The phenotypic appeal of lily (Lilium longiflorum Thunb.) prompted mutation by colchicine

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Abstract. One of the efforts to assemble high yielding varieties is to use a chemical mutation method, such as colchicine. The objective of this study was to examine the phenotypic appeal of lily convinced by colchicine to acquire a current application and interval of the period for soaked colchicine. The research was steered in the investigational research place of Institute for Vegetable Research (IIVR) at Tongkoh, Sub-District Dolat Rakyat, District Karo, North Sumatera, and Plant Tissue Culture Laboratory, Agriculture Faculty, Universitas Sumatera Utara, from July 2019 up to March 2020. The research castoff Randomized Block Design technique (Factorial-RBD) by two factorials, i.e.: application of colchicine (0, 100, 200, and 300 ppm) and the interval of time for soaked colchicine (3, 6, 9, and 12 h). The constraint experimental was the plant tallness, the span of bud after flourishing, and the width of bloom. The outcome of this study indicated the concentration of colchicine hip 300 ppm (K₃) devours pointedly influenced on the plant tallness in the first week until sixth weeks when planting and the concentration of colchicine in 100 ppm (K₁) has considerably influenced on the size of bud after flowering and the span of bloom.

1. Introduction

Lilium (Lilium longiflorum Thunb.) has many benefits, including as ornamental cut flowers, cosmetic products, medicinal ingredients, and as a perfume ingredient [1]. Data from the Directorate General of Horticulture [2] in 2014 showed that the volume of lily seeds imported into Indonesia was 2,252,176 in tuber form that is ready to be planted, while the seeds that came out were 12,960,240 in micro tubers form produced by PT Tamara Stekindo in Sumatra, Indonesia.

Good quality and quantity of seeds can be used to support increased of lily production [3]. The effort to improved high yielding varieties can be carried out through plant breeding activities and one of the aspects influencing the achievement of the superior variety assembly program is the availability of genetic diversity. Efforts to generate genetic diversity can be done through polyploidization, mutation, or other techniques and to support these breeding activities, efforts are needed to study genetic diversity [4].

The mutation is a heritable alteration that occurs in inherited substantial (DNA or RNA), in cooperation at the equal of gene arrangements (called point mutations) and at the genetic material smooth. The materials caused mutations (mutagens) are divided into three, namely: chemical
mutagens, example, colchicine and digitonin, physical material mutagens, example, ultraviolet light, radioactive, and gamma rays, and mutagens for biological materials [5].

Colchicine is a toxic natural compound and it is produced by plant metabolism in the form of secondary metabolites. Colchicine is widely used to making polyploidy plants. Colchicine inhibits chromosome segregation during meiosis in various types of plants [6]. By giving colchicine to tuberose flowers (Polianthes tuberosa L.) of 100 ppm with a length of soaking 9 h and a concentration of 300 ppm with a length of soaking 6 h resulted in a higher average number of leaves and leaf area than other treatments [7].

Polyploidy induction with colchicine in lilies has not been widely reported yet, so the playwrights are attracted to steering research on the phenotypic characters of lilies induced by mutation using colchicine to obtain an effective concentration and soaking time.

2. Materials and methods

2.1. Experimental design

This study was steered at the Research Institute of Vegetable (Balitsa) Tongkoh Village, Sub-District Dolat Rakyat, District of Karo, North Sumatra, Indonesia and at the Plant Tissue Culture Laboratory, Faculty of Agriculture, Universitas Sumatera Utara. Planting material used in this study are the bulbs of lilies, are planted on topsoil and growing media base fertilizer (a mixture of chicken manure and chaff with cow manure) in a polybag. Observation of plant height is done by measuring the height starting from one week after planting up to flower bud appear. Observation of flower length and diameter is done by measuring the flower after fully bloomed.

This research method used factorial randomized block scheme (Factorial-RBD) with two treatment aspects and three replications, namely:

The Factor I : Colchicine concentration (K):
K₀ : 0 ppm (control)
K₁ : 100 ppm
K₂ : 200 ppm
K₃ : 300 ppm

The Factor II : Length of soaking colchicine (T):
T₁ : 3 h
T₂ : 6 h
T₃ : 9 h
T₄ : 12 h

2.2. Colchicine application

Giving a colchicine solution is done by soaking the tubers to a solution of colchicine at each concentration with colchicine 0 ppm, 100 ppm, 200 ppm and 300 ppm with saturated time 3, 6, 9, and 12 h.

