RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (5) 1971-1982, 2025
http://doi.org/10.54910/sabrao2025.57.5.18
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

# COLCHICINE ROLE IN ENHANCING PHENOTYPIC VARIATIONS IN VARIOUS TRAITS OF SHALLOT (*ALLIUM ASCALONICUM* L.)

**M.A.K. SYAHRIR and D. SAPTADI***

Faculty of Agriculture, University of Brawijaya, Indonesia
*Corresponding author's email: darmawan.fp@ub.ac.id
Email address of co-author: alifkaisar012@gmail.com

**SUMMARY**

Phenotypic variation is essential for the development of shallots (*Allium ascalonicum* L.) in the selection process. This study aimed to evaluate phenotypic variation and chromosome doubling effectiveness at different colchicine concentrations in the Rubaru shallot cultivar. The experiment tested five colchicine concentrations (0, 500, 750, 1000, and 1250 ppm) with a 12-hour bulb immersion, using a completely randomized design (CRD) with six replications. The descriptive analysis of data used means, maximum and minimum, standard deviation, and coefficient of variation tests. Higher colchicine concentrations induced greater variation; however, they also reduced the survival rate. The results showed a colchicine concentration of 1000 ppm effectively increased phenotypic variation and chromosome doubling in shallot bulbs. This concentration caused the highest variation in the number of leaves, tillers, and bulbs; bulb weight; and total bulbs' weight. Additionally, tetraploidy was evident in the 1000 ppm treatment. This study can be beneficial as a reference for future breeding research to improve the Rubaru shallot cultivars.

**Keywords:** Bulbs, chromosome numbers, crossing, improvement potential, phenotyping evaluation, polyploidy induction, selection, tetraploid

**Key findings:** Research on using colchicine to increase phenotypic variation in Rubaru shallots is limited. This study demonstrated a concentration of 1000 ppm enhanced variation in several traits. Such information can serve as a foundation for future breeding efforts to improve the Rubaru shallot cultivar.

Communicating Editor: Dr. Himmah Rustiami

Manuscript received: September 28, 2024; Accepted: April 26, 2025.


**Citation:** Syahrir MAK, Saptadi D (2025). Colchicine role in enhancing phenotypic variations in various traits of shallot (*Allium ascalonicum* L.). *SABRAO J. Breed. Genet.* 57(5): 1971-1982. http://doi.org/10.54910/sabrao2025.57.5.18.

## INTRODUCTION

Shallot (*Allium ascalonicum* L.) is a horticultural plant that belongs to the family Alliaceae. Their uses are commonly as a spice and traditional medicine in Indonesia. Shallot bulbs contain carbohydrates and vitamins A, B, and C and can also reduce the risk of cancer, improve heart health, treat diabetes, alleviate allergies, and boost immunity (Shahrajabian *et al*., 2020). The shallot bulbs generally undergo processing into fried onions for flavoring in Indonesian food.

Indonesia, being a tropical country, produces shallots on a larger area. Various shallot cultivars have succeeded in development in Indonesia to meet the local community needs, including the Rubaru cultivar, which originated from Sumenep, East Java, Indonesia. The Rubaru cultivar is famous in the community, being used to make fried onions because it has a fragrant and crunchy aroma compared with other shallot varieties (Dewi, 2022). Despite its popularity, the Rubaru cultivar has the disadvantage of its small bulb size. Based on the past research finding, the Rubaru cultivar has the smallest bulb size and weight compared with two other shallot cultivars, Bima and Maja (Azmi *et al*., 2011). Therefore, due to its tiny bulb, this cultivar has the potential to be further developed by improving its bulb size. Such improvement can be attainable by increasing the phenotypic variation, which serves as a valuable basis for effective selection. Selection is an essential component of plant breeding, as it allows the evaluation of desirable traits in breeding lines.

Phenotypic variation enables the selection of high-quality genotypes for plant trait development. Methods for trait development in plants include crossing, mutation induction, polyploidy induction, and embryo culture (Anand *et al*., 2023). Shallot plants are difficult to flower and produce seeds, which causes low genetic variation (Herlina *et al*., 2019). The difficulty of shallots in flowering and producing seeds makes the implementation of crossing methods challenging. Therefore, the induction of polyploidy is the only way to increase the variation. The polyploidy induction techniques can take place by using alkaloid compounds like colchicine.

Polyploidy is a reliable approach widely used in plant improvement because of the increased number of chromosomes. Colchicine can also increase phenotypic variation, allowing for the selection of superior traits. According to Sari *et al*. (2019), soaking Trisula cultivar shallot seeds in a 0.25% colchicine solution enhanced phenotypic variation in plant height, leaf number, bulb number, and bulb weight compared with the control. Concentration is a critical factor in induction, as each colchicine concentration exerts distinct effects on phenotypic variation in different plant traits (Tammu *et al*., 2021). Research by Rahmawati *et al*. (2024) reported that soaking Srikayang cultivar shallot bulbs in a 0.1% colchicine solution for 12 hours boosted variation in plant height, whereas a 0.05% concentration enhanced variation in leaf number, bulb weight, and bulb diameter. In addition to influencing phenotypic variation, colchicine affects chromosome doubling, with its impact varying by concentration. Research on the Tawangmagu Baru garlic cultivar by Hailu *et al*. (2020) found that immersion in a 0.1% colchicine solution resulted in tetraploids, whereas a 0.8% concentration produced mixoploids.

