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Effects of Naphthaleneacetic Acid and Kinetin on Chrysanthemum Plantlets in Vitro

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Abstract. *Naphthaleneacetic acid (NAA) and kinetin play a crucial role in plant growth but must be used in correct proportions in order to produce the best outcomes. An optimal combination of both can enhance shoot, leaf, and root growth, whereas inappropriate doses may otherwise inhibit growth due to hormonal imbalance. This study examined the effects of the interaction between NAA and kinetin on the growth of chrysanthemum plants at eight weeks of age. The research was carried out using the Completely Randomized Design (CRD) in triplicate. The medium used was MS medium, supplemented with NAA at concentrations of 0–1.5 mg/L and kinetin at concentrations of 0–3 mg/L. The results indicate that NAA and kinetin positively influenced plant growth, but only when the optimal concentrations were applied. The best combination of NAA at 1.0 mg/L (N_2) and kinetin at 3.0 mg/L (K_3) produced the best results in terms of height (153 mm), number of leaves (31 leaves), number of roots (26 roots), and fresh weight (5.85 g). Increasing kinetin concentrations (K_{0-3}) generally promoted plant growth, whereas excessive NAA concentrations (> 1.0 mg/L, N_3) produced the opposite effect, likely due to toxicity or hormonal imbalance. The interaction between kinetin and NAA exhibited a synergistic effect up to a certain threshold, while excessive NAA application negatively impacted on plant development.*
Keywords: *Chrysanthemum; Kinetin; Naphthaleneacetic Acid; Plant Growth Regulators.*

Type of the Paper: Regular Article.

1. Introduction

Chrysanthemum (*Dendranthema grandiflora* Tzelev, synonym) is an ornamental flowering shrub originating from the plains of China, also commonly known as "golden flower". Chrysanthemum plants in Europe trace their origin from China and Japan, arriving for the first time in France in 1795. In 1808, Colvil from Chelsea developed eight varieties of the flower in England. While chrysanthemums were introduced to Indonesia in the 17th century, it was not until 1940 that they were developed commercially in the region. Later, chrysanthemums rose in rank as the second most economically significant floral crop globally, being among the most important ornamental plants, especially with the continued expansion of the floriculture industry [1].

Being among the most widely used ornamental plants, chrysanthemums are facing increasing demand, giving suppliers a potential source of significant financial gains. In Indonesia, the demand for chrysanthemums rises by 25% annually, compared to the overall market demand

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increase of 31.62%. Exports of chrysanthemums to various countries, including the Netherlands, Brunei, Singapore, Japan, and the United Arab Emirates (UAE), totalled 1.44 million stalks, indicating their promising prospects for development both at present and in the future. However, the industry is facing a challenge with conventional procurement of superior seeds in large quantities within a short time. One way to overcome this drawback is to carry out plant propagation through various tissue culture methods, allowing for the production of vast amounts of planting material rapidly [2].

Tissue culture is the process of growing plant parts—such as cells, tissues, or organs—*in vitro* or outside their natural environments. It is carried out under aseptic conditions using artificial media that contain all essential nutrients and growth regulators. Additionally, conditions in the culture room, including temperature and lighting, are controlled to support the growth of the plant material [3]. Tissue culture with growth regulators can also be applied for sustainable and environmentally friendly fruit production [4].

Various types of basic media can be utilized in tissue culture. For instance, Murashige and Skoog (MS) medium provides sufficient organic nutrients to satisfy the needs of different types of plant cells in culture [5]. In addition, two important groups of hormones and plant growth regulators (PGRs) can be used in tissue culture, namely, cytokinins and auxins. The former group consists of kinetin, zeatin, and benzyladenine (BA), while the latter includes compounds such as indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D).

