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RESEARCH ARTICLE
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Evaluation of Mutation Induction using Colchicine on Morphophysiological, Anatomical and Cytogenetic Characteristics of Samosir Local Shallots

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ABSTRACT

Samosir local shallots from North Sumatra are considered superior regional commodities, yet their cultivation is constrained by increasingly limited genetic resources and low productivity, primarily due to small bulb size. To address this limitation, genetic enhancement through induced mutation was explored using the chemical mutagen colchicine. This study aimed to assess the effects of colchicine on the morphophysiological, anatomical, and cytogenetic characteristics of Samosir local shallots. A factorial randomized complete block design with two factors and three replications was used. The first factor was the accession of Samosir local shallots (accessions Siunong unong Julu, Simamora 3, and Tipang 2), and the second factor involved soaking shallot bulbs in differing concentrations of colchicine solution for 24 hours, including control with distilled water, as well as concentrations of 200ppm, 400ppm, and 600ppm. The results showed that the Tipang 2 accession had higher plant length 6 weeks after planting (WAP), bulb diameter, dry weight of bulb/plant, bulb grading I, and harvest index compared to the Siunong, Unong Julu, and Simamora accessions. The 600ppm colchicine treatment tended to accelerate harvest time and increase bulb grading I. However, observations of the number and density of stomata showed non-significant differences among the Samosir shallot accessions subjected to colchicine treatment. A significant interaction was observed between Tipang 2 and 600 ppm colchicine, resulting in increased plant height. Additionally, increasing colchicine concentration from 0 to 600ppm enhanced total chlorophyll content in the Tipang 2 accession. Karyotyping and flow cytometry analysis indicated that the number of diploid chromosomes in Samosir local shallots remained stable after colchicine treatment, confirming that polyploidy induction was not observed in the M1 generation.

Keywords: Colchicine, Chlorophyll contents, Mutation, M1 generation, Samosir local shallots.

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INTRODUCTION

Shallots (*Allium ascalonicum* L.) are horticultural commodities that play an important role as flavouring agents, food industry ingredients, and biopharmaceutical sources because of their bioactive compounds such as saponins, flavonoids, essential oils, allicin, alliin and quercetin (Adeyemo et al., 2023; Hasanah et al., 2024; Ratseewo et al, 2025). Quercetin has antidiabetic, anti-osteoporotic, anticancer, antioxidant, antidiarrheal, antiallergic and antibacterial properties (Moldovan et al., 2024).

The famous shallot planting area in North Sumatra is located around Lake Toba at an altitude of 900–2000m above sea level. This area includes the Muara District, Bakti Raja District, Silalahi Village, Merek District, Haranggaol District and Samosir Island. Local variety of shallots, commonly

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called "Toba shallots" or Samosir shallots, are a type of shallot cultivated around Lake Toba. Also called "Medan shallots", Toba shallots have a long-lasting and unique aroma that distinguishes them from other varieties. Toba shallots only grow in their place; therefore, if they are planted elsewhere, their aroma will differ (Simamora et al., 2024).

Local shallot cultivation on Samosir Island has decreased because of the introduction of shallots. Therefore, efforts must be made to save the local Samosir shallots and maintain their superior properties. Local Samosir shallots are classified as superior commodities based on increasingly rare local resources, even though they have advantages such as a very distinctive taste and aroma, fragrant and more pungent, redder, and shinier colour, less water content, and high selling price in the market, but small bulb sizes. The main obstacle to shallot production is low productivity (average of 6.45tons/ha), partly due to the small size of the bulbs (Napitupulu et al., 2021; Hasanah et al., 2024).

The strategy to increase the productivity of local Samosir shallots as a local resource is carried out by increasing the size of the bulbs. Therefore, to increase productivity, genetic improvement is needed to increase the size of the bulbs, namely, through mutation techniques to obtain the desired properties. Character diversity is essential for plant populations. If the population does not have wide diversity, it is better to first increase the character diversity. In agriculture, especially plant breeding, the success of breeding depends on genetic variation. One way to increase character diversity in plants is to use plant mutation breeding methods (Yali & Mitiku, 2022; Galatal et al., 2023). Indonesian shallot consumers prefer dense and large bulbs (diameter ≥ 2.5cm), spicy taste, and fragrant (Sari et al., 2019). The characteristics of shallots can be improved by using mutation and polyploidy techniques. Mutation and polyploidy techniques are primarily applied to vegetatively propagated (Manzoor et al., 2019; Chadipiralla et al., 2020). Mutation induction improves genetic quality, with the aim of obtaining plants based on desired traits through changes in the genetic composition of plants (Sarsu, 2020; Ishtiaq et al., 2023). This causes changes in the appearance and behaviour of living organisms. If the structure or amount of genetic material is not directed, diversity increases. Therefore, diversity arises because of the mutations that produce genetic variations.

Colchicine is a chemical compound of the alkaloid group that can cause mutations and is widely used to stimulate the induction of polyploidy in plants during cell division by inhibiting chromosome segregation. Colchicine is also classified as a mutagen which plays a role in preventing the formation of microtubules and doubling the number of chromosomes; therefore, it is used to develop polyploid plants and is a mitotic inhibitor that produces many mutagenic effects in plants (Miri, 2020; Samadi et al., 2022; Wu et al., 2022). In addition, colchicine affects the diversity of plant phenotypes and genotypes (Münzbergová, 2017; Fathurrahman et al., 2023), because colchicine affects plant physiology, which causes plants to appear larger and stronger (El-Nashar and Ammar, 2016).