Colchicine induction can randomly change the morphological characteristics of plants (Tavan *et al*., 2021). These changes contribute to amplified phenotypic variation. However, limited information is available on the optimal colchicine concentration for enhancing phenotypic variation and inducing chromosome doubling in the Rubaru cultivar shallot; hence, its evaluation is necessary. Therefore, this study aimed to assess phenotypic variation and the effectiveness of chromosome doubling at various colchicine concentrations in the Rubaru cultivar of shallots.

## MATERIALS AND METHODS

The latest study commenced in 2023 at the Biotechnology and Plant Breeding Laboratories

and in the Jatimulyo experimental field of Brawijaya University, Malang, East Java, Indonesia.

**Plant materials and colchicine treatment**

In this experiment, the materials used were shallot bulbs of the Rubaru cultivar obtained from the head of the farmer group in Sumenep, East Java. The experiment included five colchicine concentration treatments: 0 ppm (P0), 500 ppm (P1), 750 ppm (P2), 1000 ppm (P3), and 1250 ppm (P4), with a 12-hour immersion duration. Each treatment consisted of 30 shallot bulbs evenly divided between six replications, with five bulbs per replication, resulting in a total of 150 bulbs used in the experiment. The preparation of colchicine solution proceeded in the biotechnology laboratory, starting with the preparation of a stock solution with a concentration of 2000 ppm by dissolving two grams of colchicine powder in one liter of distilled water. The formula used to dilute the solution follows below:

$$C_1 \times V_1 = C_2 \times V_2$$

Where $C_1$ = concentration of stock solution (ppm), $V_1$ = volume of stock solution taken (ml), $C_2$ = concentration of diluted solution (ppm), and $V_2$ = volume of diluted solution (ml).

Pouring the prepared treatment solution continued into a container with a size of 18 cm × 13 cm. The bulbs' immersion followed in the container for a predetermined period of time.

**Field experiments**

The experiment took place in a 35-$m^2$ field located in the Jatimulyo experimental field of Brawijaya University. The experimental arrangement had a completely randomized design, with all treatments randomly assigned without the use of blocks. Choosing this design enables the absence of confounding factors that would require blocking. Shallot bulbs underwent planting individually in 30 cm × 30 cm polybags before arranging them in the field with a spacing of 85 cm × 20 cm between bags.

Plant maintenance in this experiment included fertilization, pest and disease control, and weed management. The fertilizers used were urea (46% N), KCl (60% $K_2O$), and SP-36 (36% $P_2O_5$), applied 15 and 30 days after planting. Pest and disease control used a spraying technique. Pest control involved insecticides containing chlorantraniliprole and thiamethoxam at a dose of 0.4 ml/L, while disease control employed fungicides containing azoxystrobin and difenoconazole at a dose of 1 ml/L. Weed management performed was manual by pulling out weeds growing around the plants in the polybags. The variables observed included the survival rate and $LC_{50}$, plant height, the number of leaves, the number of tillers, stomata size and density, the number of bulbs, bulb diameter, bulb weight, total bulbs' weight, and the number of chromosomes. Observations on the survival rate and plant growth traits ensued during the cultivation period, while bulb trait observations occurred six days after harvest, with rotten bulbs excluded from the observation.

***Stomata observations***

Stomata observations proceeded in the plant breeding laboratory. The tools and materials used included clear cuticle polish, adhesive tape, microscope slides, and leaf samples. The selected leaves were those that grew well and showed no signs of disease in plants from each treatment group. The samples taken are shallot leaves cut along 5 cm from plants reaching 53 days after planting. The first step was to apply a thin layer of clear cuticle polish onto the shallot leaves and let it dry for 3 min. Then, taking the specimen comprised a piece of masking tape placed over the polished area, afterward carefully removed, and placed onto a microscope slide. The specimen observation utilized an Olympus BX 51 microscope (Olympus, Tokyo, Japan) with a 40× objective lens and a 10× ocular lens, resulting in a total magnification of 400×, and capturing the field of view screen to save the image. Each sample consisted of three randomly selected fields of view, each measuring 0.3545 $mm^2$. Stomata

length and width measurements engaged the ImageJ 1.54d software (National Institutes of Health, USA). Measurements came from three randomly selected stomata per field of view, with a total of nine stomata analyzed per sample. Stomata density calculation was the average number of stomata per unit area ($mm^2$).

***Chromosome observations***

Chromosome observations transpired in the plant breeding laboratory. The tools and materials used included razor blades, micropipettes, beaker glass, a measuring cylinder, microtubes, cover glasses, microscope slides, 5-mL glass vials, dropper pipettes, a refrigerator, an oven, a pencil, 45% acetic acid, absolute ethanol, 1 N hydrochloric acid, and 2% aceto-orcein.

Performing chromosome observation continued by using the squash method with some modifications (Verma *et al*., 2014). The required solution was a fixation solution prepared by mixing absolute ethanol with 45% acetic acid in a 3:1 ratio. Each sample consisted of two roots. Chromosome preparation succeeded in cutting shallot root tips to a length of 1–2 cm between 7:00 a.m. and 9:00 a.m. The roots, as placed into a microtube containing 0.1 ml of fixation solution, reached storage in the refrigerator for 14 h at ±6 °C. Afterward, the roots received a distilled-water washing before placing them into a glass vial bottle containing 1 ml of 1 N HCl. The vial, placed in an oven, sustained hydrolysis for six minutes at 60 °C.