Auxins, notably NAA, primarily promote cell elongation, callus formation, and adventitious root development while inhibiting the formation of axillary shoots [6]. This effect on cell development suggests that auxins enhance protein synthesis, supporting their role as growth-promoting factors and potential energy sources [7]. Growth regulators, including naphthalene, can be derived from fruits, suggesting their potential safe application in food, although further safety analysis is warranted [8]. However, the application of these substances may leave residues, highlighting the importance of application monitoring to ensure the quality and safety of various agricultural products for consumption [9]. Auxin-binding proteins, when combined with air-stabilized lipid films, can be used to develop portable sensors capable of rapidly detecting naphthaleneacetic acid levels in fruits and vegetables [10,11].

Plant root growth can be stimulated by rooting hormones such as auxins. These hormones help initiate root formation, increase both the quantity and quality of roots, promote uniform rooting, and enhance the success rate of root establishment. NAA, one of the hormones in this group known for its stability, is commonly used to promote root elongation [12,13]. Studies have shown that the application of NAA, along with gibberellic acid (GA_3) and 4-chlorophenoxyacetic

acid (4-CPA), to chili plants increased fruit set, yield, and quality [14]. Furthermore, NAA's application in combination with benzyl amino purine (BAP) has been found to be effective in promoting both leaf and root development [15]. The use of growth regulators, such as NAA, has been known to enhance overall biomass production [16].

Meanwhile, cytokinins promote the growth of leaf buds and stimulate cell multiplication in tissue explants. Kinetin (6-furfurylaminopurine), a type of cytokinin, regulates cell division and morphogenesis. Although effective in inducing shoot growth in tissue cultures or mother plants, this effect is often less optimal in mature plants [17]. In tissue culture, cytokinins interact with auxins to influence tissue differentiation [18].

In addition to growth regulators, sterilants play a significant role in the *in vitro* micropropagation of cassava, particularly in establishing effective tissue culture protocols [19]. Moreover, the cutting position of the nodal segment can also influence plant growth [20].

NAA and kinetin work synergistically in plant tissue culture. Maintaining an appropriate ratio between these two hormones is crucial for achieving optimal growth. Generally, higher concentrations of NAA promote root development, while increased levels of kinetin stimulate the formation of shoots and leaves. A well-balanced combination of NAA and kinetin can lead to successful plant regeneration in *in vitro* culture systems [21].

This study aimed to investigate the interaction between NAA and kinetin in stimulating the growth of chrysanthemum plants. The findings are expected to provide valuable insights for improving plant tissue culture techniques, particularly in the horticulture industry, thereby enhancing their efficiency and ultimately plant productivity.

2. Materials and Methods

This research was conducted at the Tissue Culture Laboratory of the Growth Center, LLDikti Region I, from June to August 2024. The study utilized a completely randomized design (CRD) with two factors. The first factor was the concentration of the growth regulator NAA, and the second factor was the concentration of the growth regulator kinetin. Each treatment was repeated three times. The explants used in this study were sterile chrysanthemum seedling explants.

For the shoot induction stage, MS medium was used, supplemented with NAA and kinetin. The preparation of the MS medium involved weighing and dissolving macronutrient compounds, myo-inositol, and sucrose in an Erlenmeyer flask. Subsequently, micronutrient solutions, vitamins, Fe, and Na-EDTA were added. The mixture was then heated on a hot plate with a magnetic stirrer until the solution became clear and began to boil.

Once prepared, the medium was placed into culture bottles, each with a volume of approximately 20 mL, and the appropriate combination of NAA and kinetin for each treatment

was added. The mouths of the bottles were closed with aluminum foil, covered with paper, and secured with rubber bands. The medium-filled bottles were then sterilized by autoclaving under a pressure of 1.5 atm and at a temperature of 121°C for 15 minutes. After sterilization, the bottles were stored in a room with a temperature of 23–28°C. The setup for planting the explants involved continuous 24-hour irradiation using a 36-Watt white neon light. The data obtained were analyzed descriptively by observing graphical trends to identify patterns of plant heights, leaf numbers, and root numbers across different combinations of NAA and kinetin concentrations [22].

