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Phenotypic performance of Srikayang's shallot variety M1 by colchicine induction

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Abstract. *Rahmawati ADN, Nandariyah, Parjanto. 2024. Phenotypic performance of Srikayang's shallot variety M1 by colchicine induction. Biodiversitas 25: 1297-1303.* Srikayang shallot is one of the shallot varieties from Kulonprogo Yogyakarta, and it has relatively small bulbs. Indonesian consumers prefer shallot, which has dense bulbs, is large (diameter ≥ 2.5 cm), has a spicy taste, and is fragrant. The efforts to increase shallot's diversity are important. This study aimed to evaluate the performance of Srikayang shallot M1 and evaluate Lethal Doses (LD_{50}) of colchicine. This research was conducted from September to November 2023 at Balecatur Planting Garden, Yogyakarta. Srikayang shallot bulbs were used, and colchicine concentration consisted of 0%, 0.05%, 0.075%, and 0.1%, with a soaking period of 3, 6, and 12 hours. The study was conducted using a randomized block design (RBD). Data were analyzed using descriptive analysis and probit analysis. The colchicine concentration and soaking duration affected the morphological character of Srikayang plants in leaf color, bulb skin color, bulb shape, bulb neck shape, and root tip shape. This study showed that the interaction of colchicine concentration and soaking duration improved variability on plant height, number of leaves, number of saplings, bulb weight in the cluster, main bulb weight, and main bulb diameter. The LD_{50} of Srikayang shallots was obtained at a colchicine concentration of 0.065%.

Keywords: Colchicine, performance, phenotype, Srikayang shallot

INTRODUCTION

Srikayang shallot is one of the cultivated varieties in Kulonprogo, Yogyakarta. The physical appearance of the Srikayang shallot bulb is relatively small, with a length of 2.1-2.4 cm, bulb diameter of 1.9-2.2 cm, and productivity of 10.63 T/ha (Rajiman et al. 2022). Several attempts to increase the size of onion bulbs can be made using character improvement through mutation and increasing bulb size through chromosomal manipulation. The normal chromosome number of shallot is 2n = 16 (Billa et al. 2022). Srikayang shallot plant doesn't have flowers, and no crossing can be performed. The breeding of vegetative plants is carried out by mutation to improve diversity. The mutation that applied for Srikayang shallot was induced by colchicine because the harvested part is bulbs that require a large size, which can made from polyploid. Increasing the size of local onion bulbs is expected to increase productivity and consumer preference for this local shallot. Mutations can be applied through chemical mutations, with colchicine mutagens having many advantages, such as gaining high genetic variation and being predictable. As one of the chemical mutagens, colchicine is water-soluble and easily used to induce chromosome doubling in many plant organs. Colchicine is generally a chemical mutagen because it can dissolve easily in water and changes cell chromosome number (Manzoor et al. 2019). The nature of the colchicine mutagen affects the activity of chromosome-binding threads (spindle threads), and the chromosomes that have divided will not be separated again. According to Ayu et al. (2019), 0.3 % colchicine treatment with 0 hours soaking duration in garlic Doulu variety showed the heaviest fresh bulb weight. Additionally, Dixit and Chaudhary (2014) stated that the colchicine 0.5 % induced duplication of the diploid garlic genome, darker green leaves, and increased pyruvate concentration through in vitro stem disc culture.

Indonesian consumer's preference for shallots is dense bulbs, large-sized bulbs (diameter ≥ 2.5 cm), spicy taste, and fragrant (Sari et al. 2019). Shallot characteristics can increase by mutations using a chemical mutagen, such as colchicine, and the part used as a mutation object is Srikayang bulbs. Colchicine mutagenics, when used at appropriate concentrations and exposure times, can produce polyploid individuals. Polyploid induction of garlic in vitro with 0.5% colchicine resulted in autopolyploid plants with higher biomass and secondary metabolites than diploid (Touchell 2020). Polyploidy impacts morphological changes due to increased cell size, and other impacts of polyploid are increased phenotypic plasticity, leading to stress tolerance and induced gigantism (Corneillie et al. 2019; Harrison et al. 2023). The character of polyploid plants is reflected in their morphology and anatomy and an improvement in the plant's qualities. Genetic changes in traits can be observed through plant growth. This colchicine induction technique strengthened plant morphology (Eng and Ho 2019). Therefore, by colchicine induction, larger bulbs of local shallot size can be obtained, increasing productivity. The colchicine-induced plants are larger in size than the diploid plant.

