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RESEARCH ARTICLE
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Agronomic, Cytological and Biochemical Responses of Colchicine-Induced M_1V_2 Mutants of Samosir Local Shallot (*Allium ascalonicum* L.)

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ABSTRACT

Samosir local shallot (*Allium ascalonicum* L.) is an indigenous horticultural commodity valued for its unique aroma, strong flavor, and high market price. However, its productivity remains low due to small bulb size, genetic uniformity, and susceptibility to *Fusarium oxysporum* f. sp. *cepae* (Foc), which causes Fusarium wilt leading to up to 50% yield loss. This study aims to enhance the productivity and disease resistance of Samosir shallot through genetic improvement using colchicine-induced mutation. This research was conducted using experimental design and analysis methods. Experimental research was conducted at the experimental field of the Faculty of Agriculture, Universitas Sumatera Utara and the Biotechnology Laboratory, Faculty of Agriculture, Universitas Sumatera Utara. The research used three local Samosir shallot accessions (Pasaran, Siboro, Dosroha) derived from colchicine-induced mutation (0, 200, 400, and 600 ppm) in the previous study (generation M_1V_1). Agronomic parameters observed included plant height, harvest age, bulbs number, weight of bulbs, bulbs diameter and bulb grading. Flow cytometry analysis to determine the ploidy level of individuals in the M_1V_2 population. Biochemical traits such as total flavonoid and phenolic content were analyzed spectrophotometrically. Results showed that the 400 ppm colchicine treatment significantly increased the number of leaves and bulb yield in the Siboro accession, indicating an effective induction of stable polyploidy. However, high concentrations reduced growth in sensitive accessions such as Pasaran. The induced mutants exhibited variations in bulb morphology, chlorophyll content, and quercetin levels, suggesting enhanced genetic diversity and potential resistance mechanisms. The results show that colchicine induction is an effective strategy for developing high-yielding Samosir shallot lines, contributing to the conservation of local genetic resources and supporting sustainable food production.

Keywords: *Allium ascalonicum* L., Colchicine, Genetic improvement, Metabolomics.

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INTRODUCTION

Shallot (*Allium ascalonicum* L.) is an important horticultural crop widely cultivated in Indonesia and plays a vital role as a culinary spice, food industry ingredient, and bio-pharmaceutical resource. This significance arises from its rich bioactive compounds such as saponins, flavonoids, essential oils, allicin, alliin (Mlcek et al., 2016; Hasanah et al., 2024a; Ratseewo et al., 2025; Ramadan et al., 2025) and quercetin, which exhibit various pharmacological properties including antidiabetic, antioxidant, anticancer, antibacterial, and anti-inflammatory activities (Pan et al., 2018; Moldovan et al., 2024).

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The local Samosir shallot is one of Indonesia's unique genetic resources, renowned for its distinctive aroma, intense flavor, bright red glossy bulbs, and high market value. However, its small bulb size and low productivity, averaging only 6.45 tons per hectare, limit its commercial potential (Simamora et al., 2024; Hasanah et al., 2024b). Major constraints include reduced cultivation area, poor seed quality with low germination rate, and high vulnerability to pests and diseases, particularly Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cepae* (Foc). This soil-borne pathogen is difficult to control chemically and can cause yield losses of up to 50%, significantly decreasing both yield quantity and quality (Fitriani et al., 2019; Husain et al., 2022; Marianah et al., 2024).

To overcome these challenges, genetic improvement is essential to develop high-yielding and disease-resistant Samosir shallot varieties. However, the species' vegetative propagation system restricts genetic variation, making conventional breeding methods less effective. Mutation induction through chemical mutagens, such as colchicine, offers a promising approach to increase genetic variability and improve desired traits (Manzoor et al., 2019; Husain et al., 2022). Colchicine, an alkaloid derived from *Colchicum autumnale*, disrupts spindle formation during cell division in mitosis, resulting in chromosome doubling and the formation of polyploid plants. This process is utilized in plant breeding to increase organ size, strength, and productivity (Naaz et al., 2019; Ren et al., 2018; Nett et al., 2020; Singh et al., 2025).

At the molecular level, colchicine binds to tubulin proteins, which are the main components of microtubules. This binding inhibits the polymerization of tubulin into microtubules, preventing spindle fibers from forming properly. Without spindle fibers, chromosomes cannot move and distribute normally during cell division. As a result, cells that should divide into two daughter cells remain in one cell with an increased number of chromosomes (Xia et al., 2020; Hawash, 2022; Cai et al., 2025). At the cellular level, colchicine treatment causes changes in cell size, an increase in cell nucleus size, and enlargement of plant organs. Polyploid cells are generally larger than diploid cells, which is then reflected in changes in plant morphology, such as leaf size, stem diameter, and tuber size. In addition, the increase in the number of chromosomes also affects gene expression and metabolic activity, thereby impacting changes in the physiological and biochemical characteristics of plants, including the production of secondary metabolites (Zhu et al., 2021. Fomicheva et al., 2024).

In shallots, polyploidy induction with colchicine is used to improve agronomic traits such as bulb size and weight, as well as plant genetic diversity. Polyploidy induction not only alters the genetic structure of plants but also affects primary and secondary metabolic pathways. Changes in ploidy levels lead to increased cell size, enzyme activity, and gene expression involved in secondary metabolite biosynthesis. In horticultural crops, such as shallot, polyploidy is often associated with increased levels of bioactive compounds such as flavonoids, phenols, alkaloids, saponins, and terpenoids (Madani et al., 2021; Gantait and Mukherjee, 2022). The Samosir local shallot represents a local germplasm with specific adaptations to the agroecological conditions of the highlands around Lake Toba. This variety is able to grow well in relatively low temperatures and significant climate fluctuations, demonstrating tolerance to environmental stresses such as temperature changes and water availability. Agronomically, Samosir shallots have strong vegetative growth, characterized by sturdy leaves and a large number of leaves, which support the plant's photosynthetic capacity. The bulbs have distinctive characteristics, namely medium to large size, oval shape, purplish-red skin color, and relatively thick skin, which gives them good storage capacity.