3. Results and discussion

3.1. Plant height (cm)

The results showed that colchicine concentration has a significant effect on plant tallness in 1-6 weeks afterward planting. The highest average concentration of colchicine is at 300 ppm (K₃) and the lowest average concentration of colchicine at 200 ppm at 1-2 weeks after planting (WAP) and concentration of colchicine treatment of 0 ppm (control) at 3-6 WAP. This is thought to be because colchicine can work effectively at the beginning of growth where cells are actively dividing and influencing.

The result consistent with the statement of [7] and [8], that the use of colchicine at the growing point of the plant will prevent the formation of spindle fibers and the separation of chromosomes without the formation of cell walls. This treatment can lead to an increase in the number of
chromosomes before the multiplication of chromosomes becomes apparent during certain stages of nuclear division, and increase in cell size.

Table 1. The average height of lilies at 1-6 WAP.

| WAP | Colchicine (ppm) | Soaking Time (h) | Average  |
|-----|------------------|------------------|----------|
|     |                  | (T₁) 3           | (T₂) 6   | (T₃) 9   | (T₄) 12  |          |
| 1   | K₀: 0            | 0.15 c           | 0.25 c   | 0.15 c   | 0.50 ab  | 0.26 ab  |
|     | K₁: 100         | 0.38 abc         | 0.25 c   | 0.15 c   | 0.23 c   | 0.25 b   |
|     | K₂: 200         | 0.18 c           | 0.18 c   | 0.23 c   | 0.20 c   | 0.19 b   |
|     | K₃: 300         | 0.30 bc          | 0.33 abc | 0.58 a   | 0.38 abc | 0.39 a   |
|     | Average         | 0.25             | 0.25     | 0.28     | 0.33     | 0.28     |
| 2   | K₀: 0            | 0.30             | 0.48     | 0.35     | 0.90     | 0.51 b   |
|     | K₁: 100         | 0.75             | 0.65     | 0.33     | 0.53     | 0.56 ab  |
|     | K₂: 200         | 0.40             | 0.40     | 0.45     | 0.43     | 0.42 b   |
|     | K₃: 300         | 0.70             | 0.65     | 1.05     | 0.78     | 0.79 a   |
|     | Average         | 0.54             | 0.54     | 0.54     | 0.66     | 0.57     |
| 3   | K₀: 0            | 0.55             | 0.65     | 0.65     | 1.40     | 0.81 b   |
|     | K₁: 100         | 1.38             | 1.13     | 0.55     | 1.00     | 1.01 ab  |
|     | K₂: 200         | 0.75             | 0.78     | 0.88     | 0.95     | 0.84 b   |
|     | K₃: 300         | 1.18             | 1.13     | 1.48     | 1.25     | 1.26 a   |
|     | Average         | 0.96             | 0.92     | 0.89     | 1.15     | 0.98     |
| 4   | K₀: 0            | 0.78             | 1.00     | 1.10     | 1.85     | 1.18 b   |
|     | K₁: 100         | 1.78             | 1.55     | 0.83     | 1.78     | 1.48 ab  |
|     | K₂: 200         | 1.13             | 1.23     | 1.40     | 1.50     | 1.31 b   |
|     | K₃: 300         | 1.83             | 1.63     | 2.18     | 1.78     | 1.85 a   |
|     | Average         | 1.38             | 1.35     | 1.38     | 1.73     | 1.46     |
| 5   | K₀: 0            | 1.23             | 1.55     | 1.48     | 2.35     | 1.65 c   |
|     | K₁: 100         | 2.35             | 1.98     | 1.23     | 2.78     | 2.08 b   |
|     | K₂: 200         | 1.68             | 1.70     | 2.28     | 2.15     | 1.95 bc  |
|     | K₃: 300         | 2.65             | 2.33     | 3.43     | 2.58     | 2.74 a   |
|     | Average         | 1.98             | 1.89     | 2.10     | 2.46     | 2.11     |
| 6   | K₀: 0            | 1.98             | 2.18     | 2.30     | 3.25     | 2.43 b   |
|     | K₁: 100         | 3.18             | 3.08     | 3.78     | 4.65     | 3.67 a   |
|     | K₂: 200         | 2.78             | 2.50     | 2.90     | 3.05     | 2.81 b   |
|     | K₃: 300         | 4.40             | 3.88     | 5.00     | 3.83     | 4.28 a   |
|     | Average         | 3.08             | 2.91     | 3.49     | 3.69     | 3.29     |