The mechanism of colchicine in enlarging shallot bulbs is to induce polyploidy in the form of doubling the number of chromosomes. Colchicine binds to tubulin, a protein that forms spindle fibers, thereby inhibiting spindle fiber formation during cell division, so that chromosomes cannot separate and the number of chromosomes doubles. This causes an increase in the size of cells and tissues, including shallot bulbs. Polyploid plants show characters reflected in morphology, anatomy, and improved plant quality. Observation of changes in genetic traits can be done through observation of plant growth. This colchicine induction technique strengthens plant morphology (Eng and Ho, 2019). Therefore, with the induction of colchicine mutagen, it is expected that local red onion bulbs will be larger so that productivity will increase.

At the right concentration and exposure time, the chemical mutagen colchicine can produce polyploid individuals. Previous studies have shown that 0.5% colchicine treatment of garlic in vitro produces autopolyploid plants that have higher secondary metabolites and biomass than diploids (Touchell, 2020). Similarly, Rahmawati et al. (2024) reported that a colchicine treatment of 0.05% with a 12h soaking duration produced the greatest genetic variability in tuber weight per clump and main tuber diameter, while a shorter soaking duration of 3h at the same concentration yielded the most pronounced improvements in leaf morphology. Research on the role of the chemical mutagen colchicine in morphophysiological, anatomical, and cytogenetic characteristics of Samosir local shallots has not been widely conducted. Therefore, the present study aimed to evaluate the influence of colchicine treatment on these traits in Samosir local shallots, with the objective of inducing polyploidy and enhancing their genetic potential for crop improvement.

MATERIALS & METHODS

Study Area

The research was conducted at the screen house of the Faculty of Agriculture, Universitas Sumatera Utara, to investigate the response of morphophysiological, anatomical and cytogenetic characteristic of Samosir local shallots treated by colchicine, with the expectation of inducing the formation of polyploids plants. This study was conducted between September to November 2024. The karyotype, chlorophyll and stomata analysis were conducted in the Biotechnology Laboratory, Faculty of Agriculture, Universitas Sumatera Utara, while flow cytometry analysis was conducted in the Genomic Laboratory, National Research and Innovation Agency, Cibinong, Bogor, Indonesia.

Materials and Tools

The materials used were Samosir local shallot accessions (Siunong unong Julu accession, Simamora 3 accession and Tipang 2 accession), manure, paper bags, colchicine powder, KOH to dissolve colchicine, distilled water to dilute colchicine, insecticides, and *Trichoderma harzianum* as biological fungicide, nail polish, glass slide, tape, scissors for stomatal analysis. Fungicides with the active ingredient difenoconazole and insecticides with the active ingredient propineb 70% are used to control plant

pests and diseases. The tools used included hoes, scales, scrapers, research name plates, watering buckets, stakes, spectrophotometers, microscopes, analytical scales, and several other tools used for laboratory analysis.

Procedures

The Samosir local shallot bulbs used as planting material originate from bulbs that have been harvested for approximately 3 months, measuring ± 1.50 – 1.80cm in diameter. Bulbs were obtained from bulb producers at the local shallots production centre in Humbang Hasundutan Regency (Table 1). A two-factor randomized complete block design (RBCD) was used with two factors and three replications. The first factor was the Samosir local shallots accession (Siunong unong Julu accession, Simamora 3 accession and Tipang 2 accession), and the second factor involved soaking shallot bulbs in differing concentrations of colchicine solution for 24 hours, including control with distilled water, as well as concentrations of 200, 400, and 600ppm.

Table 1: Locations for the three local Samosir shallot accessions in Humbang Hasundutan Regency

Accession	GPS	Altitude (m asl)
Siunong unong Julu	N 2º 18′ 11,803′′ E 98º 48′ 3,634′′	984,2
Simamora 3	N 2º 18′ 39,769′′ E 98º 48′ 41,160′′	933,6
Tipang 2	N 2º 20′ 55,698′′ E 98º 48′ 57,924′′	927,4

Each experimental unit was a 40 × 40cm polybag filled with a planting medium containing a mixture of soil, husks, sand, and manure with their respective ratios 1:1:0.5:1. Each experimental unit contained five single shallot bulbs that had been soaked in colchicine solution according to the treatment, cut 1/3 at the tip, and planted in polybags. Shallot bulbs per accession were planted with one bulb per polybag. Planting was performed at a depth of 1-2cm. The dose of fertilizers used ZA was applied at a rate of 150kg/ha at 7 weeks after planting (WAP); NPK (16-16-16) was applied at 200kg/ha at 14 WAP, and 250kg/ha at 28 WAP; an additional application of NPK (250kg/ha) along with KCl (187.5kg/ha) was also administered, as recommended by Hasanah et al. (2022). The biological agent as fungicide *Trichoderma harzianum* (100kg/ha) was applied one week before planting to prevent root rot disease caused by the fungus *Fusarium oxysporum* by dissolving it in water and then watering with 250 ml of a solution containing *Trichoderma harzianum*. Watering was done twice a day (morning and evening). Weeds in polybags were cleaned manually. Pest control on shallots was carried out using insecticides with the active ingredient profenofos 2ml/L. Control of fusarium wilt disease (*Fusarium oxysporum*) was carried out using fungicides with the active ingredient difenoconazole 2g/L by spraying using a sprayer. Spraying is carried out at intervals of 1-2 weeks. Shallots harvesting is carried out 70 days after planting (DAP), with the harvest criteria being when the leaves begin to fall and the bulbs emerge above the soil surface. Drying is carried out by air drying for 7 days. Morphological and agronomic traits observed included plant height, number of leaves, days to harvest, bulb diameter, and number of bulbs per plant, dry bulb weight per plant, harvest index, and bulb grading. Bulb grading was assessed in accordance with the Indonesian National Standard (SNI No. 01-3159-1992).