In addition, Samosir local shallots are known to have a sharper aroma and taste than some commercial varieties, which is related to their high organosulfur compound content. From a biochemical perspective, Samosir shallots have the potential to contain secondary metabolites such as flavonoids, phenolic compounds, and anthocyanins, which act as antioxidants and functional compounds. These adaptive, agronomic, and biochemical advantages indicate that local Samosir shallots are not only economically valuable to the local community, but also have important genetic potential as a germplasm resource in plant breeding programs and the development of location-specific superior varieties (Jabat and Ginting, 2021; Hasanah et al., 2024a; Simamora et al., 2024; Hasanah et al., 2024b).

However, like many local varieties, its genetic diversity is relatively limited, making quality improvement through mutation breeding crucial. Colchicine mutation induction in the Samosir local shallot aims to increase bulb size and yield, improve plant vigor, enhance tolerance to environmental stress (biotic and abiotic), and expand genetic diversity for selection. This aligns with research on other shallots showing that colchicine can alter phenotypic characteristics and induce polyploidy.

Polyploidization in shallots has been reported to affect phenotypic traits such as stomata size, chromosome number, and the potential for increased bulb size and weight (Yao et al., 2023; Jahanian et al., 2024; Syahrir & Saptadi, 2025). Therefore, the application of colchicine to local Samosir shallots is expected to produce superior mutant genotypes that are adaptive to the local environment and have higher productivity. Previous studies have demonstrated that colchicine-induced mutation can generate morphological and phytochemical variations in shallot plants, including increased bulb size and altered flavonoid content. In 2024, colchicine treatment on Samosir shallot successfully produced mutant lines (M_1V_1) with improved agronomic and phytochemical traits. Nevertheless, genetic stability and disease resistance of these mutants remain to be confirmed (Hasanah et al., 2025).

Therefore, the objective of the research was to evaluate the agronomic performance, phytochemical changes of colchicine-induced mutants of Samosir local shallot. The study is expected to contribute to the development of superior Samosir shallot varieties that are both high-yielding and disease-resistant, supporting the conservation of local genetic resources and advancing sustainable agricultural production in Indonesia.

MATERIALS & METHODS

Study Area

This research was conducted using experimental design and analysis methods. Experimental research was conducted at the experimental field of the Faculty of Agriculture, Universitas Sumatera Utara and the Biotechnology Laboratory, Faculty of Agriculture, Universitas Sumatera Utara. The location of the shallot sampling for M0 is shown in Fig. 1, while the climatological conditions are shown in Table 1.

Materials and Tools

The materials used were local Samosir shallot accessions induced by colchicine mutagenesis, manure, paper bags, distilled water, fusaric acid, insecticide, and *Trichoderma harzianum*. The tools used were hoes, scales, rulers, research name boards, watering buckets, stakes, spectrophotometers, microscopes, UV absorbance detectors, analytical scales, ovens, Spectrofotometry, and several tools used for laboratory analysis.

Procedures

The research used three local Samosir shallot accessions (Pasaran, Siboro, Dosroha) derived from colchicine-induced mutation (0, 200, 400, and 600 ppm) in the previous study (generation M_1V_1). In previous studies, shallot bulbs were soaked in solutions of varying concentrations of colchicine for 24 hours, including a control treatment soaked in distilled water.

Black and silver plastic mulch was installed 7 days before planting, and holes were made according to the planting distance (20 cm x 20 cm), with the number of planting holes corresponding to the number of plants per treatment (Table 2). The spacing between treatment plots was 30 cm. The biological fungicide *Trichoderma harzianum* was applied one week before planting to prevent root rot caused by the fungus *Fusarium oxysporum*. The application method was to dissolve 45 grams of the biological fungicide in 10 L of water and then water with 200 ml per planting hole.

The M_1V_1 generation shallot bulbs were cut at 1/3 of the tip of each accession and planted in the field to a depth of 1-2 cm. ZA fertilizer at 150 kg/ha was applied 7 weeks after planting (WAP). NPK (16-16-16) fertilizer at a dose of 200 kg/ha was applied at 14 days after planting (WAP) and 250 kg/ha at 28 days after planting. NPK fertilizer (16:16:16) at a dose of 250 kg/ha and KCl fertilizer at a dose of 187.5 kg/ha at 42 days after planting (Hasanah et al., 2024a).

Watering was done twice daily (morning and evening). Weeds were removed manually. Pest control was carried out by spraying an insecticide containing the active ingredient profenofos at 2 ml/L. Fusarium wilt (*Fusarium oxysporum*) is controlled by spraying the fungicide difenoconazole at 2 g/L intervals of 1-2 weeks. Harvest shallots according to harvest criteria, namely when the leaves begin to fall and the bulbs emerge above the soil surface. Post-harvest, the shallot bulbs are air-dried for 7 days.

