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Increasing ploidy level of marigold (*Tagetes patula* Sudamala Barak) using colchicine with acute and chronic methods

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[2]Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Meranti, Bogor 16680, West Java, Indonesia. Jl. Meranti, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia. Tel./fax.: +62-251-8629347, ♥♥email: syarifah@apps.ipb.ac.id

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Abstract. *Sari YN, Aisyah SI, Sukma D, Syukur M. 2025. Increasing ploidy level of marigold (*Tagetes patula *Sudamala Barak) using colchicine with acute and chronic methods. Biodiversitas 26: 2726-2734.* Marigold (*Tagetes* sp.) is a popular and potential ornamental plant in Indonesia, especially in Bali, commonly used in religious ceremonies and industry. A significant challenge in the floriculture industry is producing plants with desirable traits, such as larger flowers. Sudamala Barak has dark red flowers with small size (4,5-5,6 cm), but consumers prefer larger ones (8-10 cm). Colchicine is a chemical mutagen that disrupts spindle fiber formation during mitosis, thereby inducing chromosome doubling and resulting in polyploid cells. *Tagetes patula* is an allotetraploid species (2n = 48). This study aimed to determine the LC_{50} value and analyze the morphological, histological, and cytological characteristics of Sudamala Barak mutants. The research was conducted from May 2024 to February 2025 using two application methods: acute (seven concentrations) and chronic (four concentrations) based on LC_{50} (lethal concentration 50%). The results showed that colchicine significantly affected various plant characteristics. The LC_{50} value was found to be 0.346%. Overall, the chronic method produced greater diversity than the acute method. Colchicine treatment influenced plant height, canopy width, number of primary branches, stem diameter, flowering time, and flower diameter. It also increased the number of stomata and chloroplasts, potentially improving photosynthetic efficiency. In the acute method, the highest ploidy level was euploid-pentaploid (2n = 60) at concentrations of 0.05%; 0.1%; 0.15%; 0.25%; and 0.3%. In contrast, in the chronic method, the highest ploidy level was euploid-autoalooctaploid (2n = 96) at 10× and 20× LC_{50} dilution concentrations.

Keywords: Breeding, euploid, marigold, mutation, octoploid

INTRODUCTION

Marigold (*Tagetes* sp.) is one of the ornamental plant commodities widely cultivated in Indonesia as a landscape plant, potted flower, or cut flower (Patel 2023). Marigold belongs to the Asteraceae family, with unique flower shapes, attractive colors, and a distinct aroma. Indonesia has 22 varieties of marigold flowers, which are the result of the introduction of the flower. Marigold flowers in Indonesia are generally yellow, red-orange, and orange. Marigold cultivation is developed for ornamental plants, refugia, industry, consumption, culture, and feed (Li et al. 2020). The people of Bali are very familiar with marigold flowers, the central flower in religious ceremonies and daily offerings. *Tagetes erecta* L. variety of marigold is the most commonly used in Bali. The marigold industry in Bali is estimated to reach 100-200 billion rupiahs per year, with a daily need of 8 tons. Near Galunggung Day, the demand for marigold flowers increases and can reach 40 tons daily (Kurniati 2021).

The challenge faced in the floriculture industry is providing ornamental plants with various outstanding characteristics. *T. patula* is an allotetraploid species (2n = 48) that probably originated from hybridization or crosses between the diploid *T. erecta* (2n = 24) and *Tagetes tenuifolia* (2n = 24), or another closely related species, *Tagetes jaliscensis* (2n = 24) (Towner 1961, 1962). *Tagetes patula* Sudamala Barak is a marigold variety with dark red flowers but small in size. The current flower diameter of Sudamala Barak ranges from 4.5-5.6 cm, while consumers desire larger flowers (8-10 cm). Improvement in flower size can increase the economic value of the flower. Consumers demand the availability of new, unique, and attractive shapes and colors of ornamental plants. Plant breeding plays a significant role in producing new varieties with desired traits. One breeding technique that can be applied to improve morphological diversity is through artificial mutation or mutation induction.

