = 12.87 + 1.138x$, with an R-value of 0.995. This equation indicates that the number of leaves will rise as the concentration of kinetin increases. The physiological functions of cytokinins include promoting cell division, morphogenesis, lateral shoot growth, leaf enlargement, stomatal opening, and chloroplast formation. Similar findings were reported by Yuni [19], who stated that varying the concentrations of kinetin and IAA affected the height of the explants in each treatment.

Number of Roots

Auxin plays a crucial role in enhancing the number of roots by stimulating the formation of lateral and adventitious roots, activating root growth genes,

Table 3. Number of roots using NAA and Kinetin

Treatment Kinetin (ml L ⁻¹ water)	NAA (ml L ⁻¹ water)			Average
	N1 (0.5mL)	N2 (1mL)	N3 (1.5mL)	
K ₁ (1 mL)	8.33	7.83	8.33	8.17b
K ₂ (2 mL)	8.50	8.17	10.17	8.94b
K ₃ (3 mL)	9.50	11.00	13.83	11.44a
Average	8.78	9.00	10.77	

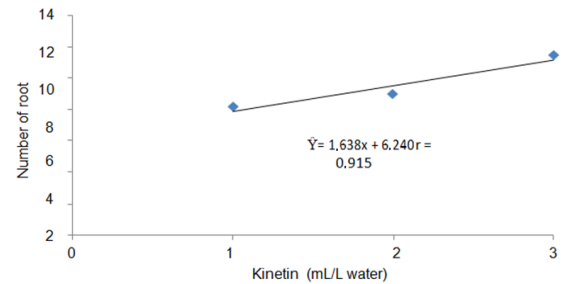


Figure 3. Relationship between PGR Kinetin and Number of Roots

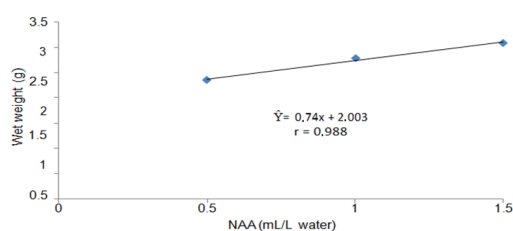
accelerating cell division, and interacting with cytokinins to achieve a balance between root and shoot growth. This hormone is essential for developing a robust and efficient root system. Conversely, high concentrations of cytokinins can inhibit root growth by suppressing the formation of lateral and adventitious roots, reducing meristem activity in the roots, increasing ethylene production, and redirecting resources towards shoot and leaf growth. The interaction between auxin and cytokinins significantly influences whether plants prioritize root growth or above-ground development [20].

Table 3 illustrates that increasing the concentrations of auxin and cytokinins can enhance plant root growth, reaching a maximum of 13.83 in treatment K₃N₃, while the lowest observed growth is 7.82 in treatment K₁N₂. The increase in concentrations of NAA (naphthaleneacetic acid) and Kinetin significantly influenced the number of roots observed in this study. This finding contrasts with the established role of cytokinins, which generally inhibit root growth. Previous studies have indicated that an increase in the concentration of Kinetin to 3 ppm reduced the average number of leaves from 8.83 to 5.17 when NAA was not present [21]. Similarly, Karjadi reported that an increase in the cytokinin BAP (benzylaminopurine) to 10 mg/L decreased the number of roots from 1.35 to 0.71 [16].

Figure 3 illustrates that increasing the concentration of kinetin results in a greater number of roots produced, demonstrating a positive linear relationship described by the equation $\hat{Y} = 6.240 + 1.638x$, with a correlation coefficient of $r = 0.915$. This indicates that higher levels of kinetin plant growth regulators (PGRs) in the media effectively stimulate cell division in the root apical

Table 4. Average Wet weight with NAA and Kinetin

Treatment Kinetin (ml L ⁻¹ water)	NAA (ml L ⁻¹ water)			Average
	N1 (0.5mL)	N2 (1mL)	N3 (1.5mL)	
K ₁ (1 mL)	2.02	2.58	2.72	2.44b
K ₂ (2 mL)	2.23	2.84	2.99	2.69ab
K ₃ (3 mL)	2.81	2.94	3.57	3.11a
Average	2.35	2.79	3.09	