Table 1: Location sample site and climate condition

Location sample site	Geographic coordinates	Altitude (m above sea level)	Climate conditions
Pasaran	2.658° N ; 98.734° E	985	• Rainfall 244 mm/month • No distinct dry season • Relative humidity 80-88%
Siboro	2.649° N ; 98.775° E	1,045	• Rainfall 230-250 mm/month • Relative humidity 80-83%
Dosroha	2.622° N ; 98.818° E	950	• Rainfall 240-250 mm/month • Relative humidity 84% • Lush vegetation and fertile soils

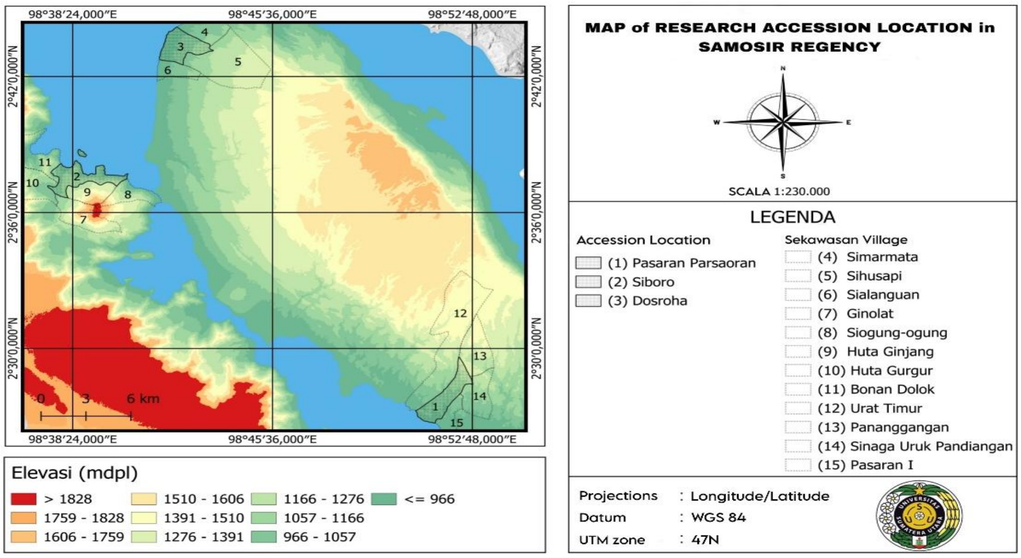


Fig. 1: Sample site of shallot for M0.

Table 2: Shallot population for each treatment

Shallot population (plant)		
S_1K_0	:	25
S_1K_1	:	40
S_1K_2	:	40
S_1K_3	:	25
S_2K_0	:	65
S_2K_1	:	25
S_2K_2	:	65
S_2K_3	:	65
S_3K_0	:	40
S_3K_1	:	65
S_3K_2	:	65
S_3K_3	:	50
Total	:	570

Agronomic parameters observed included plant height, harvest age, bulbs number, weight of bulbs, bulbs diameter and bulb grading. Flow cytometry analysis to determine the ploidy level of individuals in the M_1V_2 population. Biochemical traits such as total flavonoid and phenolic content were analyzed spectrophotometrically.

A flow cytometer (BD Accuri C6+, USA) was utilized to determine ploidy. Leaf pieces (about 0.5×0.5 cm) were dripped with 250 µL of nuclei extraction buffer and a tiny amount of polyvidon on a petri dish, then gently chopped with a razor blade. A 30 m millipore filter was employed to filter the chopped leaves' liquid. The filtrate was placed in a cuvette tube with 350 µL of staining solution, propidium iodide, and RNAse for analysis (Adabiyah et al., 2023).

Statistical Analysis

This study did not use a randomized design because the number of seeds from each treatment was small and not equal. The treatments were still in the second generation of vegetative propagation (M1V2) and were considered not yet stable enough to use a randomized design. It was suspected that there was still variation in the appearance of each mutation treatment population. The number of shallot samples for each treatment is presented in Table 2. Data is represented by mean ± standard deviation. The statistical test used is the t-test, comparing the treatment with no colchicine administration (0 ppm) at an alpha level of α = 5%.

RESULTS & DISCUSSION

In order to increase genetic diversity in plant breeding, mutation techniques can be used. Through mutation techniques, breeders can select plant genotypes according to breeding objectives. The most widely used chemical mutagen is colchicine, which is effective in changing the number of chromosomes in cells due to its water solubility. Polyploidy can occur naturally or artificially (Younas et al., 2025; Azeem et al., 2025). Colchicine can be used by applying it to seedlings at the point of growth or soaking them for a certain period of time. The concentration and duration of colchicine soaking required to cause changes in chromosome composition varies between species. Colchicine inhibits spindle fiber formation and cytokinesis, resulting in cells with a higher number of chromosomes (Zhou et al., 2017; Soomro et al., 2025).

Plant Height

Plant height varied markedly among accessions and colchicine concentrations, indicating a strong genotype-dependent response to chromosome doubling. Overall, all accessions showed a gradual increase in height with plant age; however, the magnitude and direction of response to colchicine differed substantially. Treatment with colchicine showed no significant improvement; instead, S_1K_1 and S_1K_2 maintained trajectories similar to the control, while the highest concentration (600 ppm, S_1K_3) suppressed early vegetative growth, reducing height to 12.44 cm at 2 WAP and 15.29 cm at 3 WAP (Table 3).

In the Pasaran accession (S_1), plants under control conditions exhibited normal vegetative growth throughout the observation period. Application of colchicine did not enhance plant height in this accession. Lower to moderate concentrations produced growth patterns comparable to the control, while the highest concentration caused a clear suppression of early vegetative development. Although partial recovery occurred at later stages, treated plants remained consistently shorter than untreated plants. This response suggests that Pasaran is sensitive to colchicine-induced mitotic disruption, particularly at higher concentrations, which likely interfered with cell division during early growth. In contrast, the Siboro accession (S_2) responded positively to colchicine induction, especially at the intermediate concentration Treated plants in this group exhibited consistently greater height than the control across observation periods, indicating a favorable physiological adjustment to chromosome doubling. This enhanced growth implies that Siboro possesses a higher capacity to stabilize polyploid cells following spindle disruption, allowing improved vegetative vigor and elongation.