Mutation is a sudden genetic change that an organism's descendants can inherit. It can occur naturally or artificially and may trigger changes in genes or chromosomes. Mutation techniques have produced potential genotypes in various floriculture commodities (Aisyah et al. 2023). One mutation source that breeders can use is colchicine. Research by Dewi and Pharmawati (2018) showed that marigold cell size increased with colchicine at 0.1% and 0.2% compared to no colchicine treatment. Colchicine is the most commonly used mutagenic agent in vivo and in vitro. Colchicine is a natural substance extracted from *Colchicum autumnale* L., which can increase chromosome numbers or induce polyploidy in plants (Susrama and Yuliadhi 2020). Polyploidy is the increase in the number of

genomes (chromosome sets), which leads to a rise in nucleus size, thus enlarging cell, tissue, or organ size. Natural polyploidy usually originates from unreduced gametes (2n) production due to mitosis failure. Mitosis failure occurs because the formation of spindle threads is inhibited by binding to tubulin, inhibiting the polymerization of tubulin to become microtubulin. This causes the chromosomes not to separate during the cell division process, so the cells contain multiple chromosome sets, and a polyploid organism is formed. Kushwah et al. (2018) reported that using colchicine in chrysanthemums resulted in larger, more vigorous plants, thicker leaves, and more prominent flowers with thicker petals.

The application methods of mutagens in plant breeding can include acute (short-term) and chronic (long-term) mutation techniques. Acute mutation techniques involve a single application of a high concentration of mutagen, while the chronic mutation technique involves continuously applying a lower concentration over time. Previous research by Lenawaty et al. (2022) that used marigold seeds showed that using EMS mutagen with an acute application resulted in relatively low mutation rates and limited flower forms, while the chronic application produced more variation in the mutants. Due to the generally high sterility of marigolds in Indonesia, mutation induction through cuttings represents an alternative and innovative strategy in plant breeding. To date, there is no available data on the Lethal Concentration 50% (LC_{50}) of *T. patula*, particularly when using cuttings. Therefore, it is necessary to determine the LC_{50} for *T. patula* cuttings and to analyze the morphological, histological, and cytological characteristics of the mutants. The research results are expected to produce genotypes with superior traits that can benefit the agricultural industry.

MATERIALS AND METHODS

Study area

The research was conducted from May 2024 to February 2025. The colchicine mutation induction treatment was performed at the Tissue Culture Laboratory 1, IPB University. The mutation-induced cuttings were planted at the IPB Experimental Garden, Pasir Sarongge, Ciputri Village, Pacet, Cianjur Regency, Indonesia (±1100 meters above sea level). Cytological and histological observations were conducted at the Microtechnique Laboratory, IPB University. The experiment used a Randomized Completely Block Design (RCBD) with one factor and three replications. In the first experiment, there were seven concentration levels of treatment, and in the second experiment, there were four. The experimental unit consisted of 10 plants planted in beds measuring 1m × 4m.

Procedures

Mutation induction with acute application method

Colchicine was applied to cuttings that were 1 week old or had roots in healthy and uniform condition. The shoots were then wrapped with dry cotton and wetted with a colchicine solution at seven different concentrations: 0% (control), 0.05%, 0.1%, 0.15%, 0.2%, 0.25%, and 0.3% for 6 hours (03.00-09.00 am), based on modified protocols from Avdić et al. (2013), Sajjad et al. (2013), and Dewi and Pharmawati (2018) that using seeds and other species. A transparent plastic cover was used to prevent colchicine solution evaporation during treatment. The cuttings were then rinsed with running water five times, each for 2 minutes, to remove any remaining mutagen.

Mutation induction with chronic application method

The colchicine application in the chronic method started at 03.00 am. The shoots were then wrapped with dry cotton and wetted with the diluted colchicine solution. The colchicine concentration was based on the LC_{50} obtained from the previous experiment by diluting the colchicine solution into several concentrations: no colchicine (control), 10× LC_{50} dilution (12-hour immersion), 20× LC_{50} dilution (24-hour immersion), and 30× LC_{50} dilution (36-hour immersion).

Observation parameters

The observation parameters were recorded for the M1V3 generation plants, including morphological, histological, and cytological characteristics. The morphological traits included survival percentage (%), plant height (cm), flowering time (day after pinching), canopy width (cm), flower diameter (cm), stem diameter (cm), number of primary branches, and flower color. Histological analysis determined the number, density, and size of stomata and several chloroplasts in guard cells. Stomata observation used the lower leaf surface (4-5 weeks after planting) coated with clear nail polish and allowed to dry. A piece of tape was placed on the area with nail polish and then attached to a glass slide for observation under a microscope at 40× magnification (Makin et al. 2022). The results were transferred to Image-J software for analysis.

