Cytokinins for Growth and Productivity of Polyploid Chrysanthemum on Third Generation

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Abstract. Chrysanthemum (Chrysanthemum sp) is one of the most popular cutting flower commodities in Indonesia. The beauty of colors and variations in the shape of flowers and the low level of withering cause Chrysanthemum to be in great demand. The polyploid chrysanthemum has some superior properties but the growth rate and flowering time is slow due to colchicine. The study aimed to determine the influence of the concentration of the cytokinins in the growth and productivity of chrysanthemum polyploid genotypes. The experimental design as used Split Plot Design consists of 2 factors and 2 replications. The first factors were concentration of the cytokinins (S_i, i = 1,...,4) as a main plot with different levels about 0, 1, 2, 3, 4 ml L^{-1} of cytokinins solutions, respectively. The second factors were the third generation of the polyploid chrysanthemum (G) genotype as a sub plot with 4 genotype, namely: g_0 = KRA_0, g_1 = KRA_1, g_2 = KRA_2, and g_3 = KRA_3. Based on the experiments and analysis results, it was found that the treatment of 3 ml L^{-1} of the cytokinin solution produced 16 shoots while the polyploid chrysanthemum genotype KRA_2 gave the best response to the number of shoots at 12 WAP compared to other polyploid chrysanthemum genotypes.

Keywords: Chrysanthemum, Cytokinins, Genotype, Polyploid.

1. Introduction

Chrysanthemum flower (Chrysanthemum sp), which is known by the community as chrysanthemum or gold flower, is a type of ornamental plant that is widely used and is increasingly popular in the community [1]. Chrysanthemum can be propagated sexually and vegetatively, but generally propagated by vegetative means by cutting the branches from the stem and then given growth regulators before planting in the hatchery. One of the plant breeding efforts to produce superior chrysanthemum flowers is to use the polyploidization technique with a mutagenic colchicine substance. The genotypes of KRA_0, KRA_1, KRA_2, and KRA_3 were polyploid chrysanthemum genes. In the previous test, the genotype still had some shortcomings, including longer flowering time and slower growth rate.

Optimization of chrysanthemum planting can be achieved if the factors that affect the growth and development of the plant are fulfilled. These external factors are nutrients, water, temperature, humidity, oxygen and light. The internal factors are genes and hormones. Several external and internal factors that determine are some of the factors that can be controlled by humans, among others, through fertilization and the addition of growth regulators. Plant growth regulators play an important role in controlling biological processes in plant tissue [2]; [3]. The role of growth regulators includes regulating the growth rate of each tissue and integrating these parts to produce plants. Growth regulators that can be given to chrysanthemum to overcome the above problems are by using cytokines. Application of cytokinin is intended to further stimulate shoot growth after pruning through cell division activity and cell extension, help overcome shoot dormancy, supporting photosynthesis activity the formation of chlorophyll and leaf expansion[4]. The research showed that pruning and skipping can increase the growth and productivity of Darjeeling tea [5]. According to [6], exogenous
hormones like cytokinin have an important role in regulating axillary bud growth. The results of the study showed that the use of 30 ppm kinetin can increase the number of buds that appear per sample, leaf area and chlorophyll content[7]. [8]stated that exogenously applied cytokinin stimulates the cell of elongation zone in plants. Cytokinins have implicated in diverse essential processes of plant growth and development as well as in regulation of key genes responsible for the metabolism and activities of plants.

This study aims to determine and examine the effect of cytokinin concentrations on the growth and productivity of several third generation polyploid chrysanthemum genotypes.

2. Methods
This research was conducted at the Green House of the Faculty of Agriculture, Winaya Mukti University, Tanjungsari District, Sumedang Regency with an altitude of 878 m above sea level. This trial was carried out from June to September 2019.

The equipment was used a razor blade, tray, trey, measuring glass, pipette, sprayer, camera, stationery, ruler, bucket, cup glass, bamboo, label, plastic. The materials used for this experiment were polyploidy chrysanthemum cuttings (KRA_0, KRA_1, KRA_2, KRA_3), cytokinin solution, polybag with a size of 12 cm x 25 cm, soil, husk charcoal, chicken manure, Biosugih fertilizer, Root Up, water, fungicide, bactericide, paper, and sack.

This experiment was used a Split Plot Design consists of 20 treatments and 2 replications. The treatments were without and with cytokines (1 ml L^-1, 2 ml L^-1, 3 ml L^-1, 4 ml L^-1), while for each genotype was g_0 = KRA_0 (non-polyploid chrysanthemum), g_1 = KRA_1, g_2 = KRA_2, g_3 = KRA_3. Each treatment was repeated for 2 times.