Chlorophyll content was analysed according to the method described by Hendry and Grime (1993) by using a spectrophotometric method. Chlorophyll content was determined by collecting leaf samples (0.1g) and macerating with 10mL of acetone using a mortar and pestle. The formula for determining the chlorophyll content is as follows:

Chlorophyll-a = {(12.7x A663)- (2.69 x A645)}/10
Chlorophyll-b = {(22.9 x A645)- (4.68 x A663)}/10
Chlorophyll total = {(22.9 x A649) + (20.2 x A 645)}/10

These values are expressed as µg/g fresh weight (µg/gfw). Stomatal density was determined using a clear nail polish measuring 1 × 2cm which was applied to the abaxial part of the leaf to provide an impression of the leaf surface. The samples were collected after drying and viewed under a microscope at 40×10 magnification. The unit of stomatal density used is mm^{-2}.

Flow Cytometry Analysis

Leaf pieces measuring approximately 0.5×0.5cm were placed on a petri dish and then dripped with 250µL of nuclear extraction buffer and a small amount of polyvidone before being cut carefully using a razor blade. A 30µm Millipore filter was used to filter the cut leaf fluid. The filtrate was put into a cuvette tube and 350µL of dye solution, specific fluorochromes (propidium iodide), and RNAse were added for analysis (Adabiyah et al., 2023). Flow cytometric analysis was conducted using a BD Accuri™ C6 Plus flow cytometer (BD Biosciences, USA) to determine the nuclear DNA content and assess ploidy levels, as described by Galbraith and Lambert (2012).

Calculation of the number of chromosomes carried out by the squashing method, namely observing the number of chromosomes after the bulbs are harvested, then 1 clove per treatment is taken and germinated. The tips of the roots of the treated shallot sprouts are cut and washed with running water, then wiped with tissue and fixed in the initial solution (glacial acetic acid and absolute ethanol 1:3). Then put in the refrigerator for 3h, then the roots are removed and put into a vial containing 1N HCl solution and heated at 60°C for 1-3min. Then the roots are removed and placed on a glass object and cut to a length of 2mm, then given 1 drop of 2% orcein solution, left for 10min, then covered with a cover glass and pounded with the tip of a rusty pencil until thin, then passed through a bunsen flame 3 times. The cover glass is pressed with the thumb; therefore, the edges are dipole before being photographed under a light microscope (Olympus CX 23, Japan) at a magnification of 100×10.

Grading of shallot bulbs is done based on bulb diameter, which will be grouped into several quality classes in accordance with the Indonesian National Standard 01-3159-1992 shallots as follows : grade 1 (diameter ≥ 1.7cm) ; grade II (diameter 1.3 – 1.7cm) ; grade III (diameter <1.3cm). In this study, the observation variables for bulb grading were expressed in %. The harvest index can be calculated by dividing the economic weight (dry weight of bulb) by the biological weight (dry weight of stove + dry weight of bulb).

The morphophysiological and anatomical data were analysed using analysis of variance was a significant difference then Duncan's Multiple Range Test was carried out at α = 5%.

RESULTS & DISCUSSION

Mutation techniques can be used in plant breeding to increase genetic diversity. This enables breeders to select plant genotypes for breeding purposes. Plant organs such as rhizomes, tissue culture media, roots, tubers, pollen, seeds, stem cuttings and so on. One of the most widely used and effective chemicals, colchicine, can change the number of chromosomes in cells. This is because they are readily soluble in water. Polyploidy can occur naturally or artificially (Azeem et al., 2025; Younas et al., 2025).

Colchicine can be applied to seedlings at the growing point or the seedlings can be placed in a colchicine solution for a period of time. The amount of colchicine required and the length of treatment required to change the chromosome composition vary from species to species. Colchicine stops spindle fibre formation and cytokinesis, resulting in cells with more chromosomes (Zhou et al., 2017; Soomro et al., 2025).

Chlorophyll a, Chlorophyll b and Chlorophyll Total

Based on the results (Table 2), each accession had a different pattern regarding the effect of colchicine treatment. In the Siunong Unong Julu and Simamora accessions, there was an increase in chlorophyll with colchicine treatment up to 400ppm, whereas in the 600ppm colchicine treatment, there was a decrease in chlorophyll a level. In the Tipang 2 accession, chlorophyll a decreased with increasing colchicine treatment (0-600ppm). Previous research has reported that the critical concentration also determines whether changes in the number of chromosomes occur because chromosome duplication occurs only at certain critical concentrations. The application of colchicine is more effective when colchicine solutions are soaked or incubated on dividing cells. This is because dividing cells absorb a given solution (Shariatpanahi et al., 2021; Nirala et al., 2023).