Next steps included washing the roots again with distilled water and placing them in a microtube containing 2% aceto-orcein and allowing them to stand for at least two hours. Then, transferring the roots to a microscope slide followed, with the root tips (1–2 mm) cut using a razor blade before covering them with a glass cover, gently tapping with the tip of a rubberized pencil, and pressing down with the thumb. Chromosome observation used an Olympus BX 51 microscope, with one cell from each root being examined. If cells with clearly visible chromosomes were evident, taking a screenshot of it saved the image. Chromosome counting ensued manually by analyzing the captured image. Before counting, image editing occurred using a web-based photo and graphics editor to enhance clarity. Adjustments to hue, saturation, and lightness improved chromosome visibility.

**Data analysis**

Survival rate analysis continued by comparing the number of surviving plants to the total number of plants, when calculated, used the following equation:

$$Survival\ Rate = \frac{Number\ of\ Surviving\ Plants}{Number\ of\ plants} x100$$

Then, the survival rate data helped determine the lethal concentration ($LC_{50}$) value. The $LC_{50}$ equation came from the curve fit analysis conducted on a graph based on the colchicine concentration and the survival rate of plant data. Using the $LC_{50}$ determined the optimum concentration in polyploidy experiments, characterized by 50% plant mortality in the samples (Hailu *et al*., 2020). The equation used is the four-parameter logistic (4PL) model. The four-parameter logistic (4PL) model has been recognized as a major tool to analyze the relationship between the concentrations and responses (An *et al*., 2019). Other observations engaging descriptive analysis comprised the displaying of means, maximum and minimum values, standard deviation (SD), and coefficient of variation (CV%). The standard deviation formula used was as follows:

$$S = \sqrt{\frac{\sum\left(X_i - \bar{X}\right)}{n-1}}$$

Where $X_i$ = the data value, $\bar{X}$ = the average value, and n = the number of data.

The coefficient of variation formula used was:

$$CV(\%) = \frac{S}{\bar{X}}$$

Where S = the standard deviation and $\bar{X}$ = the average value.

The criteria for the coefficient of variation were low (<10%), medium (10%–20%), and high (>20%) based on Zaki and Radwan (2022).

## RESULTS AND DISCUSSION

### Survival rate (%) and $LC_{50}$

Survival rate observation sought to determine the impact of different colchicine concentrations on plant survival (Table 1). The highest survival rate (100%) was evident in the colchicine treatment of 0 ppm, indicating that all the shallot plants survived. The survival rate decreased at a colchicine concentration of 500 ppm and remained constant at 750 ppm (97%), but further decreased at 1000 ppm (73%). A survival rate of 0% was visible in the colchicine treatment of 1250 ppm. The higher dose of colchicine causes plant death, leading to a decline in the survival rate. Colchicine is a toxic chemical that causes death in plants. Colchicine can cause fatality in plants at a higher concentration and long immersion time (Roy *et al*., 2023).

The plants' death authenticated that plants sustained induction. Based on the research conducted by Ren *et al*. (2018), a colchicine concentration of 750 µmol/L (299.57 ppm) resulted in a plant survival rate of 7.78%, while a concentration of 1250 µmol/L (499.30 ppm) recorded a survival rate of 5.56%. Another past study conducted on bananas reported a survival rate of 12% at a colchicine concentration of 2.5 mM (998.5 ppm) (Rodrigues *et al*., 2011). In this study, the $LC_{50}$ value was 1087 ppm, as shown in Figure 1. This value resulted in using the four-parameter logistic (4PL) equation:

$$y = -46.95 + \frac{98.54 - (-46.95)}{1 + \left(\frac{x}{1162.83}\right)^{10.25}}$$

with $R^2 = 0.9994$.

The $LC_{50}$ value is a useful parameter for identifying the optimal concentration required to induce mutations. The $LC_{50}$ value can be efficient as a reference to select an appropriate colchicine concentration to avoid too high concentrations, killing all plants, or too low concentrations, ineffective for induction. This helps to obtain polyploid individuals, which can later be selected for desired traits. The concentration of mutagens should be high enough to increase the probability of inducing mutation but not so high as to cause lethality to the cells or tissues (Hailu *et al*., 2021).

Compared with previous studies, Sari *et al*. (2019) reported the $LC_{50}$ value of 0.65% at the colchicine concentration of 6500 ppm using shallot cultivar Trisula seeds. Another study on shallots conducted in vitro culture by Foschi *et al*. (2013) determined an $LC_{50}$ value of 500 mg/l (500 ppm) for colchicine concentration. Previous research has shown that colchicine exhibits varying levels of sensitivity; however, the $LC_{50}$ value can vary depending on the plant species and plant parts used (Lamo *et al*., 2017).

**Table 1.** Survival rates in shallot after undergoing various colchicine treatments.

| Treatments | Number of Samples (plants) | Number of Survivors (plants) | Survival Rate (%) |
|---|---|---|---|
| Control (0 ppm) | 30 | 30 | 100 |
| 500 ppm | 30 | 29 | 97 |
| 750 ppm | 30 | 29 | 97 |
| 1000 ppm | 30 | 22 | 73 |
| 1250 ppm | 30 | 0 | 0 |