3. Results and Discussion

3.1. The effect of increasing the concentrations of NAA and kinetin on the height of chrysanthemum plants

NAA stimulates cell elongation, root growth, and apical dominance, which collectively increase plant height. In contrast, kinetin promotes cell division, lateral shoot growth, and slows leaf aging, thereby enhancing photosynthesis. A balanced combination of both substances results in optimal growth by striking a balance between plant height and the number of branches [23].

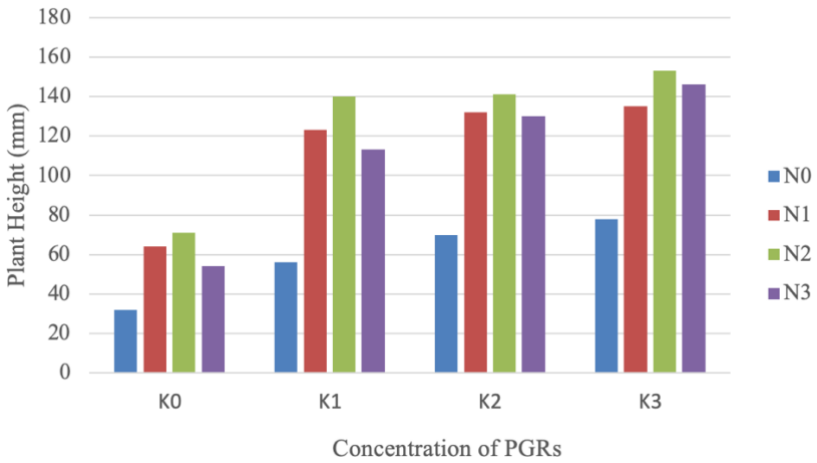

Fig. 1. The effect of PGR concentrations on plant height

Fig. 1 illustrates that the addition of NAA by up to 1.0 mg/L (N_2) could enhance plant growth with the increase in kinetin concentration. The highest growth was observed at N_2 (1.0 mg/L) combined with K_3 (3 mg/L), resulting in a plant height of 153 mm. However, growth decreased at N_3 (1.5 mg/L), likely due to toxicity or the dominance of root growth overshooting. Notably, as the concentration of kinetin increased, plant growth tended to rise. Kinetin at 3 mg/L (K_3) facilitated the highest growth across all levels of NAA concentration. Complete data can be found in Table 1.

These findings align with previous studies indicating that an increase in BAP (cytokinin) concentration could enhance plant height in chrysanthemum plants, achieving heights of up to 10 mg/L, while a higher concentration of NAA (10 mg/L) reduced growth [24]. Research by Mahadi [25] demonstrated that NAA at 0.1 mg/L resulted in maximum shoot height at 2.5 cm.

Table 1. The effect of the concentrations of NAA and kinetin on plant height (mm) at eight weeks

Kinetin/NAA	N_0 (0.0 mg/L)	N_1 (0.5 mg/L)	N_2 (1.0 mg/L)	N_3 (1.5 mg/L)
K_0 (0 mg/L)	32	64	71	54
K_1 (1 mg/L)	56	123	140	113
K_2 (2 mg/L)	70	132	141	130
K_3 (3 mg/L)	78	135	153	146

3.2. The effect of increasing the concentrations of NAA and kinetin on the number of leaves in chrysanthemum plants

NAA promotes cell elongation, root development, and apical dominance, which lead to a reduction in the formation of new shoots and leaves. In contrast, kinetin enhances cell division, increases the number of shoots and leaves, decreases apical dominance, and slows leaf senescence, allowing plants to retain their leaves for a longer period [26].

As shown in Fig. 2, increasing the concentration of kinetin (K_{0-3}) generally led to an increase in the number of leaves at each level of NAA concentration. For instance, at N_2 (1.0 mg/L NAA), the number of leaves rose from 15 (K_0) to 31 (K_3), suggesting that kinetin positively influenced leaf production. Similarly, increasing the concentration of NAA (from N_0 to N_3) also tended to enhance the number of leaves at each kinetin level. For example, at K_2 (2 mg/L kinetin), the leaf count increased from 15 (N_0) to 29 (N_2). However, there was a slight decrease at N_3, with only 22 leaves observed, indicating that high doses of NAA could negatively impact leaf growth.