Table 2: Effect of colchicine on chlorophyll a, chlorophyll b and total chlorophyll content (unit/g fresh weight of leaves) of Samosir local shallots accession

Variable observed	Colchicine (ppm)	Accession			Means
		A1 (Siunong unong Julu)	A2 (Simamora 3)	A3 (Tipang 2)	
Chlorophyll a	K_0 (0)	20.74f	25.761de	30.44a	25.65
	K_1 (200)	26.77bcde	25.22e	29.20ab	27.07
	K_2 (400)	26.46cde	29.07ab	30.16a	28.57
	K_3 (600)	22.21f	28.07abcd	28.75abc	26.35
	Means	24.05	27.03	29.64	
Chlorophyll b	K_0 (0)	9.62e	16.25cd	28.50a	18.12
	K_1 (200)	14.89cde	16.10cd	24.11ab	18.37
	K_2 (400)	15.79cd	18.74bcd	23.08bc	19.20
	K_3 (600)	13.59de	20.08bc	18.67bcd	17.45
	Means	13.47	17.79	23.59	
Chlorophyll total	K_0 (0)	30.51e	42.20cd	59.16a	43.96
	K_1 (200)	41.86cd	41.50cd	53.53ab	45.64
	K_2 (400)	42.45cd	48.03bc	53.47ab	47.99
	K_3 (600)	35.96d	48.36bc	47.63bc	43.99
	Means	37.70	45.03	53.45	

Notes: The number followed by the same letter in the same variable observed indicated not significantly different based on Duncan's Multiple Range Test at α = 5%

The chlorophyll b content of the Tipang 2 accession decreased at high colchicine concentrations (600ppm). Different responses were found in the Siunong unong Julu and Simamora 3 accessions, where increasing colchicine concentrations tended to increase the chlorophyll b content. In the Siunong unong Julu accession, 200-600ppm colchicine treatment increased chlorophyll b levels compared with the control (without colchicine treatment). In the Simamora accession, chlorophyll b tended to increase with increasing colchicine treatment, whereas in the Tipang 2 accession, it decreased with increasing colchicine treatment (Table 2). As shown in Table 3, in the Siunong Unong Julu accession, there was an increase in total chlorophyll with colchicine treatment up to 400 ppm, whereas in the 600ppm colchicine treatment, there was a decrease in the chlorophyll a level. In the Simamora 3 accession, total chlorophyll increased with increasing colchicine concentration, whereas in the Tipang 2 accession, chlorophyll a decreased with increasing colchicine treatment (0-600ppm). The results indicated that total chlorophyll was strongly influenced by the interaction between genetic factors and colchicine treatment, and that these effects varied among different accessions. Many studies have shown that colchicine concentration and application time are the main factors influencing polyploid induction (Ridwan et al., 2025). Colchicine may cause changes in the shape, colour, size, and quantity of chromosomes. Colchicine can undergo polyploidization, causing the number of chromosomes to double, resulting in a larger morphology. Colchicine-induced plants at optimal concentrations can produce polyploid plants, but a concentration that is too high or soaking for too long will result in plant death. Chlorophyll is a substance that gives colour to leaves. Colchicine-induced plants have a darker green leaf colour, and it is possible that they are polyploid plants with a higher chlorophyll content. An increase in the number of chromosomes is positively correlated with the amount of DNA in polyploid plants. This phenomenon supports faster synthesis of chlorophyll in polyploid plants (Zhang et al., 2024; Liu et al., 2025).

Table 3: Effect of colchicine on stomatal number in Samosir local shallot accessions

Variable observed	Colchicine (ppm)	Accession			Means
		A_1 (Siunong unong Julu)	A_2 (Simamora)	A_3 (Tipang 2)	
Number of stomata	K_0 (0)	23.33	18.00	13.00	18.11
	K_1 (200)	18.00	13.33	20.67	17.33
	K_2 (400)	21.00	24.00	21.33	22.11
	K_3 (600)	30.00	22.00	16.33	22.78
	Means	23.08	19.33	17.83	
Stomatal density	K_0 (0)	118.90	91.72	62.84	91.16
	K_1 (200)	91.72	67.94	105.31	88.32
	K_2 (400)	107.01	122.29	108.70	112.67
	K_3 (600)	149.53	112.10	83.23	114.96
	Means	116.79	98.51	90.02	

Notes: The number followed by the same letter in the same variable observed indicated not significantly different based on Duncan's Multiple Range Test at α = 5%

Chlorophyll *b* is characterized by its light green pigmentation, whereas chlorophyll *a* appears dark green and serves as the primary pigment for light absorption. In contrast, chlorophyll *b* functions as an accessory pigment,

capturing light energy and transferring it to chlorophyll *a* to support photosynthetic efficiency (Leister et al., 2023). Notably, chlorophyll concentration is often used as a physiological indicator of polyploidy, as polyploid plants typically exhibit increased chlorophyll content due to enhanced pro-plastid differentiation following chromosomal doubling (Kasmiyati et al., 2020).

In the present study, each accession showed a varied response to colchicine application. Treatment with up to 400ppm colchicine increased total chlorophyll levels in the Siunong unong Julu accession, whereas in the Simamora accession, the highest total chlorophyll content was found in the 600ppm colchicine treatment. The Tipang 2 accession showed the opposite response because the addition of up to 600ppm of colchicine reduced chlorophyll levels, such that the highest total chlorophyll was found in the control (without colchicine treatment).