Concentration/doses of mutagen determine the mutations that will occur. The concentration/ doses that cause death in organisms exposed to mutagens are called lethal doses. Lethal doses (LD_{50}) are the values that cause 50% of deaths in the population and are important to note because they are the optimal doses to induce diversity. This study aimed to evaluate the phenotype performance of Srikayang shallot M1 and suspect the Lethal Doses (LD_{50}) after colchicine induction.

MATERIALS AND METHODS

Experimental material

The research was conducted from September to November 2023 at the Balecatur planting garden, Sleman, Yogyakarta, and the Plant Physiology Laboratory, Faculty of Agriculture, Universitas Sebelas Maret (UNS), Surakarta, Indonesia. Srikayang shallot from Srikayang producers, Kulonprogo. Yogyakarta was selected for treatment and passed the dormancy phase (2 months after harvesting); colchicine from Sigma Aldrich catalog C9754 1 g. The polybag 25 x 30 cm, compost fertilizer, NPK, and SP-36 fertilizer were purchased from the farm shop in Yogyakarta.

Srikayang bulb treatment with colchicine

Before planting, Srikayang shallot bulbs that have been selected apply colchicine treatment and a certain soaking time. The concentration of colchicine consists of 0%, 0.05%, 0.075%, and 0.1%, with a soaking duration of 3, 6, and 12 hours. Every treatment has planted 5 Srikayang bulbs and was replicated 4 times. The control plants were without colchicine and soaking treatments. Srikayang shallot was planted 1 bulb for each polybag; it is planted 1-2 cm deep; planting condition temperature 31-34ºC, with humidity 65-75%, and soil pH 6. The watering period is twice a day in the morning and afternoon. The study was conducted in a randomized block design, and the observed variables were LD_{50} and phenotypic performance.

Assessment of LD_{50} and phenotypic performance

The LD_{50} was calculated using Probit Analysis by Microsoft Excel 2019 based on the percentage of dead plants. The percentage of alive and dead plants is calculated based on alive and dead plants until bulbs are harvested. The phenotypic performance was observed based on UPOV 2008, consisting of bulb color, leaf color, bulb shape, bulb neck shape, and root tip shape (UPOV 2008). Morphological variables of plant height, number of leaves, number of saplings, bulb weight per cluster, main bulb weight, and main bulb diameter were calculated by counting the median and variety values of M1.

RESULTS AND DISCUSSION

LD_{50} of Srikayang shallot

Each plant and variety has different lethal doses. The LD_{50} value is used to determine the appropriate dose for forming mutations. Therefore, to determine the LD_{50} value or optimum lethal doses for induced variety, we may use experimental to determine a dose that causes 50% mortality or safety doses, 50% of which plant can survive. Colchicine is a toxic chemical, and use for excessive concentration may lead to plant death. Therefore, the lethal dose amount is the main factor in obtaining mutants with the desired trait. The value of LC_{50} in garlic variety G-41 was shown at a colchicine concentration of 0.058% and used to create variability in garlic (Mahajan et al. 2015). The optimum concentration in forming mutants is estimated through a lethal value of 50. The goal is to produce new varieties without damaging their agronomic aspects, and the LD_{50} values are determined based on their mutation treatment. LD_{50} analysis on Srikayang shallots was carried out based on the dead plant using Probit Analysis in MS Excel 2019.

Based on the observations, the percentage of shallots germination treated with colchicine concentration was 0.05% in 3 and 6 hours of soaking, resulting in 80% and 75% germination. In comparison, concentrations of 0.075% and 0.1% (3, 6, and 12 hours of soaking duration) have a response to decreased germination (Figure 1). The increasing colchicine concentration and soaking duration of onion seeds were causing cell damage and decreased life chances. The relationship between the concentration of chemical mutagens and the time objects are exposed to mutagens is directly proportional.