The chloroplast number in guard cells was observed at the middle section between the vein and leaf edge. The adaxial side of the leaf was scraped with a razor blade to remove the upper epidermis layer and the underlying tissue, leaving the lower epidermis layer (abaxial side). A drop of water was added to prevent cell shrinkage. The sample was then observed under a microscope with 40× magnification (Watts et al. 2023). The results were transferred to Image-J for analysis.

Cytological analysis to determine chromosome number. Observations were made on three cells from each treatment individual. Sample preparation involved cutting 2-3 mm of the root tip from the marigold cutting in the early morning (03.00-04.00 am). The root tips were treated with a 0.1% colchicine solution for 5 hours to facilitate chromosome visualization. For observation, the root tips were soaked in HCl for 10 minutes and then stained with Aceto-orcein for 10 minutes. The root tips were covered with a cover glass and gently squashed with a pencil eraser. Chromosomes were observed under a microscope with 100× magnification (Syukur et al. 2019).

Data analysis

The LC_{50} determination was analyzed using Curve Expert 1.4 software. Quantitative character analysis was performed using Analysis of Variance (ANOVA) at a 5% significance level. If there were significant differences, further testing was conducted using the R Studio application with Duncan's Multiple Range Test (DMRT) at a 5% significance level.

RESULTS AND DISCUSSION

LC_{50} value and sensitivity level of Sudamala Barak

The sensitivity of marigold plants to colchicine can be analyzed by determining the lethal concentration. The LC_{50} value is the dose that causes the death of 50% of the plant population in a single colchicine treatment (Roy et al. 2023). The LC_{50} value can be calculated based on the percentage of surviving plants four weeks after application. The lowest survival percentage occurred at a dose of 0.30%, reaching only 57% (Table 1). This result indicates that the marigold cuttings experienced damage due to the increasing colchicine concentration. This is in line with the findings of Cabahug et al. (2021) on succulent cuttings, which showed that increased colchicine concentration caused cell damage and decreased survival chances. According to Wibisono et al. (2022), this is due to the damage of cells and tissues caused by colchicine application, which results in the plant's inability to produce new cells, making it unable to survive. The surviving marigold cuttings appeared stunted, thick and had abnormal structures.

The Curve-fit software analysis showed that the best-fitting equation is a linear curve with y = 9.789-1.383x (Figure 1). Based on this equation, the LC_{50} value for *T. patula* Sudamala Barak cuttings due to colchicine soaking is achieved at a concentration of 0.346% (Table 1). This value represents the critical point, indicating the threshold concentration of colchicine that can potentially cause the death of *T. patula* Sudamala Barak cuttings. Mutant plants from colchicine treatments around the LC_{50} are expected to have high variability with minimal damage (Aisyah et al. 2022b; Rahmawati et al. 2024). A related species, chrysanthemum, shows an LC_{50} value of 0.2% for seeds (Kushwah et al. 2018).

Morphological characteristics of Sudamala Barak mutants

The ANOVA test results in the table indicate that colchicine treatments using both acute and chronic application methods significantly affected all observation variables (Table 2). Higher colchicine concentrations in both acute and chronic applications can affect plant height. Prolonged colchicine application can inhibit plant growth (Fathurrahman et al. 2023). Both acute and chronic colchicine treatments resulted in a reduction in plant height. This finding aligns with the research of Yan et al. (2022), which showed that a colchicine treatment of 3% for 12 hours significantly reduced the height of *Buddleja lindleyana* plants. Disruption in cell division can lead to smaller organ sizes with higher ploidy levels (Mo et al. 2020). Reducing plant height through polyploidization encourages plants to become more tolerant of environmental conditions and easier to care for (Sattler et al. 2016; Ghanbari et al. 2019). Specifically, ornamental plants with shorter stature can strengthen the canopy against extreme environmental conditions.

Colchicine treatment, both acute and chronic applications, affected the canopy width of the plants. The canopy width character correlated positively with the number of branches, meaning that more expansive canopies indicate more branches. According to Eng and Ho (2019), shorter stems result in greater compactness in plant architecture. A short plant with a broad canopy is the primary goal for pot flower production due to its aesthetic value and easier handling during transportation. Both acute and chronic applications can increase the number of primary branches. A study by Aisyah et al. (2022a) also showed that applying several concentrations of colchicine significantly increased the number of branches in *Portulaca grandiflora*. The number of branches negatively correlated with plant height and canopy width, meaning taller plants have fewer branches. Acute and chronic colchicine applications showed slight increases in stem diameter. This is consistent with research by Tammu et al. (2021), where colchicine treatment at 0,1% significantly increased the stem diameter of chili plants. Stem diameter negatively correlated with plant height, meaning that the taller the plant, the smaller the stem diameter. With increased stem girth, this morphological change leads to more substantial plant growth (Prasath et al. 2022).