Colchicine interferes with cell division by inhibiting the formation of spindle threads, so that chromosomes fail to separate to different poles. Chlorophyll is a green pigment in plants which plays an important role in the photosynthesis process by capturing sunlight energy and converting it into chemical energy which is stored in the form of glucose. Although colchicine does not directly affect chlorophyll synthesis, its indirect effects on cell division can affect the number and size of chloroplasts (organelles containing chlorophyll) in plant cells. Although colchicine does not directly affect chlorophyll synthesis, its indirect effects on cell division can affect the number and size of chloroplasts (organelles containing chlorophyll) in plant cells. The mechanism underlying the relationship between colchicine and changes in chlorophyll content in shallots involves increasing chromosome number, changing cell size, and changing chloroplast structure (Herawati et al., 2020; Kasmiyati et al., 2020)

The increase in chromosome number due to colchicine treatment has the potential to increase total chlorophyll content. Polyploid cells tend to be larger than diploid cells, potentially accommodating more chloroplasts and increasing photosynthetic potential. Changes in the chloroplast structure can affect the efficiency of light capture and chlorophyll production. Indirect effects on metabolism owing to polyploidy induction can cause changes in various metabolic processes in plant cells, including the production of compounds involved in chlorophyll synthesis. Higher changes in chlorophyll content were frequently observed during polyploid induction. Higher chlorophyll content increases the photosynthetic capacity and produces higher yields than diploids (Tossi et al., 2022; Bharati et al., 2023). Chlorophyll can be reduced by mutations, and is an accurate marker for determining the sensitivity of a crop to a particular chemical (Cabahug et al., 2021; Gupta et al., 2021). It is also used to evaluate the effectiveness and efficiency of different doses of mutagens for treating plants for viable mutations (Manzoor et al., 2023).

Number of Stomata and Stomatal Density

Increasing the concentration of colchicine tended to increase the number and density of stomata in each accession compared to the colchicine concentration of 0 ppm. The Siunong unong Julu accession provided a good response compared to the other two accessions. However, the application of different colchicine concentrations did not affect the number or density of stomata in any shallots accession (Table 3). Research on the density and number of stomata showed that the size of stomata is not necessarily larger than that of normal plants. Stomatal characteristics are frequently used as indicators of plant ploidy level, and in this study, it was observed that each accession had a different response to colchicine application. Although the effect was not statistically significant, there was a tendency for the highest density and number of stomata in the 600ppm colchicine treatment group. In the Simamora and Tipang 2 accessions, the density and number of stomata only increased with the 400ppm colchicine treatment, and the density and number of stomata decreased. This was consistent with the results of several previous studies (Šmarda et al., 2023; Ræbild et al., 2024).

Stomata act as a pathway for the entry and exit of water vapour and gas in plants. Morphological markers for identifying ploidy levels in many plant species can be identified by observing stomatal density, guard cell size, and the number of plastid cells. Polyploid plants can be identified based on stomatal cells because apart from the multiplication of cells, they cause the stomata to enlarge. The induction of colchicine causes plants to experience stress and survive (Zhang et al., 2024). Ploidy testing based on stomatal cell density provides scientists with an idea for the early screening of polyploidy potential, which is precise, fast, effective, and simple, and does not waste time or space to grow large plant populations. Generally, larger stomata with a lower density level are present in polyploidy, which is a characteristic of polyploidy (Mo et al., 2020; Tammu et al., 2021; Farhadi et al., 2023). One of the most promising methods for breeders to increase crop stress tolerance is genetic modification of stomatal density. Under osmotic stress, reducing stomatal transpiration without lowering CO_2 uptake can increase water-use efficiency (Hasanuzzaman et al., 2023).

In plant breeding, colchicine treatment increases stomatal size because of an increase in chloroplasts in guard cells (Yao et al., 2023). Stomatal density is inversely proportional to stomatal size; the larger the stomata, the lower the stomatal density (Tossi et al., 2022). However, the use of colchicine to reduce stomatal density remains limited because its toxicity has a negative impact on plant germination (Kurniawan et al., 2023).

Chromosome Number

Chromosome analysis provides essential insights into the genetic and structural variations induced by colchicine treatment in Samosir shallot accessions. The karyotype analysis conducted in this study confirmed that all observed accessions retained a diploid chromosome number of 2n = 2x = 16, irrespective of colchicine treatment. Based on Table 4, Fig. 1 and 2, it can be seen that all colchicine treatments produced the number of chromosomes that were still diploid (2n=16). These results indicate that colchicine treatment at concentrations of up to 600 ppm did not

Table 4: Analysis of flow cytometric summarizes the karyotype observations for each accession under different colchicine treatments

No.	Sample	Mean PI	CV %	Ploidy level
1	A_1K_0	2260928	3.70	Diploid
2	A_1K_1	2289949	3.59	Diploid
3	A_1K_2	2136148	5.48	Diploid
4	A_1K_3	2283695	3.45	Diploid
5	A_2K_0	2258880	3.96	Diploid
6	A_2K_1	2033557	5.15	Diploid
7	A_2K_2	2207691	3.72	Diploid
8	A_2K_3	2213841	3.88	Diploid
9	A_3K_0	2187497	3.98	Diploid
10	A_3K_1	2233392	4.78	Diploid
11	A_3K_2	2222490	4.19	Diploid
12	A_3K_3	2271831	4.21	Diploid

induce polyploidy in the tested accessions. Previous studies have suggested that colchicine's efficiency in chromosome doubling varies by species and depends on factors such as treatment duration and plant genotype (Shariatpanahi et al., 2021; Nirala et al., 2023). The lack of polyploid induction in this study suggests that alternative treatment conditions or extended exposure times may be required to successfully induce chromosome doubling in Samosir shallots.