Based on probit analysis in Table 1 calculations, the lethal dose value that causes Srikayang shallot plants to die 50% is the concentration of 0.065%. The simple linear regression equation used is $y = a + bx$, then the linear regression equation is $y = 4.25\ x-6.96$, as seen in Figure 2. Table 1 shows 78 (43.33%) living plants from 180 individual treatment plants can be observed and become potential mutagenic plants. It shows that colchicine treatment and soaking duration affect Srikayang shallot growth.

The LD_{50} value indicates that 50% of plants died due to colchicine treatment, with a concentration value of 0.065%. Those calculations showed that higher concentrations of colchicine increase the probit unit. The difference in LD_{50} values in each commodity and variety can be caused by moisture conditions that affect the sensitivity of cells in responding to mutagens. Target genes, mutagen doses, and sensitivity of the target organism are several factors that affect mutation (Khursheed et al. 2019). The importance of determining lethal dose values, growth inhibitors, and chromosomal damage in large-scale mutations was to determine the frequency of optimal mutagen doses with minimal damage.

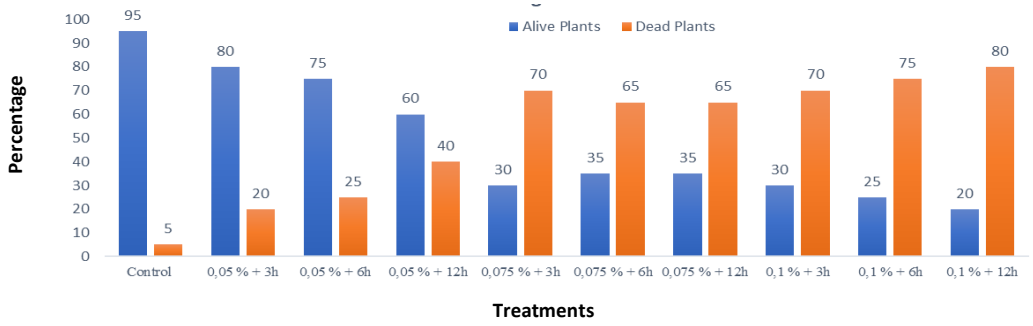


Figure 1. Percentage of living and dead plants in any colchicine concentration and soaking duration

Table 1. Assessment of colchicine treatment to Srikayang shallot with probit analysis LD_{50}

Concentration	Log_{10} (Concentration)	Mortality (%)	Total living plants (plants)	Total dead plants (plants)	Probit unit	Lethal doses
0.05%	2.69	28.33	43	17	4.43	LD_{50} = 0.065%
0.075%	2.87	66.67	20	40	5.43	
0.1%	3.00	75.00	15	45	5.67	
Total		56.67	78	102		

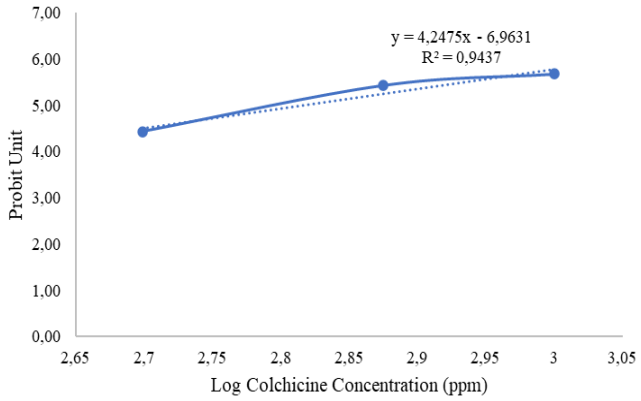


Figure 2. Linear regression graphic of LD_{50}

Phenotypic performances of Srikayang shallot M1

Colchicine applied to shallots responds differently to each individual and affects the phenotype of those plants. Some treated plants exhibited rapid height increase, wide bulb necks, extreme bulb division, and bulb color changes and tend to be lighter than controls, while others cannot form bulbs (Figure 3); the variations created through artificial mutations will be selected. Figure 3 shows that colchicine concentration and soaking duration affect individual performance and the ability to work in active cells. The wide bulb neck is affected by microtubule arrangement, doubling chromosome number. The extreme bulb division and bulb aren't produced, and they are affected by tissue growth rate and differences in cell sizes induced by colchicine activity. Colchicine can cause stunted seed growth in high concentrations (Yan et al. 2022).