Table 1. Percentage of live plants *Tagetes patula* Sudamala Barak after colchicine treatment

Concentration (%)	Percentage of plants survival (%)	Equation	LC_{50} (%)
0	97	Linear Fit y = a + bx y = 9.789-1.383x	0.346
0.05	90		
0.10	86		
0.15	79		
0.20	67		
0.25	63		
0.30	57		

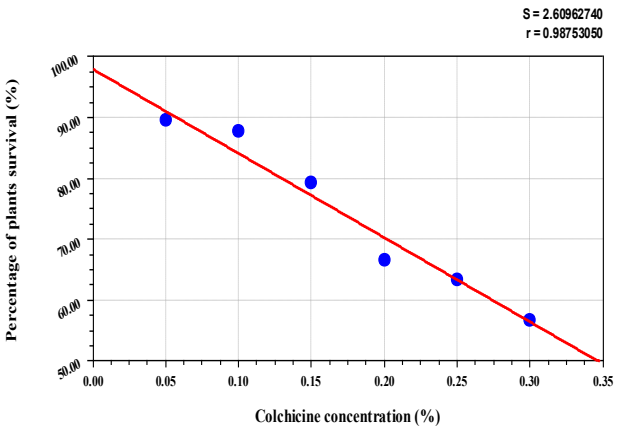


Figure 1. The curve of the percentage of survival *Tagetes patula* Sudamala Barak plants

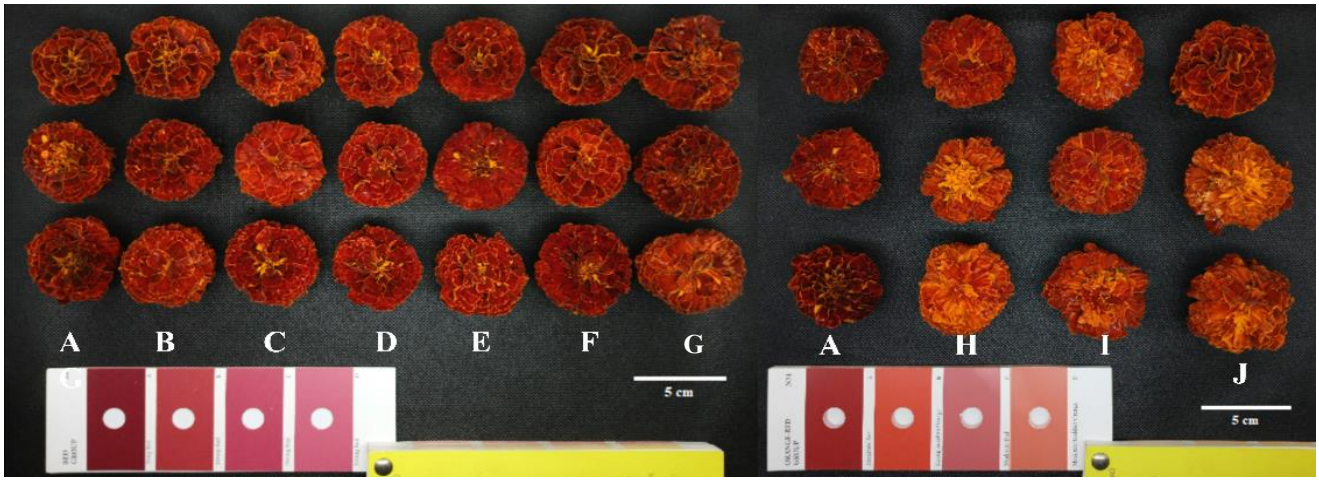


Figure 2. Flower characteristics of *Tagetes patula* Sudamala Barak mutant generation M1V3: A. Control, B. 0.05%, C. 0.1%, D. 0.15%, E. 0.2%, F. 0.25%, G. 0.3%, H. 10× LC_{50}, I. 20× LC_{50}, J. 30× LC_{50}

Table 2. Quantitative characteristics of *Tagetes patula* Sudamala Barak mutants generation M1V3