The effects of colchicine application are still apparent in the second generation of plants, and its direct effects can last for several generations. The frequency of chromosomal abnormalities at mitosis was dose dependent and its percentage varied among cultivars, but nuclear abnormalities were only observed in the M2 generation plants. The selection of individual plants in the M2 generation can be studied to observe the spectrum of variation for traits and observation of mutants (Münzbergová, 2017; Badr, 2018; Thi et al., 2020).

The stability of the chromosome number across different colchicine treatments implies that structural chromosomal changes rather than numerical alterations might play a more significant role in colchicine-induced modifications. Studies on other *Allium* species have

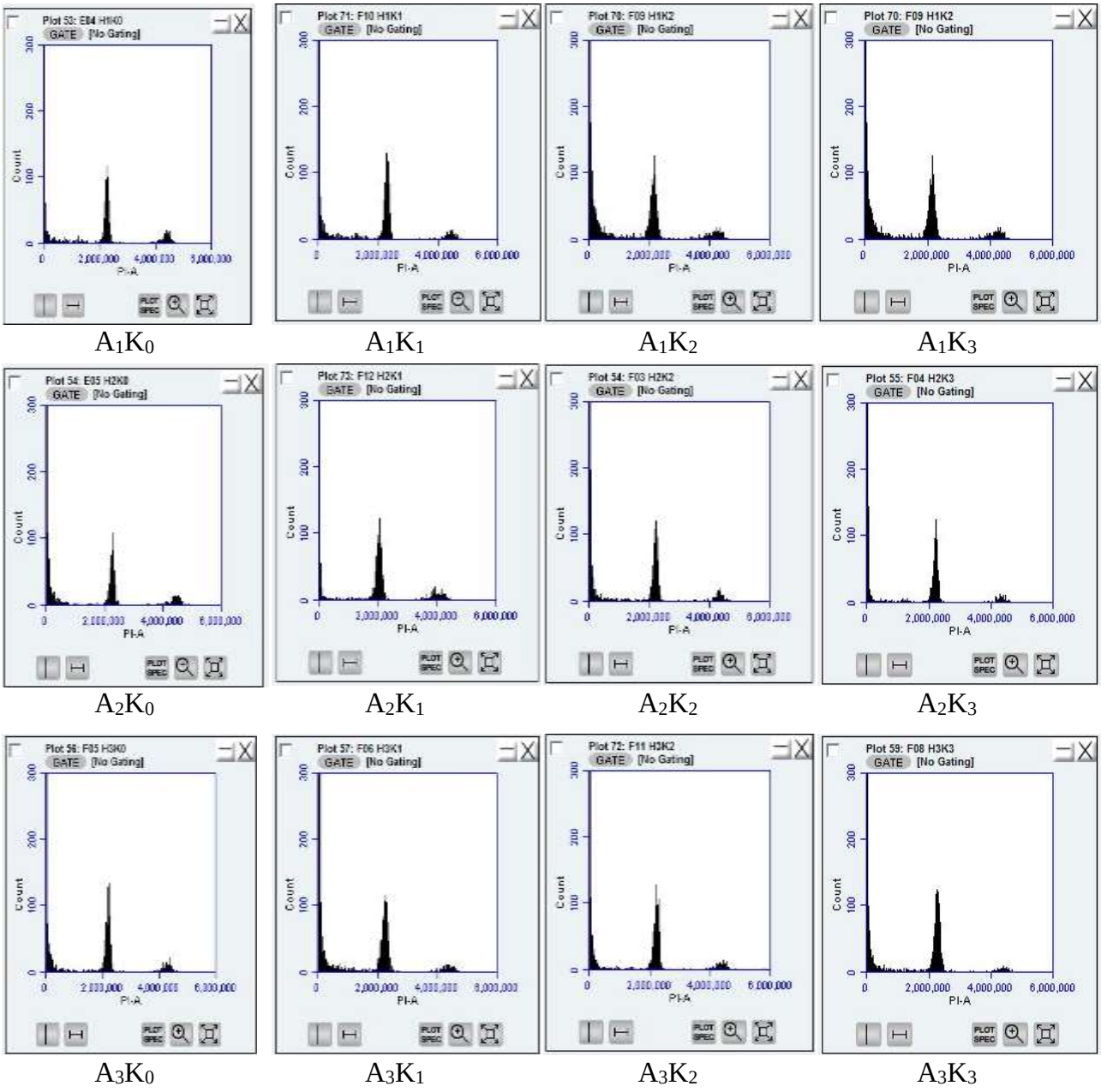

Fig. 1: Flow cytometric analysis the karyotype observations for each accession under different colchicine treatments.