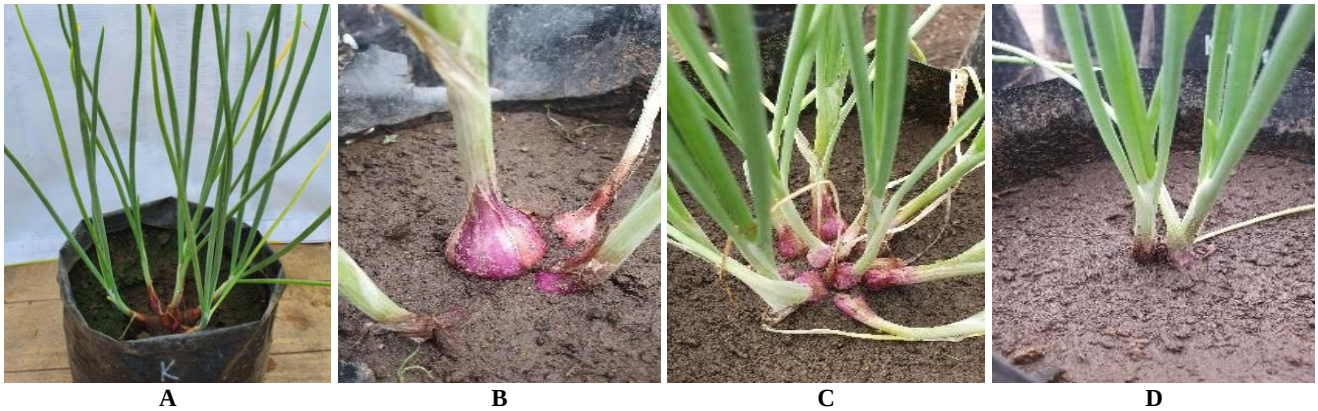


Figure 3. Morphological appearance of Srikayang shallot after induced with colchicine. A. Control, B. Wider bulb necks by colchicine 0.05 % + 3 h, C. More bulbs with small size by colchicine 0.075 % + 3 h, and D. Bulbs aren't produced by colchicine 0.1 % + 6 h

Table 2. The phenotype and morphology of Srikayang shallot M1 induced by colchicine

Treatment	Leaf color	Bulb skin color	Bulb shape	Bulb neck shape	Root tip shape
Control	137 C Yellow Green	71 A Deep Purplish Red	Broad elliptic	Rounded	Round
0.05 % + 3 h	137 A, 137 B Olive Green	71 A Deep Purplish Red	Rhombic	Slightly raised	Depressed
0.05 % + 6 h	137 B Olive Green	71 A Deep Purplish Red	Rhombic	Rounded	Depressed
0.05 % + 12 h	137 B Olive Green	71 A Deep Purplish Red	Broad ovate	Rounded	Depressed
0.075 % + 3 h	137 B Olive Green	71 A Deep Purplish Red	Circular	Rounded	Round
0.075 % + 6 h	137 B Olive Green	71 B Strong Purplish Red	Medium ovate	Slightly sloping	Round
0.075 % + 12 h	137 B Olive Green	71 D Strong Purplish Red	Rhombic	Rounded	Round
0.1 % + 3 h	137 D Yellowish Green	71 B Strong Purplish Red, N 75 D Pale Purple	Circular	Rounded	Round
0.1 % + 6 h	137 C Yellow Green	71 D Strong Purplish Red	Rhombic	Rounded	Round
0.1 % + 12 h	137 D Yellowish Green	71 B Strong Purplish Red	Medium ovate	Slightly sloping	Round

Note: Color rendering by Royal Horticulture Society color chart

Figure 4. Visualization from leaf color and bulb skin color from a treated sample of colchicine 0.05 % + 12 h