The Dosroha accession (S3) showed relatively stable growth across treatments. While some colchicine-treated plants displayed slightly reduced height at early stages, growth performance at later stages was comparable to or exceeded that of the control. The highest colchicine concentration produced the tallest plants at the final observation, suggesting effective recovery and possible benefits of polyploidization in this genotype. These results indicate that Dosroha has moderate tolerance to colchicine, with sufficient cellular resilience to maintain normal growth dynamics. Overall, the differential responses among Samosir local shallot accessions in the M1V2 generation highlight the complex interaction between genotype-specific cellular stability and the physiological effects of induced polyploidization. Colchicine treatment can either suppress or enhance plant height depending on the accession's ability to tolerate spindle disruption and establish stable polyploid cells. Reduced growth in sensitive genotypes, such as Pasaran, is consistent with the cytotoxic nature of colchicine, which inhibits microtubule polymerization and disrupts mitosis (Hasanah et al., 2025). Conversely, enhanced growth observed in Siboro supports the concept that successful chromosome doubling can increase cell size, metabolic capacity, and overall vegetative performance (Abubakar et al., 2025). Dosroha represents an intermediate response, reflecting partial tolerance and adaptive recovery following mitotic stress.

Table 3: Plant height (cm) of local Samosir shallots originating from Samosir Regency, generation M_1V_2, results of colchicine mutation induction

Treatment	Plant height (WAP)				
	2	3	4	5	6
S_1K_0	14.77 ± 2.70	17.76 ± 3.81	20.57 ± 5.19	22.88 ± 5.84	25.44 ± 6.84
S_1K_1	13.55 ± 2.92^{ns}	16.40 ± 3.75ns	19.19 ± 5.02^{ns}	22.05 ± 6.43^{ns}	25.06 ± 7.78^{ns}
S_1K_2	14.51 ± 2.24^{ns}	17.03 ± 2.29^{ns}	19.22 ± 2.83^{ns}	21.64 ± 3.82^{ns}	23.98 ± 4.94^{ns}
S_1K_3	12.44 ± 2.75*	$15.29 \pm 3.18^{*}$	18.19 ± 3.95^{ns}	21.06 ± 4.86^{ns}	23.93 ± 5.90^{ns}
S_2K_0	11.74 ± 2.47	14.00 ± 2.69	16.94 ± 4.14	19.13 ± 5.04	20.98 ± 6.02
S_2K_1	13.39 ± 2.07*	15.33 ± 2.41^{ns}	17.19 ± 2.14^{ns}	18.99 ± 2.47^{ns}	20.96 ± 2.85^{ns}
S_2K_2	$19.09 \pm 2.55^{*}$	$21.28 \pm 2.82^{*}$	$23.52 \pm 3.55^{*}$	$25.78 \pm 4.48^{*}$	$27.98 \pm 5.52^{*}$
S_2K_3	$14.93 \pm 2.81^{*}$	$17.28 \pm 3.04^{*}$	$19.69 \pm 3.62^{*}$	$22.05\pm 4.38^{*}$	$24.41 \pm 5.29^{*}$
S_3K_0	19.76 ± 3.29	22.42 ± 3.09	25.01 ± 3.31	27.65 ± 3.82	30.52 ± 4.43
S_3K_1	19.30 ± 2.84^{ns}	21.97 ± 2.94^{ns}	24.59 ± 3.26^{ns}	27.23 ± 3.85^{ns}	29.92 ± 4.42^{ns}
S_3K_2	16.76 ± 2.23^{ns}	$20.22 \pm 2.90^{*}$	$23.64\pm 3.05^{*}$	27.15 ± 4.34^{ns}	30.59 ± 5.69^{ns}
S_3K_3	19.95 ± 3.16^{ns}	22.93 ± 2.76^{ns}	$25.89\pm 2.82^{*}$	28.94 ± 3.21^{ns}	31.86 ± 3.88^{ns}

Data are presented as mean ± SD. The symbol * indicates a significant difference from the control (treatment K0), while the symbol ^{ns} indicates no significant difference from the control, based on an independent t-test at a significance level of 5% (α = 0.05).

Yield Components: Harvest Age, Bulb Number, Fresh and Dry Weight, Bulb Diameter

Yield components exhibited pronounced variation among accessions and colchicine concentrations, indicating a strong interaction between genotype and induced polyploidization. Each accession responded differently in terms of bulb formation, biomass accumulation, and bulb size, reflecting contrasting physiological adjustments to colchicine-induced mitotic stress. In the Pasaran accession (S1), colchicine treatment did not improve yield performance. Although a slight increase in bulb number was observed at moderate concentration, this was accompanied by a marked reduction in bulb biomass and diameter. These results indicate that colchicine disrupted assimilate allocation in Pasaran, leading to the formation of more but smaller and lighter bulbs (Table 4). Such a response suggests that this accession has limited tolerance to mitotic disturbance, resulting in reduced sink strength and inefficient storage organ development under polyploidization pressure.

In contrast, the Siboro accession (S_2) showed a highly positive response to colchicine, particularly at the intermediate concentration. Plants in this treatment produced substantially more bulbs with greater fresh and dry biomass compared to the control, demonstrating enhanced yield capacity. Even at higher concentration, Siboro maintained superior bulb production relative to untreated plants, indicating strong adaptability and genomic stability following chromosome doubling. This response suggests that induced polyploidization in Siboro effectively enhanced sink capacity and assimilate partitioning toward bulb development. The Dosroha accession (S3) displayed a contrasting pattern. Despite high productivity under control conditions, colchicine application consistently reduced bulb number and overall biomass. This decline implies that the inherently high yield potential of Dosroha may be sensitive to genomic imbalance caused by chromosome duplication. Polyploid instability in this accession likely disrupted coordinated growth and carbohydrate allocation, leading to reduced bulb formation under colchicine treatment.