Method	Concentration	PH	CW	NPB	SD	FT	FD	FC
Acute	0%	103.5^{a}	77^{abc}	69^{b}	2.1^{b}	39^{b}	4.6^{f}	RHS 53A
	0,05%	93.7^{d}	71.6^{d}	7.6^{a}	2.6^{a}	42^{a}	5^{e}	RHS 53B
	0,1%	96^{cd}	76.9^{bc}	7.5^{ab}	2.7^{a}	40^{ab}	5.1^{d}	RHS 53B
	0,15%	96.7^{cd}	76.6^{bc}	7.8^{a}	2.7^{a}	39^{b}	5^{de}	RHS 53B
	0,2%	97.3^{cd}	75.4^{cd}	7.6^{a}	2.6^{a}	42^{a}	5.2^{c}	RHS 53B
	0,25%	101.5^{ab}	81.1^{a}	8.1^{a}	2.6^{a}	39^{b}	5.3^{b}	RHS N34A
	0,3%	98.5^{bc}	79.8^{ab}	7.7^{a}	2.5^{a}	42^{a}	5.8^{a}	RHS N34A
Chronic	0%	103.2^{a}	77.6^{b}	7.1^{b}	$2.^{c}$	41^{c}	4.7^{c}	RHS 53A
	10x LC_{50}	101.9^{a}	81.9^{a}	8^{a}	2.5^{a}	44^{b}	5.7^{b}	RHS 53B
	20x LC_{50}	103.6^{a}	80.4^{ab}	8^{a}	2.3^{b}	45^{b}	5.6^{b}	RHS N34A
	30x LC_{50}	99^{b}	79.9^{ab}	8.1^{a}	2.2^{c}	49^{a}	6^{a}	RHS N34A

Note: Numbers followed by the same letter for each parameter indicate no significant difference at the 5% significance level in the Duncan's Multiple Range Test (DMRT). PH: Plant Height (cm), CW: Canopy Width (cm), NPB: Number of Primary Branches, SD: Stem Diameter (cm), FT: Flowering Time (day after pinching), FD: Flower Diameter (cm), FC: Flower Color

Flowering time was recorded after the last pinching, 6 weeks after planting. Flowering time varied with each concentration. This aligns with research by Samadi et al. (2022), which observed that colchicine at different concentrations delayed flowering in *Crocus sativus* plants. Kushwah et al. (2018) revealed that colchicine treatment resulted in slightly slower growth but stronger and larger plants with larger flowers. Polyploidized plants usually exhibit slower growth rates, leading to slower or more extended flowering periods than their diploid counterparts (Sattler et al. 2016). Changes in the nucleus to cytoplasm ratio explain the growth inhibition in some plants, facilitating ecological adaptation in certain species (Fakhrzad et al. 2023).

Flower color variation differs based on the colchicine concentration applied (Figure 2). Without colchicine, the flowers are deep red (RHS 53A). Applying colchicine using the acute method at concentrations of 0.05-0.2% changes the flower color to an intense red (RHS 53B). The flowers turn softer red or moderate red at higher concentrations (0.25-0.3%) (RHS N34A). In the chronic application method, flowers at higher concentrations generally shift to moderate red (RHS N34A). This indicates that higher concentrations lead to softer red flowers than the intense red seen at lower concentrations. Flowers' shape, size, and color are key factors in determining flower quality and, primarily, the commercial value of ornamental plant species (Cristina and Sala 2023). Additionally, flower color is essential in ecology, particularly in attracting pollinating insects. According to Buchori et al. (2024), deep red flowers indicate a high anthocyanin accumulation in the petals. Anthocyanins produce pink to deep purple hues, while carotenoids are more dominant in producing yellow to red hues (Kishimoto et al. 2019; Yamagishi 2020). Combining anthocyanins and carotenoids results in red coloration (Li et al. 2022). In *T. patula*, red flowers are caused by the presence of xanthophyll and anthocyanin compounds, such as cyanidin-3-galactoside, cyanidin-3-glucoside, and cyanidin-3-sophoroside (Teixeira et al. 2023). The ornamental plant industry highly values new qualitative traits, such as dwarf form and striking flowers. Generally, acute and chronic applications at higher concentrations result in larger flower diameters, which may indicate an increase in ploidy levels. Research by Bhattarai et al. (2021) found that size flowers of tetraploid *Gerbera* were more significant than those of diploid *Gerbera*. Larger flowers are a primary result of the gigantism phenomenon

and are highly sought after for their ornamental advantages. Polyploidy leads to larger organs, such as flowers and leaves (Iannicelli et al. 2020).