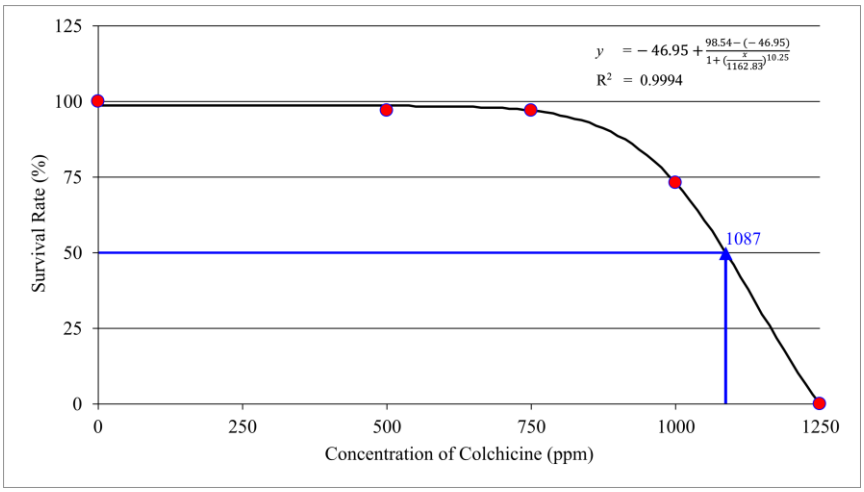


**Figure 1.** Determination of median lethal concentration (LC50) of colchicine concentration of shallot cultivars. The use of $LC_{50}$ (lethal concentration) measured the colchicine concentration required to kill half of a test population (Hailu *et al*., 2021).


**Figure 2.** Shallot plants' condition under different colchicine treatments. Each individual represents a sample from each treatment group. The treatments are P0 (0 ppm); P1 (500 ppm); P2 (750 ppm); P3 (1000 ppm); and P4 (1250 ppm). No plants survived in treatment P4, as all plants died.

**Plant growth traits**

The plant growth variables comprised plant height, the number of leaves, and the number of tillers. The shallot plant's physical conditions in each treatment appear in Figure 2. The results revealed colchicine concentrations caused a decrease in the average values of plant height and the number of leaves and tillers (Table 2). The control treatment (0 ppm) has the highest average values for the plant growth traits. In this study, the topmost

**Table 2.** Observations on growth traits in shallot plants with colchicine treatments at 49 days after planting.

| Traits | Treatments | Avg. | Max | Min | SD | CV (%) | Criteria |
|---|---|---|---|---|---|---|---|
| Plant height (cm) | Control (0 ppm) | 46.08 | 47.96 | 45.18 | 1.04 | 2.26 | Low |
| | 500 ppm | 44.41 | 49.12 | 40.14 | 3.13 | 7.05 | Low |
| | 750 ppm | 40.39 | 42.64 | 37.58 | 1.99 | 4.93 | Low |
| | 1000 ppm | 42.25 | 46.65 | 33.85 | 5.49 | 12.99 | Moderate |
| | 1250 ppm | - | - | - | - | - | |
| Number of leaves | Control (0 ppm) | 62.90 | 71.20 | 51.80 | 7.26 | 11.54 | Moderate |
| | 500 ppm | 56.81 | 68.20 | 46.25 | 8.91 | 15.68 | Moderate |
| | 750 ppm | 55.67 | 66.40 | 50.00 | 6.37 | 11.44 | Moderate |
| | 1000 ppm | 53.71 | 68.50 | 33.33 | 14.96 | 27.85 | High |
| | 1250 ppm | - | - | - | - | - | |
| Number of tillers | Control (0 ppm) | 11.30 | 12.40 | 10.00 | 1.05 | 9.29 | Low |
| | 500 ppm | 10.77 | 13.40 | 9.00 | 1.57 | 14.58 | Moderate |
| | 750 ppm | 9.62 | 12.00 | 8.50 | 1.39 | 14.45 | Moderate |
| | 1000 ppm | 10.11 | 13.00 | 6.50 | 2.81 | 27.79 | High |
| | 1250 ppm | - | - | - | - | - | |

The "-" symbol indicates that all plants died, hence, no data obtained. The criteria of CV are low (<10%), medium (10%–20%), and high (>20%).

concentration caused the dead plants. The colchicine treatment (1000 ppm) caused moderate variations in plant height, while it affected maximum variations in the number of leaves and tillers. Research conducted by Rahmawati *et al*. (2024) found that colchicine immersion at a concentration of 0.1% (1000 ppm) for 12 hours in shallot bulbs of the Srikayang cultivar resulted in a moderate variation in plant height but high variation in the number of leaves. Another study reported that colchicine immersion at a concentration of 400 ppm in shallot bulbs of the Sakato cultivar revealed a high variation in plant height and the number of leaves (Zulfahmi *et al*., 2024).

Each colchicine treatment resulted in varied plant growth and response, with phenotypic variations observed in shallot plants. However, the higher colchicine concentrations led to greater phenotypic variations. Higher variation suggests that selection activities should proceed to identify individual plants with promising trait values. The application of colchicine can lead to substantial variations in the morphological traits of crop plants (Mangena and Mushadu, 2023).

Colchicine can inhibit plant growth traits, leading to a decrease in plant height and the number of leaves and tillers in shallot plants. This was due to colchicine as an anti-mitotic agent, which can cause failure to form spindle fibers, causing the rate of cell division to decrease, which inhibits plant growth (Kumar *et al*., 2019). Similar findings appeared when soaking garlic bulbs in colchicine at concentrations ranging from 0.05% (500 ppm) to 0.45% (4500 ppm), causing a decrease in both plant height and the number of leaves (Mahajan *et al*., 2015). Colchicine immersion at a concentration of 0.1% (1000 ppm) also initiated a decline in the number of tillers and leaves in Javanese cardamom shoots (Komala *et al*., 2022).