Bulb diameter further highlighted accession-specific responses to colchicine. In Pasaran, bulb size declined across all colchicine concentrations, reflecting impaired cell expansion and reduced storage tissue development. Conversely, Dosroha exhibited an increase in bulb diameter at the highest concentration, despite a reduction in bulb number. This suggests that colchicine selectively promoted cell enlargement within individual bulbs, potentially through enhanced endoreduplication or localized stable polyploid formation in bulb tissues. Siboro showed relatively stable bulb diameter, indicating balanced cell division and expansion accompanying increased bulb number. Variation in yield components underscores that colchicine-induced polyploidization can either enhance or suppress agronomic performance depending on genotype-specific tolerance. Siboro demonstrated successful polyploid induction, resulting in increased bulb number and biomass, consistent with previous findings that polyploid tissues often possess enlarged storage cells and improved photosynthate utilization (Miri, 2020; Rahmawati et al., 2020). In contrast, Pasaran and Dosroha experienced yield penalties at medium to high colchicine concentrations, likely due to genomic instability, aneuploidy, or mosaic polyploidy, which are known to disrupt metabolic coordination and carbohydrate transport. These findings emphasize that colchicine application must be carefully optimized for each genotype to maximize its potential benefits in shallot improvement.

Bulb Grading

Quality grading revealed clear accession-specific responses to colchicine induction, indicating that bulb marketability was strongly influenced by the stability of induced ploidy rather than by yield magnitude alone. Changes in grade composition across treatments reflected differences in the ability of each genotype to maintain uniform bulb development under mitotic stress. In the Pasaran accession (S1), colchicine application generally reduced bulb quality. The most pronounced deterioration occurred at 400 ppm, where a significant decline in Grade I bulbs was accompanied by a marked increase in Grade III bulbs. This shift indicates severe disruption in bulb uniformity at this concentration, suggesting that intermediate colchicine levels induced unstable or mosaic ploidy, resulting in heterogeneous bulb sizes. Lower and higher concentrations did not produce significant improvements in grade distribution, confirming that Pasaran is poorly suited to colchicine-induced polyploidization (Table 5).

Table 4: Harvest age, number of bulbs, wet weight of bulbs, dry weight of bulbs and diameter of bulbs of local Samosir red onion generation M1V2 with colchicine mutation induction treatment

Treatment	Harvest age (WAP)	Number of bulbs (bulbs)	Wet weight of bulbs (g)	Dry weight of bulbs (g)	Bulb diameter (mm)
S_1K_0	77	2.43 ± 0.80	7.71 ± 6.97	6.90 ± 6.31	16.63 ± 5.63
S_1K_1	79	3.15 ± 2.18^{ns}	5.77 ± 3.90^{ns}	4.80 ± 3.47^{ns}	13.78 ± 4.58*
S_1K_2	69	3.05 ± 1.83^{ns}	3.80 ± 3.99*	2.57 ± 1.61*	11.15 ± 2.88*
S_1K_3	77	2.24± 0.79*	5.66± 3.51*	5.37± 1.33^{ns}	12.44 ± 2.53*
S_2K_0	77	3.76 ± 1.87	8.74 ± 5.96	7.77 ± 5.51	15.49 ± 5.48
S_2K_1	79	3.57 ± 2.03^{ns}	2.38 ± 2.47*	6.64 ± 1.13*	12.48 ± 1.89*
S_2K_2	69	10.65 ± 3.41*	15.43 ± 8.13*	13.02 ± 7.31*	12.17 ± 2.45*
S_2K_3	77	6.35 ± 2.48*	14.84 ± 9.20*	12.54 ± 7.97*	14.87 ± 4.15^{ns}
S_3K_0	96	13.88 ± 6.86	17.64 ± 8.33	15.87 ± 7.45	11.06 ± 1.45
S_3K_1	96	9.68 ± 6.20*	16.03 ± 8.30^{ns}	14.30 ± 7.41^{ns}	11.95 ± 2.01*
S_3K_2	98	10.77 ± 4.52*	10.06± 4.32*	8.86 ± 3.90*	9.85 ± 1.41*
S_3K_3	98	8.72 ± 3.35*	14.27± 5.43*	12.95 ± 5.01*	14.80 ± 10.85*

Data are presented as mean ± standard deviation. The symbol * indicates a significant difference from the control (treatment K_0), while the symbol ^{ns} indicates no significant difference from the control, based on an independent t-test at a significance level of 5% (α = 0.05).

Table 5: Quality grading of local Samosir red onion bulbs of the M_1V_2 generation with colchicine mutation induction treatment

Treatment	Grade (%)		
	I	II	III
S_1K_0	43.61 ± 38.11	23.61 ± 25.65	32.78 ± 42.71
S_1K_1	27.36 ± 38.73^{ns}	24.13^{tn} ± 29.55^{ns}	48.51^{tn} ± 40.63^{ns}
S_1K_2	6.92 ± 19.19*	25.81^{tn} ± 32.44^{ns}	67.27* ± 38.86*
S_1K_3	23.44 ± 33.23^{ns}	29.07^{tn} ± 23.15^{ns}	47.50^{tn} ± 36.09^{ns}
S_2K_0	40.45 ± 38.50	19.67 ± 22.19	39.88 ± 40.08
S_2K_1	0	9.18^{tn} ± 26.23^{ns}	90.82± 26.23*
S_2K_2	11.00 ± 17.42*	26.77^{tn} ± 21.89^{ns}	58.21 ± 33.32*
S_2K_3	32.96 ± 30.08^{ns}	29.46* ± 23.15*	37.58^{tn} ± 31.40^{ns}
S_3K_0	2.81 ± 5.16	22.52 ± 15.94	74.67 ± 17.93
S_3K_1	8.67 ± 13.22*	27.66^{tn} ± 21.08^{ns}	60.64 ± 26.37*
S_3K_2	0.60± 3.50*	13.73* ± 14.96*	85.67 ± 15.64*
S_3K_3	8.53 ± 14.79*	30.88* ± 18.33*	60.99 ± 21.94*

Data are presented as mean ± standard deviation. The symbol * indicates a significant difference from the control (treatment K_0), while the symbol ^{ns} indicates no significant difference from the control, based on an independent t-test at a significance level of 5% (α = 0.05).