Histological characteristics of Sudamala Barak mutant

ANOVA results presented in the table show that colchicine treatment using the acute application method significantly affected the number of stomata, stomatal density, stomatal length, and the number of chloroplasts (Table 3). Chronic colchicine application significantly affected stomatal length, stomatal width, and the number of chloroplasts (Table 3). The number of stomata correlates positively with stomatal density and negatively with stomatal size (Figure 3). An increasing number of stomata results in higher density and smaller stomatal size, and vice versa. A similar finding was observed by Bat et al. (2021) in pepper and eggplant, where an increase in chromosome number led to larger cell sizes while the number of stomata per field of view decreased.

Stomatal density negatively correlates with stomatal size. As ploidy levels increase, stomatal density decreases while stomatal size increases (Kruthika et al. 2023). This is likely due to the larger cell size in polyploids, which may require a lower stomatal density to maintain adequate gas exchange (Afifah et al. 2020). Additionally, larger stomata in polyploids may facilitate more efficient water absorption, as polyploids often have higher water requirements than diploids (Sanaei-Hoveida et al. 2024). Research by Karagöz et al. (2024) on *Silene compacta*. plants found that tetraploid plants had larger stomatal sizes than the diploid control plants. The parameter for the number of chloroplasts in guard cells is one of the most stable parameters. According to Ansari et al. (2022), calculating chloroplasts in guard cells is an indirect and accurate method to evaluate the ploidy level of a plant. In general, the number of chloroplasts tends to increase with the increase in the ploidy level of a plant. Additionally, chloroplast numbers positively correlate with stomatal size. A similar study by Chinnathambi et al. (2024) on marigolds in haploid condition showed an average of 8 chloroplasts, tetraploids with an average of 16 chloroplasts, and polyploids with an average of 18 to 20 chloroplasts in guard cells. A more significant number of chloroplasts allows an increase in photosynthetic capacity and energy production needed to support more considerable cellular activity (Farhadi et al. 2023).

Cytological characteristics of Sudamala Barak mutant

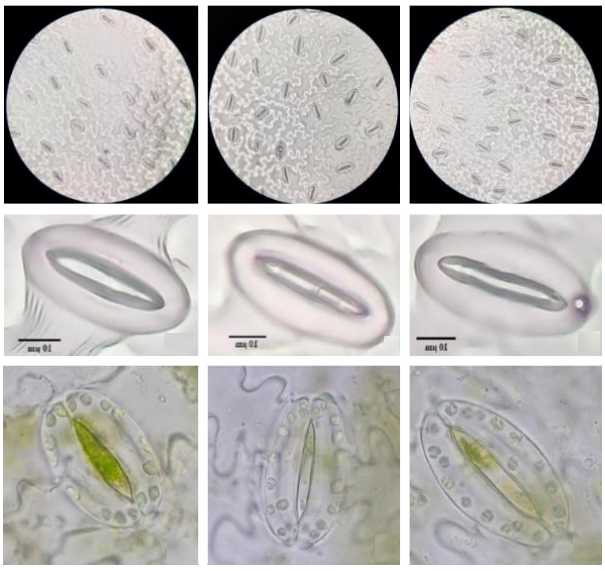
Cytological analysis results show that colchicine treatment causes variations in chromosome numbers in several genotypes. Colchicine is an alkaloid compound that binds to tubulin and prevents its polymerization into microtubules, which play a role in mitosis, resulting in polyploid cells with a doubled chromosome number (Haring et al. 2023). According to Gupta et al. (2021), mutations caused by polyploidization involving gene mutations result in overall genomic modifications, creating extensive phenotypic variations. Polyploidy is when a cell or organism gains one or more additional sets of chromosomes (Udofia et al. 2024). Polyploidy is classified into euploid and aneuploid based on chromosome composition (Syukur et al. 2019; Yirga 2021).

Euploid is a chromosome state where the number is a multiple of the basic chromosome number, e.g., diploid, triploid, tetraploid, etc. Based on genomic composition, euploids can be classified into autopolyploid and allopolyploid. Autopolyploid contains multiple copies of the essential (×) chromosome set from the same genome, while allopolyploid is a combination of genomes from different species (Manzoor et al. 2019; Yirga 2021).