The implementation of the experiment includes preparation of planting media, preparation of seeds, soaking of seeds in Root-Up solutions, preparation of cytokinin solutions, spraying seeds with cytokinins, maintenance which includes embroidery, weeding, pest and disease control, watering and fertilizing, and observing plant height. stem diameter, number of leaves, number of internodes, number of shoots, length of stalk, number of flowers, diameter of flower crowns, age of flowering. The research data were analyzed using the F test at the 5% real level and Duncan's Advanced Test at the 5% real level.

3. Results and Discussions

3.1 Plant Height, Stem Diameter, Number of Leaves, and Number of Internodes
Statistically results showed that there was no effect of the cytokinin concentrations on the plant height of the polyploid chrysanthemum genotypes and there was no difference between the polyploid chrysanthemum genotypes on the plant height.

The stem diameter was influenced by the administration of the cytokines at low concentrations (1 ml L^-1), but at higher concentrations it tended to decrease the stem diameter. This is presumably because growth regulators such as cytokinins are only effective in low concentrations, and when given in high concentrations they can become inhibitors and even cause poisoning. Meanwhile, between different genotypes did not show a difference in stem diameter.

The cytokinin concentrations showed no significant effect on the number of leaves, while between genotypes did not give a difference in the number of leaves. This was thought to be due to environmental factors, namely the temperature of the air around the greenhouse which was hot, so that the plants experience shedding of leaves quickly. Environmental factors that influence leaf growth and development include light intensity, air temperature, water availability, and nutrients [9].

The cytokinin concentrations showed no significant effect on the number of internodes, while between genotypes showed differences in the number of segments (KRA_1 and KRA_2 genotypes had more internodes than other genotypes). All observations indicate that vegetative growth of plants really requires nutrients, especially nitrogen (N). In addition to nitrogen availability, genetic factors also affect the growth of a plant (changes that occur in plants due to colchicine can vary) [10]. It can
be assumed that the number of internodes could be affected by colchicine administration. Overall, the effect of cytokinin concentrations on plant height, stem diameter, number of leaves, and number of internodes can be seen in Table 1. The average number in Table 1 of treatments followed by the same letter in the same column shows insignificant differences according to the Least Significant Difference Test at the 5% level.

| Treatments (ml L⁻¹ solution) | Height (cm) | Diameter (mm) | Leaves (sheet) | Internodes |
|-----------------------------|-------------|---------------|----------------|------------|
| Cytokinins (Sᵢ)            |             |               |                |            |
| 0                           | 26,16 a     | 3.41 b        | 23.25 a        | 27.25 a    |
| 1                           | 26,13 a     | 3.37 b        | 27.38 a        | 23.50 a    |
| 2                           | 21,94 a     | 3.27 a        | 24.31 a        | 21.38 a    |
| 3                           | 22,03 a     | 3.14 a        | 25.88 a        | 25.00 a    |
| 4                           | 20,13 a     | 3.16 a        | 25.06 a        | 20.75 a    |
| Genotype (G)                |             |               |                |            |
| g₀ = KRA₀                   | 22,95 a     | 3.18 a        | 27.70 a        | 22.00 a    |
| g₁ = KRA₁                   | 24,35 a     | 3.32 a        | 24.80 a        | 24.80 b    |
| g₂ = KRA₂                   | 23,40 a     | 3.33 a        | 22.25 a        | 26.20 b    |
| g₃ = KRA₃                   | 22,40 a     | 3.24 a        | 25.15 a        | 21.30 a    |

3.2 Number of Shoots

There was an effect of the cytokinin concentrations on the number of shoots of various Chrysanthemum genotypes. The cytokinin concentrations with a level of 3 ml L⁻¹ solution and KRA₂ polyploid chrysanthemum genotype showed the highest number of shoots compared to the cytokinin concentrations in other chrysanthemum genotypes. This is consistent with the statement [12] that the concentration of BA (cytokinin) had a significant effect on the number of shoots and the number of leaves, but was not significantly different on shoot height. The effect of the cytokinin concentrations and various polyploid chrysanthemum genotypes on the number of shoots is shown in the Table 2. The average number of treatments marked with lowercase letters (vertical direction) and capital letters (horizontal direction) shows insignificant differences according to the Least Significant Difference Test at the 5% level.