**Figure 4.** Relationship between NAA and *Chrysanthemum* Wet Weight

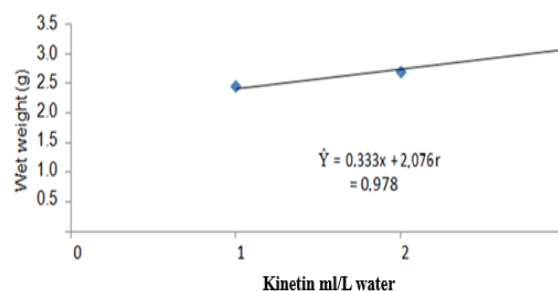
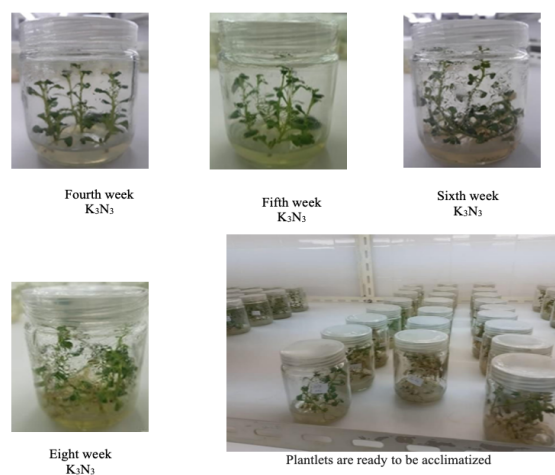
meristem, compared to lower concentrations. Additionally, noted that varying growth rates can be achieved by applying auxin and cytokinin in different ratios. Specifically, when the concentration of cytokinin exceeds that of auxin, it promotes the formation of new roots [22].

Wet Weight

Auxin and cytokinin work together to enhance plant growth. Auxin primarily promotes cell elongation, root development, and tissue differentiation, whereas cytokinin emphasizes cell division, shoot and leaf growth, and the delay of aging in plants. The balance and interaction between these two hormones are crucial for developing plants with robust root systems and productive shoots and leaves, which ultimately supports optimal overall growth [23].

Table 4 illustrates the relationship between auxin and cytokinin concentrations and their impact on plant growth rates. This is evident as wet weight increases with higher concentrations of these plant growth substances. The highest wet weight, measured at 3.57 grams, is observed in the K3N3 combination, while the lowest wet weight of 2.02 grams is found in the K1N1 combination.

Figure 4 illustrates the relationship between the wet weight of chrysanthemum shoots and the administration of NAA, resulting in a positive linear equation represented as $\hat{Y} = 2.003 + 0.74x$, with an r value of 0.988. This equation indicates that the wet weight increases as the dosage of NAA increases. Auxin plays a crucial role in cell division and enlargement in shoots and also stimulates root formation. Additionally, auxin is known to induce callus formation, inhibit the effects of

**Figure 5.** Relationship between Kinetin and *Chrysanthemum* Wet Weight**Figure 6.** Plantlet growth

chlorophyll cytokinin in callus, and suppress callus embryogenesis. By promoting the formation of roots or shoots, auxin encourages the embryogenic process. Consequently, as the growth improves due to the administration of NAA and Kinetin, the wet weight of chrysanthemum shoots increases correspondingly.

Figure 5 illustrates the relationship between the wet weight of chrysanthemum seedlings and the administration of kinetin, resulting in a positive linear equation represented by $\hat{Y} = 2.076 + 0.333x$, with an r value of 0.978. This equation indicates that as the concentration of kinetin increases, the wet weight of the seedlings also increases. Kinetin is classified as a cytokinin hormone, which is a type of growth regulator that plays a crucial role in cell division [5].

The concentration of kinetin combined with NAA in the culture medium affects the formation of shoots. When the amount of cytokinin exceeds that of auxin, it promotes shoot and leaf growth. Conversely, if cytokinin levels are lower than auxin levels, this leads to enhanced root growth. However, maintaining a balanced ratio of cytokinin to auxin results in the balanced development of shoots, leaves, and roots.[24]

NAA encourages the formation of axillary shoots in plants. Furthermore, the combination of auxin and cytokinins can stimulate callus growth and promote cell

division. The increase in kinetin concentration, up to 3 ml L⁻¹, along with an increase in NAA concentration, up to 1.5 ml L⁻¹, is still considered balanced, as evidenced by Figure 5.

The development of young plant growth from 4 weeks to 8 weeks until ready for acclimatization. with a concentration of K3N3 with a concentration composition of 3ml L⁻¹ Kinetin and 1.5ml L⁻¹ can be seen in Figure 6.

CONCLUSION

In general, the interaction of NAA and kinetin has a positive effect on plant height, number of leaves, number of roots and wet weight up to a concentration of 1.5 ml L⁻¹ in NAA and 3 ml L⁻¹ in Kinetin. The balance of the concentration of the combination of auxin (NAA) and cytokinin (Kinetin) can increase the growth rate of ornamental Chrysanthemum plants until the eighth week after planting.

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