Table 3. Histological characteristics of *Tagetes patula* Sudamala Barak mutant generation M1V3

Method	Concentration	SN	SD	SL	SW	CN
Acute	0%	28^{b}	139.9^{b}	39^{c}	22	16^{d}
	0.05%	26^{b}	132.8^{b}	46.7^{b}	23	20^{c}
	0.1%	23^{c}	118.2^{c}	48.6^{ab}	25.6	22^{ab}
	0.15%	26^{b}	134.1b	46.3^{b}	23.7	21^{b}
	0.2%	22^{c}	112.2c	49.6^{a}	25	22^{a}
	0.25%	32^{a}	161.3^{a}	46.2^{b}	23	22^{ab}
	0.3%	29^{b}	145^{b}	46.7^{b}	23.4	21^{ab}
Chronic	0%	22	112.1	42.7^{b}	23.8^{b}	16^{b}
	10x LC_{50}	22	111	52.5^{a}	26.7^{a}	21^{a}
	20x LC_{50}	23	114.6	49.2^{a}	25.3^{ab}	21^{a}
	30x LC_{50}	20	102.5	52.1^{a}	25.9^{a}	21^{a}

Note: Numbers followed by the same letter within each parameter indicate no significant difference in the DMRT test at the 5% level. SN: Stomatal Number; SD: Stomatal Density (/μm²); SL: Stomatal Length (μm); SW: Stomatal Width (μm); CN: Chloroplast Number



A B C

Figure 3. Cross-section of *Tagetes patula* leaves showing stomata and chloroplasts; upper (stomatal density at 40× magnification), middle (stomatal size at 40× magnification), lower (number of chloroplast at 100× magnification). A. Control, B. Acute treatment with 0.2% concentration, C. Chronic treatment with 10× LC_{50} concentration