The Siboro accession (S_2) exhibited a more complex response pattern. At 200 ppm, bulb quality declined sharply, with a significant increase in Grade III bulbs and the near absence of Grade I bulbs, indicating strong instability in early polyploid formation. However, at 600 ppm, Siboro showed a notable recovery in quality, characterized by a higher proportion of Grade I and Grade II bulbs and a reduction in Grade III bulbs. This improvement suggests that higher colchicine concentration promoted more uniform and stable chromosome doubling, allowing consistent cell expansion and bulb development. Dosroha (S3) displayed a relatively low proportion of Grade I bulbs under control conditions, reflecting its inherent tendency toward smaller or less uniform bulbs. Colchicine treatment at 600 ppm significantly improved bulb quality in this accession, as evidenced by an increased proportion of Grade I and Grade II bulbs and a concurrent reduction in Grade III bulbs. Despite overall yield reductions reported in other parameters, this result indicates that high colchicine concentration selectively enhanced bulb uniformity in Dosroha, possibly through stable polyploid formation in specific tissues.

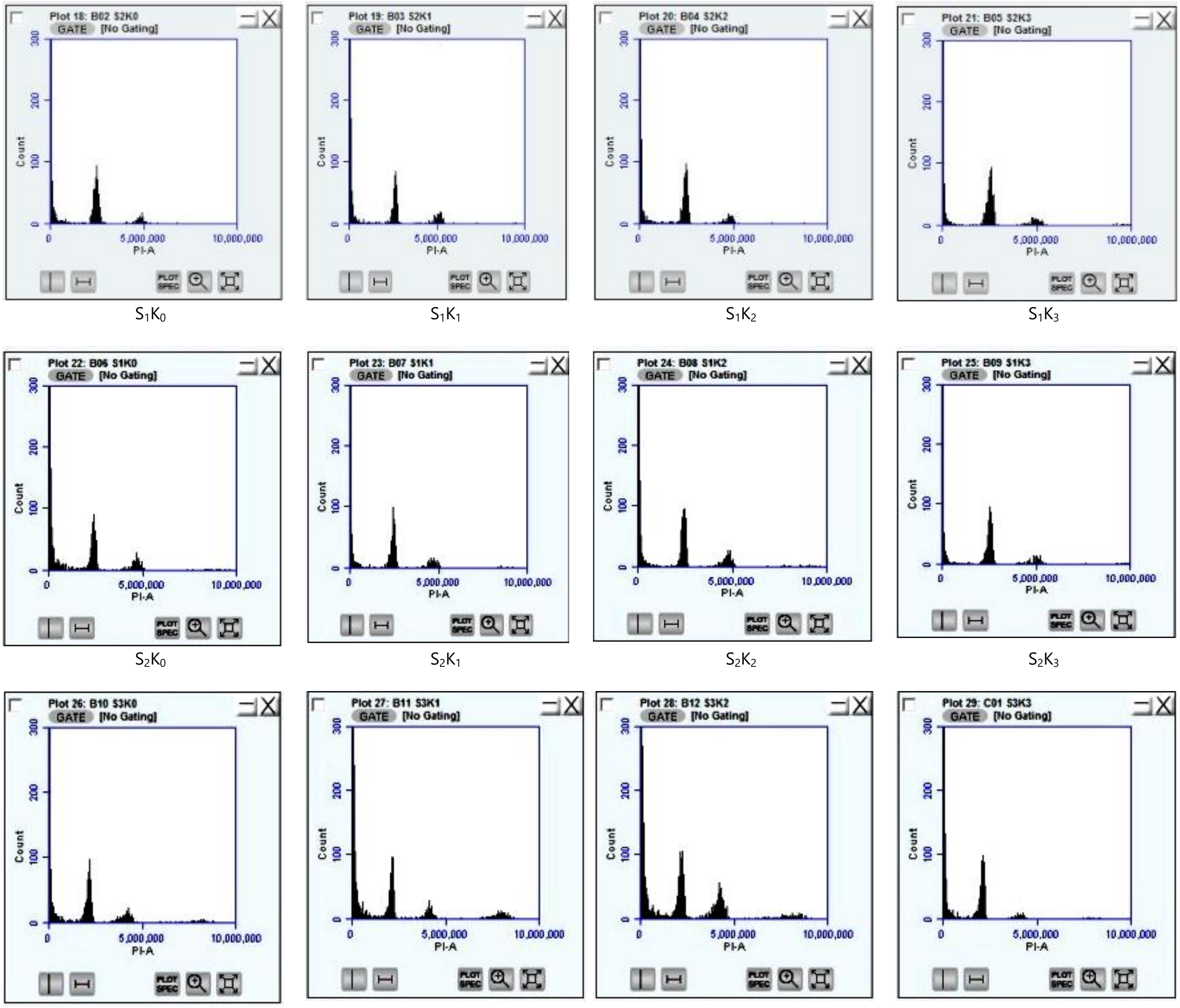
The grading patterns demonstrate that colchicine concentration critically determines whether induced polyploidization leads to quality deterioration or improvement. Intermediate concentrations tended to generate heterogeneous bulb populations with unstable ploidy, particularly in Pasaran and Siboro, resulting in lower commercial grades. In contrast, higher concentration (600 ppm) in Siboro and Dosroha appeared to favor more stable polyploidization, improving bulb uniformity and marketable quality. These findings emphasize that successful colchicine induction depends not merely on chromosome duplication but on the establishment of stable polyploid cell lineages, which ultimately governs morphological consistency and commercial value.