Colchicine applied to Srikayang shallots causes the leaf color to turn 1 level darker than the control (according to the description of Srikayang shallots). It was found at concentrations of 0.05% and 0.075%, while at concentrations of 0.1%, leaf color tended to be the same and lighter than controls (Table 2). In bulb skin color, 0.05% (3, 6, and 12 h) and 0.075 % + 3 h gave the same bulb skin color response as the control, while at 0.075% (6 and 12 h) and 0.1% colchicine concentrations gave bulb skin color response 1-2 levels lighter than the control. The concentration of colchicine and soaking duration affect the character of the shallot bulb (Table 2). In bulb shape, only the control has a broad elliptic, while in 0.05% (3 and 6 hours), 0.075% + 12 hours, and 0.1%+ 6 hours, found that bulb shapes are rhombic. Colchicine treatment 0.05% + 12 hours has broad ovate in bulb shape. Circular bulb shape was found in colchicine treatment at 0.075% + 3 hours and 0.1% + 3 hours, while medium ovate bulb shape was found in 0.075% + 6 hours and 0.1% + 12 hours colchicine treatment. The bulb neck shape is mostly rounded as the control. The colchicine treatment 0.05% + 3 hours has slightly raised, and 0.1% + 12 hours has slightly sloping, in bulb neck shape. The root tip shape is mostly round as the control, while in colchicine treatment, 0.05% (3, 6, and 12 hours) showed a depressed root tip shape.

Based on the *Allium* descriptor, the bulbs' character was observed after harvest (UPOV 2008). This study found that the bulb shape of colchicine treatment consists of rhombic, broad ovate, medium ovate, and circular shape (Figure 5). The bulb neck was rounded, slightly raised, and slightly sloping (Figure 6). The root tip shape is found to be round and depressed, as seen in Figure 7. The phenotype performance of the Srikayang bulb has changed by colchicine induction. Many variations of bulb shape, bulb neck, and root tip shape from this study can be caused by organic matter cells changing. Colchicine increases protein, vitamins, total weight, and cell number (Farhadi et al. 2022).

The morphological changes by ploidy level are one way to adapt plants to different environmental conditions. Generally, polyploid plants are more tolerant of adverse environmental conditions than diploid plants (Farhadi et al. 2022). Colchicine can affect plant cells, resulting in chromosomal gene structure changes. Colchicine is commonly used as an antimitotic agent due to its better penetration and faster mobility in cells than other chemical mutagens such as trifluralin, oryzalin, and methyl-amiprophos (Gantait and Mukherjee 2021).

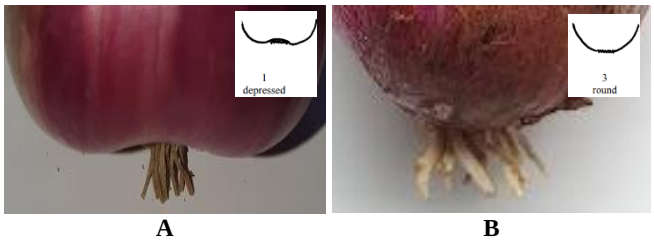
The mutagen effects of colchicine and EMS are reported to effectively provide morphological variation and alter cell metabolism in *Crocus sativus* L (Samadi et al. 2022). Application of colchicine to *Cucumis melo* "Chammel" resulted in tetraploid plants whose seeds were wider and heavier than their diploid (Woo et al. 2021). Colchicine is also used as a mutagen in gladiolus, petunias, calendula, and chrysanthemums to increase the aesthetic value of such ornamental plants. Colchicine-induced *Echeveria Peerless* houseplants show thicker leaves, darker flowers, and better leaf compactness (Cabahug et al. 2021).

A **B** **C** **D** **E**

Figure 5. Variation of bulb shape: A. Rhombic, B. Broad ovate, C. Medium ovate, D. Circular, E. Broad elliptic (control)

A **B** **C**

Figure 6. Variation of bulb neck shape: A. Rounded, B. Slightly raised, C. Slightly sloping



A **B**

Figure 7. Variation of root tip shape: A. Depressed, B. Round

Colchicine concentration between 0.025-0.1 % with 24 hours soaking duration produced mixoploid plants and affected the plant height, fruit flesh thickness, and the number of Katokkon fruits (Tammu et al. 2021). Diploid and polyploid plants have different morphological forms. These different morphologies are the response of cells to colchicine induction, resulting in genetic material changes. Phenotypic and genetic changes in plants caused by polyploidy occur due to increases in cell size, allele diversity (heterozygosity), gene dose effects, or interactions between epigenetics and genetics (Manzoor et al. 2019).