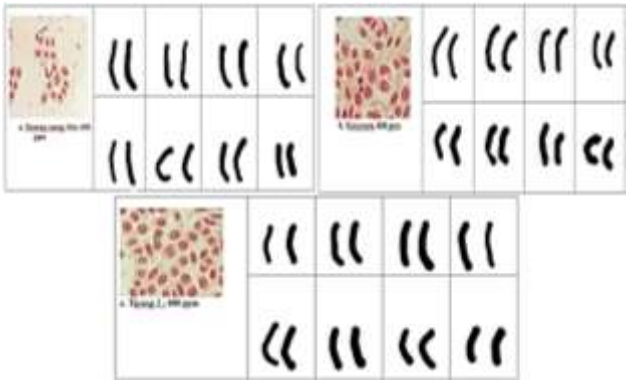

Fig. 2: (a-b-c): Number of chromosomes, (a) Siunong-unong Julu; colchicine 600ppm, diploid 2n = 16; (b) Simamora; colchicine 600ppm, diploid 2n = 16, and (c) Tipang 2 ; colchicine 600ppm, diploid 2n = 16.

reported that colchicine can lead to alterations in chromosome structure, affecting gene expression related to morphological traits (Zhang et al., 2024). Future cytogenetic analysis, such as fluorescence in situ hybridization (FISH) or flow cytometry, may be necessary to detect subtle chromosomal variations beyond numerical changes. Although no numerical chromosomal changes were observed, the colchicine treatment had a notable impact on the physiological characteristics of the shallots, including alterations in leaf chlorophyll content and stomatal density, as presented in the main study. The correlation between chromosome integrity and morphological traits suggests that colchicine's primary effect may be through epigenetic modifications or structural chromosomal changes rather than whole-genome duplication (Blasio et al., 2022).

Morphological/Agronomic Characteristics

Based on Table 5, it can be seen that there are significant differences in several Samosir local shallot accessions regarding plant length 5-6 WAP, number of leaves 6 WAP, harvest time, bulb diameter, number of bulbs per plant, dry weight of bulbs per plant, grading of quality bulbs I, grading of quality bulbs III and harvest index. This is due to the genetic factors of each accession, Samosir local shallots are usually cultivated in the highlands (> 900 m asl, Table 1) but in this study it was carried out in the lowlands (32 m asl), so that environmental factors are also influencing factors. This is in line with Yeshiwas et al. (2023); Hasanah et al. (2022) that each accession has different yield potentials and characteristics due to the genetic potential of each plant. Each plant genotype can have different responses and characteristics to various environmental conditions. Several agronomic characters of plant yield components are influenced by genetic factors and can also be influenced by environmental variables. Each accession also has different characteristics; this is due to the ability of the plant to translocate photosynthate to the bulb section.

Based on Table 6, it can be seen that the colchicine concentration treatment had no significant effect on the length of the plant 6 WAP, the number of leaves 6 WAP, bulb diameter, number of bulbs per plant, the dry weight of bulbs per plant, the grading of bulb quality I, the grading of bulb quality II, the grading of bulb quality III, and the harvest index.

This phenomenon is likely attributable to the suboptimal concentrations of colchicine applied, which were insufficient to induce stable polyploidy or significantly alter the morphological and agronomic characteristics observed in this study. As reported by Madani et al. (2021) and Kashtwari et al. (2022), colchicine acts by inhibiting spindle fiber formation during mitosis, thereby preventing chromosome separation and promoting chromosome doubling, which can lead to polyploidy. When applied at appropriate concentrations and developmental stages, colchicine-induced polyploidy is often associated with increased cell size, enhanced biomass, and enlarged vegetative or reproductive organs. However, the success of this induction is highly dependent on the dosage, exposure duration and sensitivity of the plant genotype. In some cases, colchicine treatment may induce unintended mutations

Table 5: Growth and Production of Local Samosir Shallots Accesion with Application of Cholchicine

Treatment	Plant length 6 WAP	Leaf number 6 WAP	Harvest time (DAP)	Bulb Diameter (mm)	Number of bulb/plants	Dry weight of bulb/plant (g)	Bulb Grading I (%)	Bulb Grading II (%)	Bulb Grading III (%)	Harvest Index
Accesion (H)										
A_1 (Siunong unong Julu)	23.83c	23.62a	73.87b	11.26b	9.58a	11.38b	6.22b	27.93	66.13ab	0.90b
A_2 (Simamora 3)	26.39b	19.56b	72.37ab	11.38b	8.56ab	10.89b	2.49b	28.52	69.77a	0.92ab
A_3 (Tipang 2)	29.85a	22.06ab	72.12a	17.90a	6.87b	22.10a	52.53a	27.55	12.45c	0.95a
Cholchicine (K)										
K_0 (0 ppm)	25.09	20.11	73.33	12.44	7.61	12.03	14.18	23.07	52.66	0.91
K_1 (200 ppm)	28.25	23.38	72.5	14.04	9.47	17.39	24.32	31.26	46.53	0.93
K_2 (400 ppm)	26.06	21.77	73.16	13.61	8.05	14.16	17.77	30.32	51.71	0.93
K_3 (600 ppm)	27.36	21.72	72.16	13.95	8.22	15.58	25.40	25.38	46.93	0.93
A x K										
A_1K_0	23.20d	21.41abc	74.00	9.22	8.33	7.94	11.39	13.54	76.73	0.87
A_1K_1	24.45cd	24.66a	74.50	12.76	10.08	13.61	6.15	31.62	62.43	0.92
A_1K_2	23.62d	26.33a	73.50	11.18	9.66	11.29	0.69	35.20	63.41	0.92
A_1K_3	24.04cd	22.08abc	73.50	11.87	10.25	12.66	6.67	31.35	61.98	0.91
A_2K_0	25.16cd	20.91abc	73.50	11.76	8.33	12.14	5.54	27.25	68.60	0.91
A_2K_1	28.50bc	23.50ab	72.00	11.42	10.91	12.38	0.00	35.84	65.95	0.92
A2K2	27.66bcd	17.58bc	72.00	11.21	8.16	11.36	3.50	25.66	70.83	0.94
A_2K_3	24.25cd	16.25c	72.00	11.12	6.83	7.69	0.93	25.35	73.73	0.91
A_3K_0	26.91cd	18.00bc	72.50	16.36	6.16	16.01	25.61	28.42	12.64	0.95
A_3K_1	31.79ab	22.00abc	71.00	17.94	7.41	26.18	66.82	26.31	11.22	0.95
A_3K_2	26.91cd	21.41abc	74.00	18.44	6.33	19.83	49.11	30.10	20.87	0.92
A_3K_3	33.79a	26.83a	71.00	18.86	7.58	26.38	68.60	25.38	5.09	0.97