**Stomata**

In shallot plants, the stomatal observations focused on the length, width, and stomatal density. The stomata pictures in each treatment are visible in shallot plants in Figure 3. Based on the data in Table 3, colchicine treatments resulted in an increase in the average length and width of the stomata. The colchicine treatments (500 and 1000 ppm) caused moderate variations in the stomatal width in shallot plants. Similarly, Bambara groundnut plants treated with 0.1% colchicine exhibited a moderate variation in stomatal length and width (Alisha *et al*., 2024).

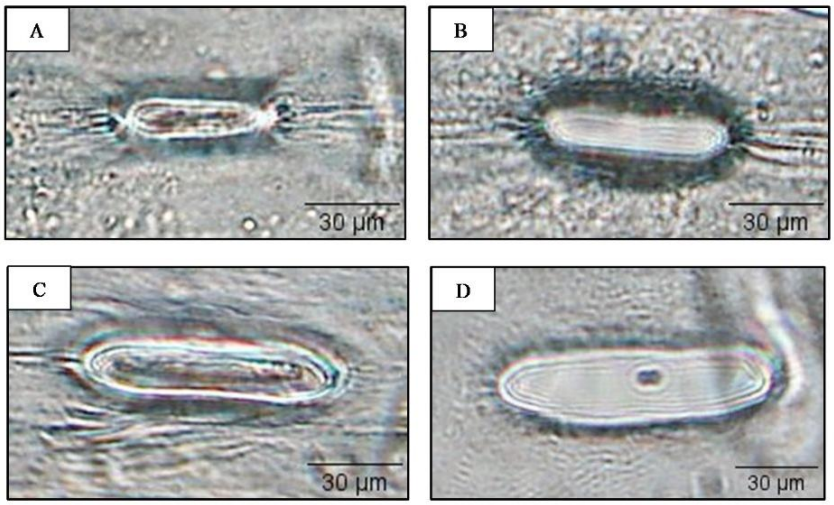


**Figure 3.** Stomata size in shallot plants with various colchicine treatments: A) Control (0 ppm), B) 500 ppm, C) 750 ppm, and D) 1000 ppm.

**Table 3.** Observations on stomata in shallot plants with colchicine treatments.

| Traits | Treatments | Avg. | Max | Min | SD | CV (%) | Criteria |
|---|---|---|---|---|---|---|---|
| Stomata length (µm) | Control (0 ppm) | 61.09 | 63.21 | 58.65 | 1.63 | 2.67 | Low |
| | 500 ppm | 62.46 | 65.05 | 60.12 | 1.97 | 3.15 | Low |
| | 750 ppm | 64.72 | 67.57 | 61.10 | 2.25 | 3.48 | Low |
| | 1000 ppm | 66.67 | 71.16 | 60.06 | 4.58 | 6.87 | Low |
| | 1250 ppm | - | - | - | - | - | |
| Stomata width (µm) | Control (0 ppm) | 13.82 | 15.64 | 11.81 | 1.26 | 9.12 | Low |
| | 500 ppm | 14.86 | 17.12 | 12.87 | 1.54 | 10.36 | Moderate |
| | 750 ppm | 15.60 | 18.49 | 14.42 | 1.60 | 10.26 | Moderate |
| | 1000 ppm | 16.58 | 19.34 | 14.10 | 1.88 | 11.34 | Moderate |
| | 1250 ppm | - | - | - | - | - | |
| Stomata density ($mm^2$) | Control (0 ppm) | 20.72 | 22.00 | 18.80 | 1.09 | 5.26 | Low |
| | 500 ppm | 19.37 | 22.56 | 16.74 | 2.35 | 12.13 | Moderate |
| | 750 ppm | 19.85 | 22.19 | 18.10 | 1.62 | 8.16 | Low |
| | 1000 ppm | 21.45 | 24.91 | 18.10 | 2.81 | 13.10 | Moderate |
| | 1250 ppm | - | - | - | - | - | |

The "-" symbol indicates that all plants died, hence, no data obtained. The criteria of CV are low (<10%), medium (10%–20%), and high (>20%).

Overall, the colchicine concentrations produced an increase in stomatal size, with higher concentrations resulting in larger stomata. Colchicine can increase stomatal length and width, making it a potential indicator of polyploidized individuals (Baby *et al*., 2023). An enlargement in cell size is the most important thing about polyploidy due to the addition of chromosomes (Manzoor *et al*., 2018). Comparing the treatments, the average values of stomata density decreased in the colchicine treatment of 500 ppm and increased at the concentration of 1000 ppm. Azizan *et al*. (2021) reported similar findings that colchicine immersion at 0.5% (5000 ppm) in stevia plants in vitro culture resulted in a stomatal density of 93 µm², whereas the control had a density of 83 µm². The increased stomatal density may be due to an increased number of cells caused by colchicine. Likewise, an upsurge emerged in the number of cells that undergo symmetrical division in *Brassica napus*

plants applied with colchicine (Zaki and Dickinson, 1995). The colchicine treatments at 500 and 1000 ppm triggered moderate variations versus the concentrations of 0 and 750 ppm for all the traits in shallot plants. Previous studies have also reported that variations in stomatal size and density may be because of genetic factors in crop plants (Bertolino *et al*., 2019).