Table 3 shows that applying colchicine at different concentrations and soaking durations had different diversity responses to variable plant height, number of leaves, number of saplings, the weight of bulbs per cluster, the main bulb weight, and the main bulb diameter. There is an increase in the variety value of all variables observed, proving that colchicine treatment in Srikayang shallot effectively increases plant diversity. Based on the observations on plant height, number of leaves, and saplings of shallots Srikayang population M1 shows the variation of characters observed by the range of values. One indicator of the success of mutation induction can be evaluated by estimating the mean and range of values. Estimation of flattening is carried out through observations on each M1 individually; on the plant height variable, the highest range of values was shown in the treatment of 0.1% colchicine + 6 hours soaking duration, with variety values 48.82. In comparison, the number of leaves, the highest range of values, was shown in the treatment of 0.05% colchicine + 3 hours soaking duration, with variety values of 30.51. Compared to the variable number of saplings, the largest range of values is shown at a colchicine concentration of 0.075% + 3 hours soaking duration, with a variety value of 6.66. This shows that applying colchicine mutagen can increase the diversity in Srikayang shallots M1. The different ranges of values between treatments indicate mutational events by chemical mutagens, which are randomly distributed in the genes of exposed organisms.

The observed variable of bulb weight per cluster had the highest variety value shown in colchicine concentration 0.05% + 12 hours soaking duration, with 178.74 variety value. The highest variety value of main bulb weight is 7.26, obtained at a colchicine concentration of 0.05% + 12 hours soaking duration. The highest main bulb diameter variety values of 28.17 were shown on colchicine concentration of 0.05% + 12 hours soaking duration. The increased colchicine concentration and soaking duration improve the variety of responses to the bulb weight per cluster, the weight of the main bulb, and the main bulb diameter significantly more than the control. Larger colchicine concentration and soaking duration induced a larger variety value, but it was decreased on colchicine concentration of 0.1% (Table 3).

Table 3. Median and variety values of Srikayang shallot M1

Treatment	PH (cm)		NL (leaves/plant)		NS (saplings/plant)		BWC (g)		MBW (g)		MBD (mm)	
	X	σ^2	X	σ^2	X	σ^2	X	σ^2	X	σ^2	X	σ^2
Control	29.5	4.68	12	14.22	6	2.16	26.9	87.58	6	3.35	21.1	9.72
0.05 % + 3 h	30.5	29.20	10	30.51	5	1.40	16.3	171.68	4.4	6.83	20.3	19.79
0.05 % + 6 h	30.1	15.96	7	3.83	5	1.63	15.8	13.93	3.5	3.16	18.7	9.41
0.05 % + 12 h	27.3	10.04	7,5	28.90	5	1.29	18.5	178.74	5.7	7.26	22.5	28.17
0.075 % + 3 h	26.6	11.14	10	24.70	5.5	6.66	18.4	130.22	7.1	2.85	24.5	23.62
0.075 % + 6 h	24.5	8.49	8	1.23	6	1.33	10.3	5.89	2.7	5.14	15	4.19
0.075 % + 12 h	26	6.97	9	14.66	5	1.57	19	31.48	4.6	1.10	20.3	8.66
0.1 % + 3 h	27.9	7.41	5	4.26	5	0.96	11	20.66	3.6	1.03	19.2	9.98
0.1 % + 6 h	23.4	48.82	5	11.30	3	4.70	9.3	49.44	3.5	3.50	16.2	17.44
0.1 % + 12 h	22.7	16.24	5	3.66	4.5	1.66	5.1	37.90	1.7	1.04	13.2	5.73

Note: PH: plant height, NL: number of leaves, NS: number of saplings, BWC: Bulb weight per cluster, MBW: main bulb weight, MBD: main bulb diameter, X: median, σ^2: variety values