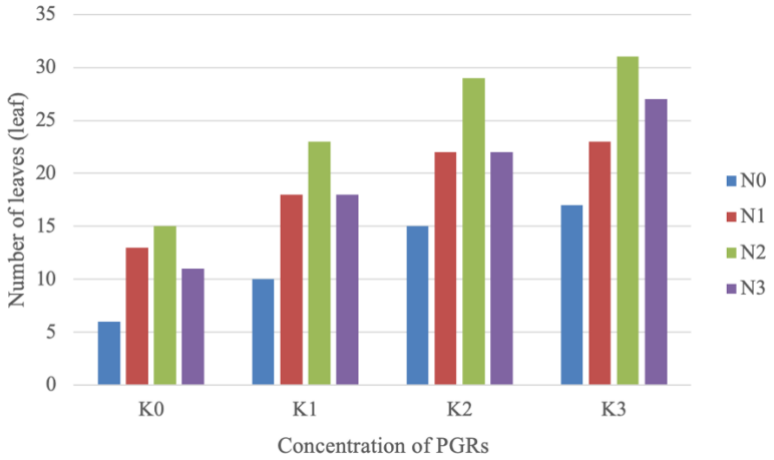

Fig. 2. The effect of PGR concentrations on the number of leaves

Overall, the interaction between kinetin and NAA displayed a synergistic effect, but only up to a certain point, after which the number of leaves began to decline. The most effective combination for increasing the number of leaves, yielding the highest leaf count of 31, was composed of kinetin at 3 mg/L (K_3) and NAA at 1.0 mg/L (N_2). Beyond this combination, there was a slight decrease in leaf number, which may be attributed to toxic effects or an imbalance in

growth hormones. Administering growth hormones at specific levels could significantly enhance leaf production, which was in contrast to the control group (without hormones). Complete data can be found in Table 2.

Table 2. The effect of the concentrations of NAA and kinetin on the number of leaves (pieces) at eight weeks

Kinetin/NAA	N_0 (0.0 mg/L)	N_1 (0.5 mg/L)	N_2 (1.0 mg/L)	N_3 (1.5 mg/L)
K_0 (0 mg/L)	6	13	15	11
K_1 (1 mg/L)	10	18	23	18
K_2 (2 mg/L)	15	22	29	22
K_3 (3 mg/L)	17	23	31	27

Kinetin and NAA typically promote the increase in the number of leaves in chrysanthemum plants, although the effects are not always consistent. When NAA is applied at excessively high doses (specifically N_3), it may result in a decrease in leaf number, likely due to negative impacts on the plant's physiology. Meanwhile, research indicates that increasing kinetin up to a concentration of 3 mg/L can increase the number of leaves in chrysanthemum plants [27,28]. From this it can be inferred that the number of leaves produced on each explant is influenced by the balance and interaction between endogenous (naturally present in the explant) and exogenous (absorbed from the media) levels of PGRs.

3.3. The effect of increasing the concentrations of NAA and kinetin on the number of roots in chrysanthemum plants

Increasing the concentration of kinetin leads to a higher number of roots. The hormone contributes to cell division, which promotes root growth. Additionally, increasing the concentration of NAA, an auxin known for its role in stimulating root development, also enhances root formation, but only up to a certain level.

The combination of kinetin at 3 mg/L and NAA at 1.0 mg/L (referred to as K_3N_2) resulted in the highest number of roots [26]. In contrast, the combination of kinetin at 3 mg/L and NAA at 1.5 mg/L (K_3N_3) led to a slight decrease in the number of roots [21]. This indicates that increasing NAA beyond 1.0 mg/L may not always yield positive outcomes for root formation, possibly due to toxic effects or inhibition of growth at higher doses.