**Bulb traits**

Observations on bulb traits consisted of the number of bulbs, bulb diameter, bulb weight, and total bulbs' weight. The images showing the bulb condition for each treatment are available in Figure 4. A higher concentration of colchicine led to decreased average values with the highest variations (Table 4). The colchicine


**Figure 4.** Shallot bulb traits after subjected to various colchicine treatments. The treatments are P0 (0 ppm); P1 (500 ppm); P2 (750 ppm); P3 (1000 ppm); and P4 (1250 ppm).

**Table 4.** Observations on bulb traits in shallots with colchicine treatments.

| Traits | Treatments | Avg. | Max | Min | SD | CV (%) | Criteria |
|---|---|---|---|---|---|---|---|
| Number of bulbs | Control (0 ppm) | 10.77 | 12.00 | 9.60 | 0.93 | 8.64 | Low |
| | 500 ppm | 10.75 | 13.00 | 9.75 | 1.34 | 12.47 | Moderate |
| | 750 ppm | 9.30 | 12.25 | 6.33 | 1.99 | 21.40 | High |
| | 1000 ppm | 9.36 | 11.50 | 6.50 | 2.34 | 25.00 | High |
| | 1250 ppm | - | - | - | - | - | |
| Bulb diameter (cm) | Control (0 ppm) | 3.41 | 3.70 | 2.99 | 0.27 | 7.92 | Low |
| | 500 ppm | 3.23 | 3.67 | 2.62 | 0.39 | 12.07 | Moderate |
| | 750 ppm | 3.15 | 4.05 | 1.59 | 0.90 | 28.57 | High |
| | 1000 ppm | 3.04 | 3.86 | 2.15 | 0.73 | 23.92 | High |
| | 1250 ppm | - | - | - | - | - | |
| Bulb weight (g) | Control (0 ppm) | 9.18 | 10.82 | 7.77 | 1.15 | 12.53 | Moderate |
| | 500 ppm | 8.47 | 10.24 | 7.33 | 1.06 | 12.51 | Moderate |
| | 750 ppm | 8.08 | 10.13 | 5.60 | 1.69 | 20.92 | High |
| | 1000 ppm | 7.60 | 9.76 | 5.37 | 1.64 | 21.58 | High |
| | 1250 ppm | - | - | - | - | - | |
| Total bulbs' weight (g/plant) | Control (0 ppm) | 98.85 | 125.48 | 80.83 | 15.66 | 15.84 | Moderate |
| | 500 ppm | 90.91 | 104.49 | 71.52 | 14.31 | 15.74 | Moderate |
| | 750 ppm | 75.41 | 88.67 | 35.42 | 20.16 | 26.73 | High |
| | 1000 ppm | 71.40 | 97.55 | 40.36 | 23.99 | 33.60 | High |
| | 1250 ppm | - | - | - | - | - | |

The "-" symbol indicates that all plants died, hence, no data obtained. The criteria of CV are low (<10%), medium (10%–20%), and high (>20%).

treatment at 1000 ppm caused the maximum variations in the number of bulbs, bulb weight, and total bulbs' weight. Research by Rahmawati *et al*. (2024) found that colchicine immersion at a concentration of 0.1% (1000 ppm) for 12 hours in shallot bulbs of the Srikayang cultivar resulted in moderate variation in bulb diameter but high variation in bulb weight. Another study reported colchicine immersion with a concentration of 400 ppm on shallot bulbs of the Sakato cultivar had high variations in the number of bulbs, bulb diameter, and bulb weight (Zulfahmi *et al*., 2024).

Each shallot plant responded differently to colchicine treatment, revealing considerable variations. Polyploidization activities in crop plants can enhance the genetic variation marked by modification of traits, and the fruitful selection can succeed in selecting the genotypes with desirable traits (Fox *et al*., 2020). With the highest variations, selection activities can take place in mutants with the desired traits. The selection process depends on genetic distinctions to obtain genotypes with superior traits.

The colchicine treatment (0 ppm) has the supreme average value for the bulb characteristics, while colchicine treatment 1000 ppm showed the lowest average values for bulb-related characters. The results revealed that higher colchicine concentrations caused lower average yield traits than the control. The decrease in traits was due to colchicine toxicity that inhibits plant growth. Colchicine is a toxic substance that can deter growth traits in crop plants (Manzoor *et al*., 2018). Based on the results of a study by Rahmawati *et al.* (2024), treatment with 0.1% (1000 ppm) colchicine for 12 hours in shallots of the Srikayang cultivar manifested a reduction in the average bulb diameter and weight compared with the control treatment. Widoretno *et al*. (2023) reported that colchicine control treatments showed better shoot multiplication in *Amorphophallus muelleri* plants than the colchicine concentration of 120 mg/l (120 ppm). In another study on *Acacia crassicarpa*, colchicine immersion at a concentration of 0.08% (800 ppm) caused a decrease in the number of shoots (Sinuraya *et al*., 2023).

**Chromosome number**

The observations on the chromosome number aimed to determine the chromosome count in shallot plants with each treatment of colchicine (Figure 5). According to the results, some shallot bulbs emerged with the chromosome doubling (Table 5). Chromosome duplication occurred in some bulbs from the samples as a result of colchicine treatment. Out of the 58 bulbs selected as samples, 10 experienced chromosome doubling. However, most of the chromosome doublings were notable in the colchicine treatments at 750 and 1000 ppm. Colchicine inhibits the formation of the spindle, making the chromosomes fail to separate during anaphase, resulting in more chromosomes than before (Yana *et al*., 2023).