| Treatments with Cytokinins (ml L⁻¹ solution) | Number of Age Shoots (12 WAP) |
|---------------------------------------------|--------------------------------|
|                                              | g₀ = KRA₀  | g₁ = KRA₁  | g₂ = KRA₂  | g₃ = KRA₃  |
| 0                                           | 10.25 a    | 11.25 a    | 11.50 a    | 11.50 a    |
|                                             | A          | B          | B          | B          |
| 1                                           | 11.75 c    | 12.00 b    | 11.75 a    | 11.75 a    |
|                                             | A          | A          | A          | A          |
| 2                                           | 11.00 b    | 12.00 b    | 12.00 a    | 13.00 b    |
|                                             | A          | B          | B          | C          |
| 3                                           | 13.50 d    | 14.75 c    | 16.00 b    | 12.75 b    |
3.3 Flower Stalk Length, Flower Crown Diameter, Number of Flowers and Flowering Age

The results of the study in Table 3 show that cytokinin concentrations have no effect on flower stalk length, flower crown diameter, flower number, and flowering age, while polyploid chrysanthemum genotypes indicate that flower stalk length, flower crown diameter, number of flowers, and flowering age have no differences.

This result was strongly suspected due to a lack of lighting factors so that the plants flower quickly and have short stalks. In accordance with the statement [13] on cut chrysanthemum plants, the addition of 4 and 5 hours of artificial light at night can increase the length of flower stalks of the same length by 54.82% and 55.46%, respectively. The average number of treatments followed by the same letter in the same column shows insignificant differences according to the Least Significant Difference Test at the 5% level.

Table 3. Effect of Cytokinin Concentrations on Flower Stalk Length, Flower Crown Diameter, Number of Flowers and Flowering Age of Various Polyploid Chrysanthemum Genotypes.

| Treatments | Stalk Length (cm) | Diameter (cm) | Total Interest | Flowering Age (WAP) |
|------------|-------------------|---------------|----------------|---------------------|
| Cytokinins (S) |                  |               |                |                     |
| 0          | 9.59 a            | 3.24 a        | 4.50 a         | 8.75 a             |
| 1          | 11.25 a           | 3.75 a        | 5.06 a         | 8.75 a             |
| 2          | 9.94 a            | 3.72 a        | 4.69 a         | 8.75 a             |
| 3          | 8.94 a            | 3.44 a        | 4.44 a         | 9.00 a             |
| 4          | 8.44 a            | 2.66 a        | 4.19 a         | 9.00 a             |
| Genotype (G) |                  |               |                |                     |
| g0 = KRA0   | 10.95 a           | 3.55 a        | 4.55 a         | 8.90 a             |
| g1 = KRA1   | 9.28 a            | 3.12 a        | 4.60 a         | 8.90 a             |
| g2 = KRA2   | 8.38 a            | 3.33 a        | 4.75 a         | 8.80 a             |
| g3 = KRA3   | 9.93 a            | 3.45 a        | 4.40 a         | 8.80 a             |

The result was thought to be due to the lack of colchicine soaking time. According to [14] treatment of colchicine concentration of 300 ppm and soaking time for 9 hours resulted in the largest flower diameter compared to treatments including without colchicine. This is consistent with the results of experiments conducted by [15] that the 12 hour colchicine immersion treatment produced the largest flower diameter, while the smallest diameter was produced at 24 hours treatment, the flower size in the 24 hour treatment was smaller than the flower size for the control.

According to [14] that the concentration and immersion of colchicine had no significant effect on the morphological changes in the number of flowers per stem in tuberose plants. According [16], the treatment of plant immersion time on the colchicine mutagen has an insignificantly different effect on growth and production parameters. The prolonged immersion treatment has a positive effect on plant growth and production so that it has not been able to convert the plant into polyploid. Cytokinin concentration has no effect on the number of flowers, presumably because the function of cytokinins affects the senescence process. The concentration of cytokinins in the crown of roses and carnations decreases with age, and the addition of exogenous cytokines can slow down the aging process. However, not all cut flowers will respond to exogenous cytokinins to overcome the effects of ethylene.
produced by these flowers. Cytokinin response depends on the type of flower, the rate of flower development and the concentration of cytokinins [17].

4. Conclusions
Based on the experimental results regarding the effect of cytokinin concentrations on growth and the results of several third-generation polyploid chrysanthemum genotypes, it was found that the interaction between cytokinin concentration and genotype was found. The $s_0$ concentration (without cytokinins) in the KRA$_1$ genotype affected the plant height at 8 WAP while $s_3$ (3 ml L$^{-1}$ cytokinin solution) in the KRA$_2$ genotype affected the number of shoots at 12 WAP. Giving a concentration of 3 ml L$^{-1}$ cytokinin solution on the KRA$_2$ genotype gave the highest yield of 16.00 shoots.

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