Table 3. The effect of the concentrations of NAA and kinetin on the number of roots at eight weeks

Kinetin/NAA	N_0 (0.0 mg/L)	N_1 (0.5 mg/L)	N_2 (1.0 mg/L)	N_3 (1.5 mg/L)
K_0 (0 mg/L)	5	8	10	7
K_1 (1 mg/L)	7	14	17	14
K_2 (2 mg/L)	10	19	23	18
K_3 (3 mg/L)	11	21	26	21

The interaction between kinetin and NAA is synergistic in nature up to a specific concentration, particularly at 1.0 mg/L NAA (N_2). While kinetin at 3 mg/L yielded the best results for root growth, the effect was contingent upon the NAA concentration. NAA at 1.5 mg/L appears to inhibit root growth, potentially due to hormonal imbalances. Complete data can be found in Fig. 3 and Table 3.

Administering kinetin and NAA into the media significantly affected the number of roots observed at four weeks. It is believed that increasing the level of cytokinin (kinetin) may lead to a reduction in the number of roots since the hormone tends to stimulate shoot formation [29]. Additionally, the concentration of MS medium impacts on root development, with optimal results achieved at a 1x concentration of MS medium [30].

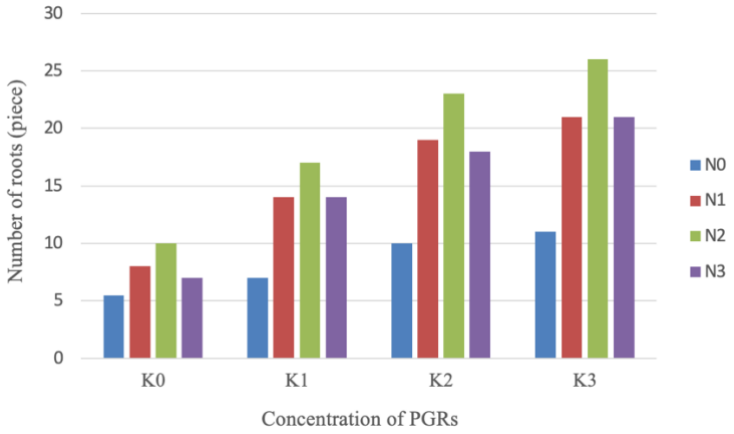

Fig. 3. The effect of PGR concentrations on the number of roots

3.4. The effect of increasing the concentrations of NAA and kinetin on the fresh weight of chrysanthemum plants

The fresh weight of roots is influenced by both the number of roots and their length during the cutting period. The dry weight of the roots indicates the amount of water they have absorbed. Generally, longer roots contribute to a higher fresh weight, and so does an increased number of roots. This is because longer and more numerous roots can store greater amounts of nutrients and water [29]. Similarly, shoot biomass has been reported to be positively correlated with root biomass, root length, root surface area, root volume, root tip number, root fork number, and root crossings in ratoon crops [31].