Flow Cytometry Analysis

Flow cytometric analysis showed that colchicine treatment, even at the highest tested concentration, was generally unable to induce stable polyploidization in Samosir local shallot accessions. Most samples displayed uniform nuclear DNA profiles characteristic of diploid plants, indicating that chromosome doubling did not occur consistently across treatments. This response was clearly observed in Pasaran (S1) and Siboro (S2), which maintained single, stable fluorescence peaks at all colchicine concentrations, reflecting strong diploid stability and limited sensitivity to colchicine-induced mitotic disruption (Table 6 and Fig. 2).

Table 6: Flow cytometry results of local Samosir shallots of the M_1V_2 generation with colchicine mutation induction treatment

Treatment	Mean PI	CV%	Ploidy Level
S_1K_0	2.373.332	5.10	Diploid
	4.710.901	3.78	
S_1K_1	2.409.827	5.48	Diploid
	4.692.240	4.43	
S_1K_2	2.406.987	5.05	Diploid
	4.777.893	4.02	
S_1K_3	2.507.289	5.58	Diploid
	5.069.285	3.53	
S_2K_0	2.441.337	5.81	Diploid
	4.797.600	3.92	
S_2K_1	2.606.727	4.29	Diploid
	5.096.286	3.76	
S_2K_2	2.422.440	5.44	Diploid
	4.829.869	3.59	
S_2K_3	2.491.198	6.62	Diploid
	5.013.614	4.15	
S_3K_0	2.094.005	7.08	Diploid
	4.129.111	5.77	
S_3K_1	2.086.603	5.81	Diploid
	4.119.385	4.95	
	7.968.795	3.97	
S_3K_2	2.130.952	7.05	Mixoploid
	4.218.386	5.26	
S_3K_3	2.077.545	5.77	Diploid
	4.080.516	4.67	



S_1K_0 S_1K_1 S_1K_2 S_1K_3

S_2K_0 S_2K_1 S_2K_2 S_2K_3

S_3K_0 S_3K_1 S_3K_2 S_3K_3

Fig. 2: Flow cytometry data of local Samosir shallots of the M1V2 generation resulting from colchicine-induced mutation.

A notable exception was detected in the Dosroha accession (S3) at the intermediate colchicine concentration of 400 ppm. At this level, a distinct double-peak fluorescence pattern was observed, indicating the presence of mixoploid cell populations. This pattern suggests that colchicine at 400 ppm partially disrupted spindle formation, leading to chromosome doubling in only a subset of meristematic cells, while the remaining cells retained their diploid state. Such partial responses are typical of colchicine treatments in non-uniform tissues, particularly bulb crops, where colchicine penetration and mitotic activity vary spatially.

The reliability of these flow cytometry results is supported by coefficient of variation values that remained within acceptable thresholds for accurate ploidy determination, as outlined by Soni and Henry (2025). The occurrence of mixoploidy specifically at intermediate concentration aligns with previous reports that colchicine often exerts uneven effects on microtubule polymerization, resulting in asynchronous mitosis and mosaic chromosomal states rather than uniform polyploidy (Gupta et al., 2024). Similar patterns of colchicine-induced mixoploidy have been documented in shallot and garlic, where partial chromosome doubling frequently occurs in early generations following treatment (Hailu et al., 2021; Fomicheva et al., 2024).

These findings indicate that the colchicine doses applied in this study were insufficient to generate stable tetraploid plants in the M_1V_2 generation. Instead of inducing uniform polyploidization, colchicine primarily caused transient or localized chromosomal instability, as evidenced by mixoploidy in Dosroha at 400 ppm. This outcome underscores the importance of genotype-specific sensitivity, exposure duration, and meristem accessibility in determining the success of polyploid induction. Without optimization of these factors, colchicine treatment is more likely to produce unstable chromosomal configurations than agronomically useful polyploid lines.

Total Flavonoid and Phenolic Content

Flavonoid accumulation showed response patterns that closely paralleled changes in total phenolic content, reinforcing the role of colchicine as a strong modulator of secondary metabolism. Across accessions, increasing colchicine concentration generally suppressed flavonoid biosynthesis, indicating that mitotic disruption and associated cellular stress limited the allocation of resources toward secondary metabolite production.

Based on Table 7, it can be seen that in the Pasaran accession (S1), flavonoid content declined significantly with colchicine application, with the most pronounced reduction occurring at 600 ppm. This marked decrease suggests that high colchicine concentrations strongly inhibited flavonoid biosynthesis in Pasaran, likely due to metabolic downregulation associated with spindle inhibition. A similar trend was observed in the Siboro accession (S2), where flavonoid levels decreased progressively with increasing colchicine concentration, and significant reductions were evident at higher doses. These consistent declines across both accessions align with the general phenomenon of metabolic suppression under colchicine-induced mitotic stress, where cellular energy is preferentially directed toward survival and repair rather than secondary metabolite synthesis (Celik et al., 2020). In contrast, the Dosroha accession (S3) exhibited a distinct and genotype-specific response. Although flavonoid levels initially declined at the lower colchicine concentration (200 ppm), a significant increase was observed at 400 ppm, followed by a sharp enhancement at 600 ppm. This recovery pattern mirrors the response observed for phenolic compounds and antioxidant activity in the same accession, suggesting the activation of compensatory metabolic mechanisms under higher stress levels. Such enhancement may be mediated by stress-induced upregulation of flavonoid biosynthetic genes or altered carbon allocation associated with changes in ploidy integrity (Koziara-Ciupa & Trojak-Goluch, 2025).