Most of the chromosome doubling events, which led to tetraploid genotypes, were prevalent in the colchicine treatment at 1000 ppm in shallot plants. Hailu *et al*. (2020) reported the immersion of garlic cultivar Tawangmangu Baru in colchicine at concentrations of 0.02% (200 ppm) and 0.08% (800 ppm) produced the mixoploids, while a

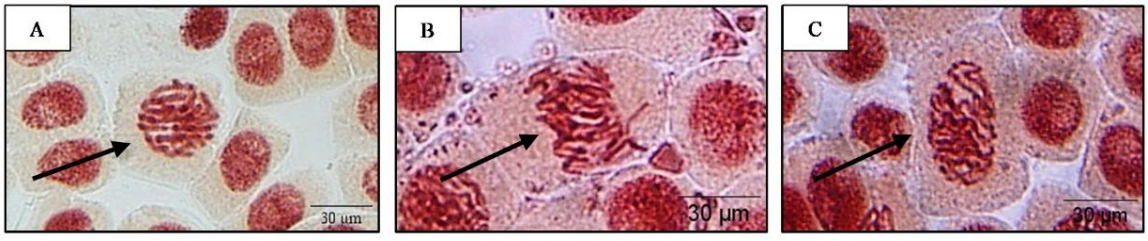


**Figure 5.** Number of chromosomes in shallot plants: A) Diploid (2n = 2x), B) Tetraploid (2n = 4x), and C) Mixoploid.

**Table 5.** Observations on chromosome number with colchicine treatments in shallots.

| Colchicine treatments | Number of samples | Diploids (2x) | Tetraploids (4x) | Mixoploids |
|---|---|---|---|---|
| Control (0 ppm) | 15 | 15 | - | - |
| 500 ppm | 15 | 13 | - | 2 |
| 750 ppm | 14 | 10 | - | 4 |
| 1000 ppm | 14 | 10 | 1 | 3 |
| Total | 58 | 48 | 1 | 9 |

Ploidy level is determined by the number of chromosome sets in a cell. The number of basic chromosomes in a set in shallot is 8 (x = 8).

concentration of 0.1% (1000 ppm) produced the tetraploids. Moreover, some shallot bulb samples did not experience the chromosome doubling. The effect of colchicine in inducing mutation was random, and diploid individuals (2n) were still present in the population (Suminah *et al*., 2002).

## CONCLUSIONS

Colchicine treatment at 1000 ppm is effective in increasing phenotypic variation and inducing chromosome doubling. The colchicine concentration at 1000 ppm resulted in the maximum phenotypic variations in the number of leaves, tillers, and bulbs; bulb weight; and total bulbs' weight in shallot plants. Furthermore, a tetraploid individual appeared in the 1000 ppm treatment.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the funding support from PNBP 2023, Faculty of Agriculture, Brawijaya University.

## REFERENCES

Alisha TS, Saptadi D, Damanhuri (2024). Evaluation of morphological and cytological variations in colchicine-induced M1 and M2 generations of Bambara groundnut (*Vigna subterranea* L.). *Agrikultura* 35(2): 284–297.

An H, Landis JT, Bailey AG, Marron JS, Dittmer DP (2019). dr4pl: A stable convergence algorithm for the 4 parameter logistic model. *The R. J.* 11(2): 171–190.

Anand A, Subramanian M, Kar D (2023). Breeding techniques to dispense higher genetic gains. *Front. Plant Sci.* 13:1076094.

Azizan NI, Shamsiah A, Hasan NA, Hussein S (2021). Morphological characterization of colchicine-induced mutants in *Stevia rebaudiana*. *IOP Conf. Ser.: Earth Environ. Sci*. 757: 1–7.

Azmi C, Hidayat IM, Wiguna G (2011). Pengaruh varietas dan ukuran umbi terhadap produktivitas bawang merah. *J. Hort*. 21(3): 206–213.

Baby J, Ganesan NM, Ganesan KN, Sivakumar SD, Chandrasekhar CN, Sobhakumari VP (2023). Stomatal studies on colchicine treated bajra napier hybrids (*Pennisetum glaucum* × P. *purpureum*). *Agric. Sci. Dig.* 43(5): 610–615.

Bertolino LT, Caine RS, Gray JE (2019). Impact of stomatal density and morphology on water-use efficiency in a changing world. *Front. Plant Sci*. 10: 225.

Dewi IR (2022). Inovasi Teknologi Budidaya dan Penanganan Hasil Bawang Merah Varietas Rubaru. Balai Pengkajian Teknologi Jawa Timur, Malang.

Foschi ML, Martínez LE, Ponce M, Galmarini CR, Bohanec B (2013). Effect of colchicine and amiprophos-methyl on the production of in vitro doubled haploid onion plants and correlation assessment between ploidy level and stomatal size. *Rev. Fac. Cienc. Agrar*. 45(2): 155–164.

Fox DT, Soltis DE, Soltis PS, Ashman TL, Van-de-Peer Y (2020). Polyploidy: A biological force from cells to ecosystems. *Trends Cell Biol*. 30(9): 688–694.

Hailu MG, Mawcha KT, Nshimiyimana S, Suharsono S (2021). Garlic micro-propagation and polyploidy induction in vitro by colchicine. *Plant Breed. Biotechnol.* 9(1): 1–19.

Hailu MG, Wiendi NMA, Dinarti D (2020). Increasing ploidy level of garlic (*Allium sativum* L.) Tawangmangu Baru in-vitro using colchicine. *J. Trop. Crop Sci*. 7: 122–136.