Table 4. The range value of Srikayang shallot M1

Treatment	PH (cm)		NL (leaves/plant)		NS (saplings/plant)		BWC (g)		MBW (g)		MBD (mm)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Control	26.3	33.8	7	19	4	9	11.3	44.4	2.7	9.8	14.6	27.8
0.05 % + 3 h	25	45	4	26	3	8	8.6	57.9	2.5	12.7	14.6	30.2
0.05 % + 6 h	21	37.1	4	11	3	7	7.7	20.9	2.1	8.7	14.1	24.7
0.05 % + 12 h	22.2	32.7	3	20	3	7	8.8	45.6	2.4	9.8	15.1	28.2
0.075 % + 3 h	25.7	32.8	4	19	2	9	16.3	45.8	2.9	11	17.8	30.5
0.075 % + 6 h	20.3	29.3	6	9	5	8	7.9	14.2	2.2	8.3	13.5	19.5
0.075 % + 12 h	23	30.8	4	15	3	7	8.3	22.3	2.1	5.1	14.6	22.5
0.1 % + 3 h	22.5	30.2	3	9	3	6	10.5	22.1	2.6	5.4	13.9	21.7
0.1 % + 6 h	22.6	39	4	12	3	8	5.7	23.8	1.7	5.5	12.2	22.6
0.1 % + 12 h	22	30.4	3	15	3	6	4.5	17.2	1.6	3.7	10.9	16.7

Note: PH: plant height, NL: number of leaves, NS: number of saplings, BWC: Bulb weight per cluster, MBW: main bulb weight, MBD: main bulb diameter

Table 4 shows the minimum and maximum (range value) from Srikayang shallot M1 induced by colchicine. Plant height ranges value is from 20.3 to 45 cm, the number of leaves ranges from 3 to 26 leaves/plant, the number of saplings is 2 to 9 saplings/plants, bulb weight per cluster ranges value from 4.5 to 57.9 g, main bulb weight ranges value from 1.6 to 12.7 g, and main bulb diameter range value from 10.9 to 30.5 mm. Using colchicine in high concentrations may lead to stunted plant growth. Variation changes occur in Srikayang shallot plants because few organs were mutated. The bulb component is the most important parameter in onion cultivation activities, so selecting the right concentration and soaking duration of colchicine is expected to produce polyploidy individuals characterized by a large bulb size. Colchicine acts on cells by binding to α-dimers and β-tubulins that inhibit microtubule polymerization during the mitotic cycle, doubling chromosomes. This cytostatic agent is able to bind to tubulin dimers to prevent spindle formation, shorten the length of the spindle fibers, and cause temporary inactivation of spindle cleavage; this affects the individual plants' appearances. Tetraploid plants show an increase in organ size, stomata size, trichome density, and the content of secondary metabolites and their biomass (Parsons et al. 2019).

Moreover, the plant-induced colchicine size is larger than the diploid plant. Colchicine concentration of 0.25%-0.5% with a soaking duration of 24, 36, and 48 hours effectively induces basal meristem in garlic discs in vitro, and it is expected to produce ploidy individuals with large bulb sizes and increase production yield (Hailu et al. 2021). The induction of colchicine affects the phenotype of plants due to polyploidization in cells, so the effect on plant organs becomes greater. Treatment of various concentrations of colchicine and soaking time were able to increase the phenotype of *Vigna unguiculata* var. *sesquipedalis* on variables of plant height, pod weight, number of pods, and weight of 100 seeds (Fathurrahman et al. 2023); those variability components are used in plant breeding programs. There is a pattern of increased variety values starting from colchicine concentration 0.05% + 3 hours soaking duration, then a decrease in variety values starting from 0.1% concentration + 3 hours soaking duration. The decrease in variety value is related to the number of plant deaths, as the concentration of colchicine increases and the soaking duration is influenced by lethal doses (LD_{50}). The highest genetic variability is produced in LC_{20}-LC_{50} or LD_{20}-LD_{50} (Sari et al. 2019).

In conclusion, lethal doses (LD_{50}) for Srikayang shallot were found in 0.065 % of colchicine concentration.

Colchicine treatment can improve the variability and phenotype performance of Srikayang shallot. The concentration of colchicine and soaking duration affected the phenotype performance of Srikayang M1 in leaf color, bulb skin color, bulb shape, bulb neck shape, and root tip shape. This study showed that colchicine concentration at 0.05% + 12 hours soaking duration has the highest genetic variability on bulb weight per cluster, main bulb weight, and main bulb diameter. Colchicine concentration of 0.05% + 3 hours of soaking duration gives the highest response to several leaves parameters. Colchicine concentration of 0.075% + 3 hours has the highest genetic variability on the number of saplings parameter, while 0.1% + 6 hours soaking duration had the highest variability on plant height.

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