The interface among the concentration of colchicine in 300 ppm and the interval of time for saturated colchicine on 9 h (K₅T₃) has considerably effect happening the plant tallness in 1 WAP, with the highest average 0.58 cm and the lowest average was in treatment K₀T₁, K₀T₃, and K₃T₃ with an average of 0.15 cm (table 1). This is presumably because at the beginning of plant growth (at vegetative growth points, ex. in seeds, sprouts and the tips of plant stems), the cells are actively dividing and when given colchicine immersion treatment, the cells are getting bigger because of the failure of chromosome separation [9].
3.2. Flower length after bloom (cm)

The results showed that colchicine concentration has a significant effect on flower length after blooming. The highest average concentration of colchicine was 100 ppm (K1) with an average length of 14.79 cm and the lowest was in control (K0) with an average of 9.98 cm (table 2).

It shows that the effective colchicine given affects the phenotype of lilies so that it produces larger flowers than the control plants. This is consistent with [10] which states that one of the techniques to create a giant orchid or greater than normal is to double the chromosomes (polyploid). Chromosome multiplication can be helped with colchicines.

| Table 2. The average length of lilies after blooming. |
|-----------------------------------------------|
| **Colchicine (ppm)** | **Soaking Time (hour)** | **Average** |
|                  | (T1) | (T2) | (T3) | (T4) |       |
|-------------------|------|------|------|------|-------|
| Average           |      |      |      |      |       |
| **K0**: 0         |      |      |      |      |       |
| 9.73              | 10.25| 10.00| 9.93 | 9.98b|
| **K1**: 100       |      |      |      |      |       |
| 15.63             | 14.68| 13.50| 15.35| 14.79a|
| **K2**: 200       |      |      |      |      |       |
| 14.15             | 13.75| 12.23| 13.3 | 13.36a|
| **K3**: 300       |      |      |      |      |       |
| 12.58             | 13.30| 15.68| 14.85| 14.10a|
| **Average**       |      |      |      |      |       |
| 13.02             | 12.99| 12.85| 13.36| 13.05 |

Description: The number tailed by the similar note in the same row and column are not significantly dissimilar by Duncan's Multiple Assortment Test at 5% equal.

3.3. Flower diameter (cm)

The results showed that the colchicine concentration has a significant effect on flower diameter, which produced larger flowers than control plants. The highest average was found in the colchicine concentration (K1) with an average of 9.71 cm and the lowest average was in control (K0) with an average of 4.87 cm (table 3). According to [11,12], stated that increasing the length and width of the flower crown, automatically increases the overall size of the flower. This means that the size of the tetraploid flower is larger than the size of the diploid flower and the doubling of the numeral of chromosomes has an important outcome on the size of the flower.

| Table 3. The average diameter of lilies. |
|----------------------------------------|
| **Colchicine (ppm)** | **Soaking Time (hour)** | **Average** |
|                  | (T1) | (T2) | (T3) | (T4) |       |
|-------------------|------|------|------|------|-------|
| **Average**       |      |      |      |      |       |
| **K0**: 0         |      |      |      |      |       |
| 5.08              | 4.83 | 5.35 | 4.23 | 4.87 c|
| **K1**: 100       |      |      |      |      |       |
| 10.38             | 9.85 | 9.20 | 9.40 | 9.71 a|
| **K2**: 200       |      |      |      |      |       |
| 8.63              | 7.83 | 7.90 | 7.30 | 7.91 b|
| **K3**: 300       |      |      |      |      |       |
| 7.60              | 7.90 | 10.05| 9.50 | 8.76 ab|
| **Average**       |      |      |      |      |       |
| 7.92              | 7.60 | 8.13 | 7.61 | 7.81  |

Description: The number tailed by a similar note in the same row and column are not significantly dissimilar by Duncan's Multiple Range Test at 5% equal.

4. Conclusions

Colchicine concentration of 300 ppm has a significant effect on plant height at 1-6 weeks after planting and colchicine concentration of 100 ppm significantly affected the length and diameter of
flowers after blooming. Colchicine soaking time did not affect all variables of observation. Interactions of colchicine concentration of 300 ppm and soaking time of colchicine for 9 h has a significant effect on plant height at first week after planting.

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