Herlina L, Reflinur R, Sobir S, Maharijaya A, Wiyono S (2019). The genetic diversity and population structure of shallots (*Allium cepa* var. aggregatum) in Indonesia based on R gene-derived markers. *Biodiversitas* 20(3): 696–703.

Komala N, Aisyah SI, Nurcholis W (2022). Mutasi induksi dengan kolkisin pada kapulaga jawa (*Amomum compactum* Soland. Ex Maton) generasi MV1. *Indonesian J. Agron*. 50(2): 234–241.

Kumar R, Jha K, Sengupta S, Misra S, Mahto C, Chakravarty M, Saha D, Narayan S, Yadav M (2019). Effect of colchicine treatment on plant growth and floral behaviour in cape gooseberry (*Physalis peruviana* L.). *J. Pharmacogn. Phytochem*. 8(5): 405–411.

Lamo K, Ji Bhat D, Kour K, Singh Solanki SP (2017). Mutation studies in fruit crops: A review. *Int. J. Curr. Microbiol. Appl. Sci.* 6(12): 3620–3633.

Mahajan V, Devi A, Khar A, Lawande KE (2015). Studies on mutagenesis in garlic using chemical mutagens to determine lethal dose ($LD_{50}$) and create variability. *Indian J. Hortic.* 72(2): 289–292.

Mangena P, Mushadu PN (2023). Colchicine-induced polyploidy in leguminous crops enhances morpho-physiological characteristics for drought stress tolerance. *Life* 13(10): 1966.

Manzoor A, Ahmad T, Bashir MA, Baig MMQ, Quresh AA, Shah MKN, Hafiz IA (2018). Induction and identification of colchicine induced polyploidy in *Gladiolus grandiflorus* white prosperity. *Folia Hortic*. 30(2): 307–319.

Rahmawati AAN, Nandariyah N, Parjanto P (2024). Phenotypic performance of Srikayang's shallot variety M1 by colchicine induction. *Biodiversitas* 25(3): 1297–1303.

Ren J, Wu X, Song C, Liang Y, Gao W, Wang Y (2018). Induction of polyploid tillered onion using colchicine and pendimethalin. *Sains Malays.* 47(11): 2617–2624.

Rodrigues FA, Soares JDR, dos Santos RR, Pasqual M, e Silva S (2011). Colchicine and amiprophos-methyl (APM) in polyploidy induction in banana plant. *Afr. J. Biotechnol.* 10(62): 13476–13481.

Roy S, Thakur A, Singh R, Kumar A, Yadav S (2023). Determination of semi-lethal dose of colchicine on in vitro grown callus of *Azadirachta indica* genotypes. *Appl. Biol. Chem. J*. 4(1): 28–33.

Sari Y, Sobir, Syukur M, Dinarti D (2019). Induksi poliploid TSS (True Shallot Seed) bawang merah Varietas Trisula menggunakan kolkisin. *J. Hort. Indonesia*. 10: 145–153.

Shahrajabian MH, Sun W, Cheng Q (2020). Chinese onion and shallot, originated in Asia, medicinal plants for healthy daily recipes. *Notulae Sci. Biol*. 12: 197–207.

Sinuraya F, Roslim DI, Deviona D, Suharyanto S (2023). The effect of colchicine concentration and immersion time on growth and morphological characters of *Acacia crassicarpa* A. Cunn. Ex Benth in-vitro explants. *J. Biol. Tropis*. 23(3): 238–247.

Suminah, Sutarno, Setyawan AD (2002). Polyploid induction of *Allium ascalonicum* L. by colchicine. *Biodiversitas* 3(1): 174–180.

Tammu RM, Nuringtyas TR, Daryono BS (2021). Colchicine effects on the ploidy level and morphological characters of Katokkon pepper (*Capsicum annuum* L.) from North Toraja, Indonesia. *J. Genet. Eng. Biotechnol.* 19(1): 31.

Tavan M, Sarikhani H, Mirjalili MH, Rigano MM, Azizi A (2021). Triterpenic and phenolic acids production changed in *Salvia officinalis* via *in vitro* and *in vivo* polyploidization: A consequence of altered genes expression. *Phytochemistry* 189.

Verma AS, Das S, Singh A (2014). Laboratory Manual for Biotechnology. Chand Publisher, New Delhi, India.

Widoretno W, Azriningsih R, Sukmadjaja D, Rosyidah M (2023). In vitro induction and identification of polyploid *Amorphophallus muelleri* Blume plants by colchicine treatment. *AGRIVITA J. Agric. Sci.* 45(1): 87–97.

Yana DV, Ernawiati E, Agustrina R, Handayani TT (2023). Colchicine effect of mitosis root tip by banana kepok yellow (*Musa paradisiaca* L.) polyploid explants. *J. Ilm. Biol. Eksper. Keanekaragam. Hayati*. 10(1): 1–6.

Zaki HEM, Radwan KSA (2022). Estimates of genotypic and phenotypic variance, heritability, and genetic advance of horticultural traits in developed crosses of cowpea (*Vigna unguiculata* [L.] Walp). *Front. Plant Sci.* 13: 987985.

Zaki M, Dickinson H (1995). Modification of cell development in vitro: The effect of colchicine on anther and isolated microspore culture in *Brassica napus*. *Plant Cell Tissue Organ Cult*. 40: 255–270.

Zulfahmi, Mahmuzar, Affandy D, Rosmaina (2024). Evaluating genetic variability and selection criteria in shallot M1V1 mutant induced by colchicine treatment. *SABRAO J. Breed. Genet.* 56(5): 1883–1894.