Table 7: Antioxidant activity, total flavonoids, and total phenols in local Samosir red onions of the M1V2 generation resulting from colchicine mutagenesis induction

Treatment	Antioxidant activity (µg/mL)	Total of flavonoid (QE/g extract)	Total of fenol (GAE/g extract)
S_1K_0	131.11 ± 0.66	8.72 ± 0.05	4.57 ± 0.09
S_1K_1	134.01 ± 0.75*	7.52± 0.13*	3.26 ± 0.06*
S_1K_2	147.16 ± 1.16*	3.45 ± 0.03*	0.08 ± 0.01*
S_1K_3	150.80 ± 1.14*	2.22 ± 0.04*	0.94 ± 0.02*
S_2K_0	126.90 ± 0.85	10.87 ± 0.19	5.66 ± 0.06
S_2K_1	133.65 ± 0.83*	8.05 ± 0.02*	3.80 ± 0.04*
S_2K_2	135.88 ± 0.76*	7.21 ± 0.17*	2.98 ± 0.03*
S_2K_3	138.59 ± 1.00*	6.68 ± 0.34*	2.55 ± 0.02*
S_3K_0	146.76 ± 0.97	3.40 ± 0.03	1.04 ± 0.01
S_3K_1	150.64 ± 1.08*	3.14± 0.05*	0.79 ± 0.03*
S_3K_2	144.32 ± 0.97*	5.05 ± 0.13*	1.61 ± 0.02*
S_3K_3	130.24 ± 0.80*	9.76 ± 0.23*	4.96 ± 0.02*

Data are presented as mean ± standard deviation. The symbol * indicates a significant difference from the control (treatment K_0), while the symbol ^{ns} indicates no significant difference from the control, based on an independent t-test at a significance level of 5% (α = 0.05).

The functional importance of this response is underscored by the role of flavonoids as key reactive oxygen species (ROS) scavengers (Mlcek et al., 2016; Pan et al., 2018). The restoration and enhancement of flavonoid content at 600 ppm in Dosroha likely contributed to its improved antioxidant capacity, indicating a coordinated defense response against colchicine-induced oxidative stress. This highlights Dosroha's unique ability to metabolically rebound under high colchicine pressure, unlike Pasaran and Siboro, which remained suppressed.

Total phenolic content showed a generally similar but more uniformly suppressive response across accessions. In Pasaran and Siboro, phenolic levels declined significantly with increasing colchicine concentration, with the strongest suppression occurring at the highest dose. This pattern suggests that phenylpropanoid metabolism in these accessions is highly sensitive to cytogenetic interference. The inhibitory effect of colchicine on phenolic synthesis is likely linked to reduced activity of phenylalanine ammonia-lyase (PAL), a key enzyme in the phenylpropanoid pathway, under conditions of disrupted mitosis (Gantait & Mukherjee, 2021).

Comparable reductions in phenolic accumulation following colchicine treatment have been reported in other Allium species (Wen et al., 2022; Abubakar et al., 2025).

Dosroha displayed a contrasting response, with phenolic content increasing significantly at 400 ppm and reaching its highest level at 600 ppm. This rebound suggests that high colchicine concentration triggered a shift toward stress-related secondary metabolism, possibly reflecting enhanced sink–source reallocation or compensatory activation of phenylpropanoid pathways. Such behavior is characteristic of tissues experiencing unstable or partial polyploidization, where metabolic plasticity may increase in response to genomic imbalance (Eng & Ho, 2019).

Overall, these results demonstrate that flavonoid and phenolic metabolism are highly sensitive to colchicine-induced cytogenetic stress, but the direction and magnitude of the response are strongly genotype-dependent. While Pasaran and Siboro primarily exhibited metabolic suppression at medium to high colchicine concentrations, Dosroha showed a unique capacity to recover and even enhance secondary metabolite production under high-dose treatment. This finding emphasizes that successful manipulation of secondary metabolites through colchicine induction depends not only on concentration but also on accession-specific stress tolerance and metabolic flexibility.

Conclusion

Agronomic evaluation of the M_1V_2 population showed strong genotype-dependent responses to colchicine treatment. Siboro exhibited the most favorable agronomic performance, with increased plant height, higher bulb number, and improved bulb weight and grading at moderate concentrations, indicating good physiological tolerance. Pasaran showed growth suppression and reduced bulb quality at higher colchicine doses, demonstrating high sensitivity, while Dosroha displayed mixed responses with improvements in bulb diameter but instability in yield traits. Flow cytometry confirmed that most individuals remained diploid across treatments, with only one mixoploid case detected in Dosroha at 400 ppm, indicating that colchicine up to 600 ppm did not induce stable polyploidization. Biochemical profiles showed that total flavonoid and phenolic contents generally decreased with increasing colchicine concentration, except in Dosroha at high dosage where partial recovery occurred. Overall, colchicine produced significant variation in agronomic and biochemical traits but did not successfully induce stable tetraploid lines in the M_1V_2 generation.

These findings suggest that Siboro is the most suitable accession for advancement in mutation breeding programs due to its agronomic stability and positive yield response under colchicine treatment. Dosroha may serve as a valuable source for improving bulb size and certain biochemical traits but requires cytological stabilization because of the mixoploid indication. Pasaran is less recommended for colchicine-based induction due to its high sensitivity and consistent performance decline. The lack of stable polyploid formation indicates the need for optimizing colchicine concentration, exposure duration, or alternative induction methods. For future breeding, integrating agronomic selection with cytological screening and biochemical profiling will be essential to identifying superior lines with enhanced productivity, improved bulb quality, and desirable phytochemical content.

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