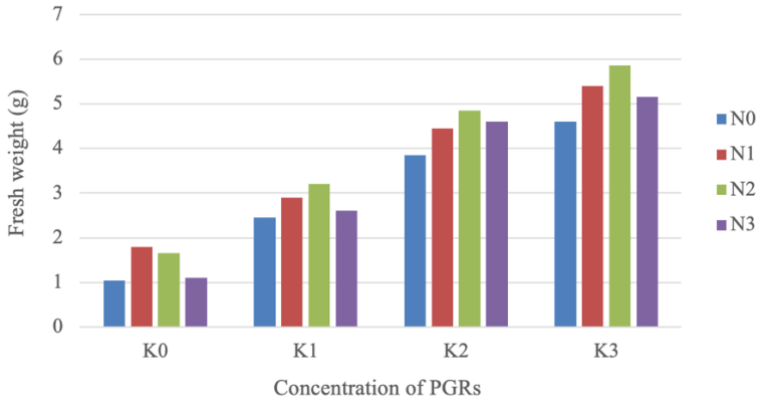

Fig. 4. The effect of PGR concentrations on fresh weight

Kinetin is a cytokinin that stimulates cell division and promotes shoot growth, ultimately leading to increased plant biomass (fresh weight). As shown in Fig. 4, the fresh weight of the plants increased consistently with higher concentrations of kinetin (K_{0-3}). Notably, the highest fresh weight was achieved at K_3 (3 mg/L kinetin) across all concentrations of NAA. Kinetin significantly contributed to increased fresh weight, particularly at concentrations ranging from 2 to 3 mg/L. This aligns with established findings that high levels of cytokinin promote shoot formation, whereas high levels of auxin inhibit it [32]. Conversely, despite its crucial role in facilitating root growth and differentiation, excessive NAA can inhibit shoot formation and reduce leaf biomass—at low to medium concentrations (0.5–1.0 mg/L) NAA enhanced fresh weight, but at higher concentrations (1.5 mg/L), fresh weight declined across all kinetin levels.

On the other hand, despite its critical role in facilitating root growth, cell differentiation, and stem elongation, excessive application of NAA can inhibit shoot formation and reduce leaf biomass, which negatively affects fresh weight. At low to medium concentrations (0.5–1.0 mg/L), NAA increased fresh weight, but at higher concentrations (1.5 mg/L), fresh weight began to decline across all kinetin levels.

The optimal combination for achieving maximum fresh weight was composed of NAA at 1.0 mg/L (N_2) and kinetin at 3.0 mg/L(K_3), resulting in a fresh weight of 5.85 g (Table 4). High levels of NAA (1.5 mg/L) tended to decrease fresh weight, especially when not balanced with an adequate amount of kinetin. Previous research reported that the application of IBA 100 ppm produced a relatively high yield from 19 days after planting until 21 days after planting, with a fresh plant weight of 1.0433 grams [33].

Table 4. The effect of the concentrations of NAA and kinetin on fresh weight at eight weeks

Kinetin/NAA	N_0 (0.0 mg/L)	N_1 (0.5 mg/L)	N_2 (1.0 mg/L)	N_3 (1.5 mg/L)
K_0 (0 mg/L)	1.05	1.8	1.65	1.1
K_1 (1 mg/L)	2.45	2.9	3.2	2.6
K_2 (2 mg/L)	3.85	4.45	4.85	4.6
K_3 (3 mg/L)	4.6	5.4	5.85	5.15

4. Conclusion

The addition of naphthaleneacetic acid (NAA) and kinetin generally promotes the growth of chrysanthemum plants, but only up to a certain concentration. The optimal combination for achieving the best growth—measured by height, number of leaves, number of roots, and fresh weight—consists of NAA at 1.0 mg/L (N_2) and kinetin at 3.0 mg/L (K_3). Increasing the concentration of kinetin (from K_{0-3}) tends to enhance plant growth. However, raising NAA levels above 1.0 mg/L (to N_3) can actually hinder growth, likely due to toxicity or hormonal imbalances. The interaction between kinetin and NAA is synergistic only up to a specific point. Therefore, chrysanthemum plants should be grown with the optimal combination of kinetin and NAA (K_3N_2) to achieve maximum yield.

Abbreviations

NAA	Naphthaleneacetic acid
PGRs	Plant growth regulators

Data availability statement

Data will be shared upon request by the readers.

CRediT authorship contribution statement

Saipul Sihotang: Conceptualization, Methodology, Resources, Formal Analysis, Investigation, Data curation. **Magdalena Saragih**: Writing – Review & Editing. **Denny Akbar Tanjung**: Writing – Original Draft, Validation. **Dini Puspita Yanty** & **Elli Efrida**: Data Curation, Formal Analysis, Conceptualization. **Swati Sembiring** & **Riduan Sembiring**: Conceptualization, Supervision, Project Administration.

Declaration of Competing Interest

The authors declare no conflict of interest. Greetings to the Growth Center of LLDikti Region 1 for providing the necessary facilities for the successful implementation of this research.

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