Notes: The number followed by the same letter in the same variable observed indicated not significantly different based on Duncan's Multiple Range Test at α = 5%

or cellular abnormalities. These may result from mitotic disturbances, such as delayed or incomplete chromosome segregation during anaphase, leading to lagging chromosomes, micronuclei formation, or chromosomal instability. Such aberrations can compromise normal development, potentially resulting in malformed phenotypes or reduced fertility. Therefore, while colchicine is a valuable tool for inducing polyploidy, careful optimization of its application is essential to avoid cytological abnormalities and ensure the stability of desired traits in subsequent generations.

However, other researchers have reported that that colchicine concentrations between 0.025-0.1% with a soaking time of 24h produced mixoploid plants and affected plant height, fruit flesh thickness, and the number of Katokkon fruits (Tammu et al., 2021). Manzoor et al. (2019) stated that diploid and polyploid plants have different morphological forms which are cell responses to colchicine induction which results in changes in genetic material. Polyploidy causes phenotypic and genetic changes in plants with increased cell size, allelic diversity (heterozygosity), gene dose effects, or interactions between epigenetics and genetics. The interaction between several Samosir local shallot accessions from Humbang Hasundutan regency and colchicine concentration treatment had significant effect on plant length 6 WAP and number of leaves 6 WAP. There are differences in the interaction pattern between colchicine and Samosir local shallot accessions on plant length 6 WAP. This is because shallots have wide genetic variation among different accessions. Each accession has a different sensitivity to colchicine. Each local Samosir shallot accession has a unique genetic composition. These genes can influence how to respond to the presence of colchicine. Siunong unong Julu and Simamora 3 accessions were less responsive so that giving colchicine 0-600 ppm resulted in a different but not significant length of the 6 WAP plant. Meanwhile, the Tipang 2 accession was more responsive to colchicine application, resulting in an increase in colchicine, especially at a colchicine concentration of 600 ppm.

The rate of cell division and metabolic activity between accessions differs in relation to the mechanism of action of colchicine in disrupting cell division. The Tipang 2 accession is thought to have a faster rate of cell division, making it more responsive to colchicine. In contrast, the Siunong unong Julu and Simamora 3 accessions are thought to be less responsive to colchicine and have the ability to detoxify colchicine. Another factor is the potential for polyploidy induction in the form of a doubling of the number of chromosomes because colchicine induction in each accession is different. The Tipang 2 accession is thought to be more susceptible to polyploidy, causing a greater effect on cells and organs, including plant length and number of leaves.

The phenomenon is in line with Roy et al. (2020) that colchicine works by inhibiting the formation of microtubules, which are essential for cell division. Plants that respond better to colchicine will have a more effective mechanism for inhibiting microtubule formation. The use of colchicine can also affect plant morphology. Parsons et al. (2019) and Rahmawati et al. (2024) state that shallot bulbs are the most important component in shallot cultivation activities. Determining the right concentration and duration of colchicine soaking is expected to produce polyploid individuals, which are characterized by large bulb sizes. Colchicine works on cells by binding to α-dimers and β-tubulins that inhibit microtubule polymerization during the mitotic cycle, chromosome duplication. This cytostatic agent acts by binding to tubulin dimers to prevent spindle formation, shortening the length of spindle fibers, and causing temporary inactivation of spindle division, thus affecting the appearance of each plant.

Conclusion

The findings of this study indicate that colchicine treatment of Samosir shallot accessions affects the chlorophyll a, chlorophyll b, total chlorophyll content, plant length 6 WAP and leaf number 6 WAP. The Siunong Unong Julu accession showed an increase in total chlorophyll with colchicine treatment of up to 400ppm, whereas with 600ppm colchicine treatment, there was a decrease in chlorophyll a level. In the Simamora accession, total chlorophyll increased with increasing colchicine concentration, whereas in the Tipang 2 accession, chlorophyll a decreased with increasing colchicine treatment (0-600ppm). Each shallot accession responded differently to colchicine treatment. Observations of the number and density of stomata did not show any differences between each Samosir local shallot accession treated with colchicine. The Siunong unong Julu accession provided a good response compared to the other two accessions. However, the application of different concentrations of colchicine did not affect the number or density of stomata in any shallot accession. The karyotype analysis and flow cytometry confirmed that the diploid chromosome number of Samosir shallots remained stable under colchicine treatment, indicating no successful induction of polyploidy. The interaction between several Samosir local shallot accessions and colchicine concentration treatment had significant effect on plant length 6 WAP and number of leaves 6 WAP. Tipang 2 accession was more responsive to colchicine application, resulting in an increase in colchicine, especially at a colchicine concentration of 600ppm. These findings suggest that alternative colchicine application methods or extended treatment durations may be necessary to achieve polyploidization.

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