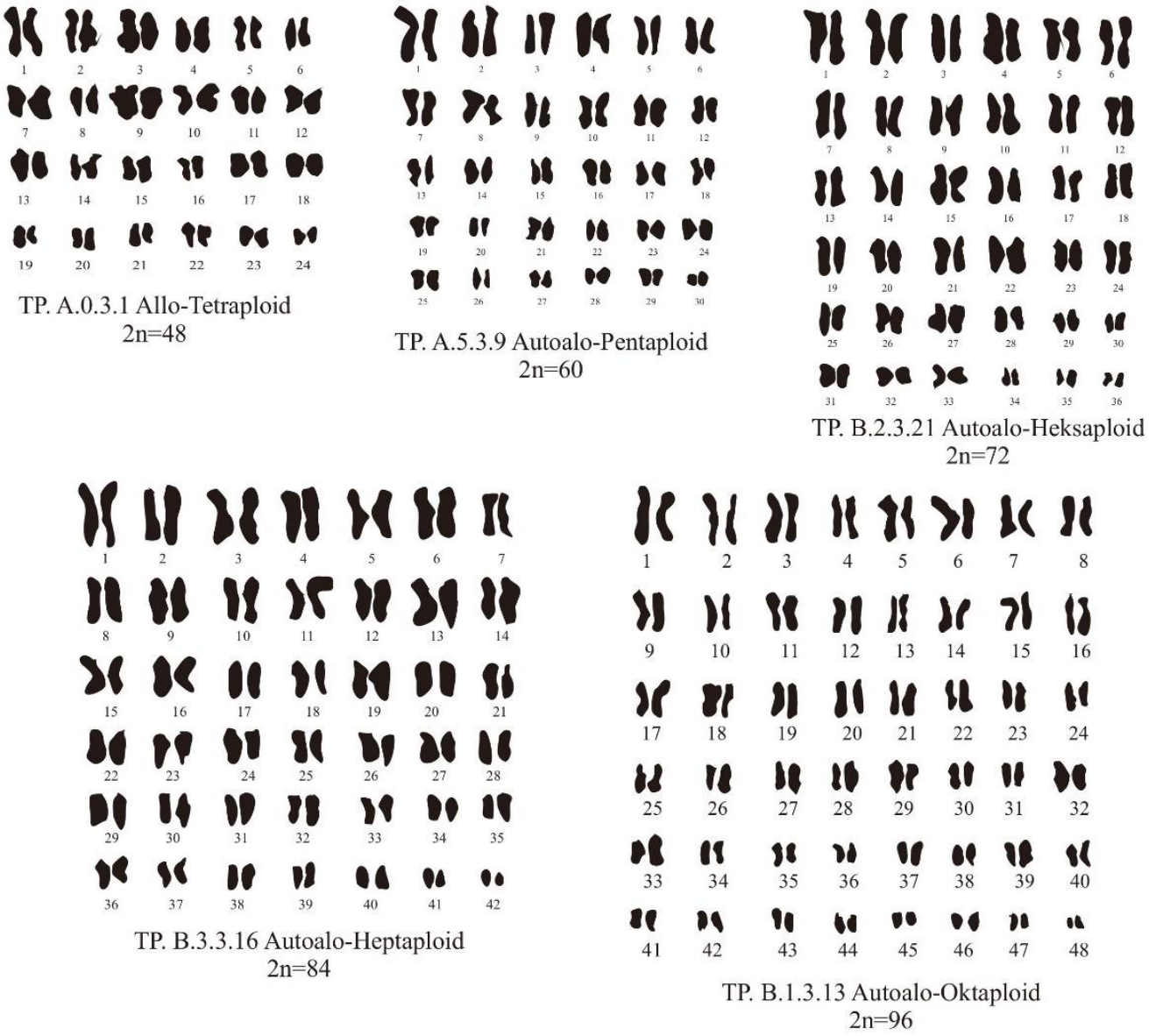


Figure 4. Karyotype analysis of several *Tagetes patula* Sudamala Barak genotypes

Aneuploid refers to chromosome imbalance, whether there is an excess or reduction from the normal diploid state, such as nullisomic (2n-2), monosomic (2n-1), trisomic (2n+1), and tetrasomic (2n+2) (Yirga 2021). Aneuploidy occurs due to failure in spindle fiber attachment to the poles, causing one chromatid not to separate during metaphase, known as non-disjunction, leading to aneuploidy, where one chromosome lacks a pair (Ji et al. 2022; Song et al. 2024). Chromosomal differences between cells within the same individual are also possible, referred to as mixoploid, indicating the occurrence of chimeras, which are unstable mutants (Widoretno et al. 2023).

Tagetes patula generally has a basic chromosome number of 2n = 4× = 48 (autotetraploid) (Towner 1961, 1962). In the acute method, several ploidy levels are observed, both euploid and aneuploid (Figure 4). There are 13 genotypes of euploid-autoallopentaploid (2n = 60) and seven genotypes showing chromosomal imbalance or mixoploid. Furthermore, there are 11 genotypes of aneuploid-tetrasomic (2n+2 = 50), nine genotypes of aneuploid-double tetrasomic (2n+2+2 = 52), and four genotypes of aneuploid-triple tetrasomic (2n+2+2+2 = 54). A concentration of 0.05% results in more variation in chromosome numbers in *T. patula* mutants. The highest ploidy level, euploid-pentaploid (2n = 60), is observed at concentrations of 0.05%; 0.1%; 0.15%; 0.25%; and 0.3%.

More diverse mutants are produced in the chronic method compared to the acute method, which includes both euploid and aneuploid ploidy levels (Figure 4). This is in line with Karagöz et al. (2024) who stated that at low concentrations with longer exposure times, the toxic effects are reduced, and polyploid plant formation rates are increased. There are 13 genotypes of euploid-autoallopentaploid (2n = 60), seven genotypes of euploid-

autoallohexaploid (2n = 72), two genotypes of euploid-autoalloheptaploid (2n = 84), two genotypes of euploid-autoallooctaploid (2n = 96), and six genotypes showing chromosomal imbalance or mixoploid. Additionally, there are three genotypes of aneuploid-tetrasomic (2n+2 = 50), three genotypes of aneuploid-double tetrasomic (2n+2+2 = 52), five genotypes of aneuploid-triple tetrasomic (2n+2+2+2 = 54), and two genotypes of aneuploid-quadruple tetrasomic (2n+2+2+2+2 = 56). The dilution concentration of 10x LC_{50} results in more variation in chromosome numbers in *T. patula* mutants. The highest ploidy level, euploid-autoallooctaploid (2n = 96), is observed at 10× and 20× LC_{50} dilution concentrations.

Colchicine-induced acute and chronic mutations with various concentrations resulted in several genotypes with different ploidy levels. Based on Table 6, there is an increase in ploidy levels due to colchicine mutation induction. A study by Mahanta et al. (2023) on *Gerbera* plants treated with 0.1% colchicine for 4 hours resulted in tetraploid plants with double the chromosomes (2n = 4× = 100) compared to their diploid counterparts (2n = 2× = 50). This is consistent with research by Anggraeni et al. (2023) on Artemisia cina treated with 200 ppm colchicine for 24 hours, which resulted in one diploid plant (2n = 18), one pentaploid (2n = 45), one hexaploid (2n = 54), and one octaploid (2n = 64). A study by Bhattarai et al. (2021) found that a colchicine concentration of 0,1% was more efficient in inducing in vivo tetraploid *Gerbera*.

In this study, an increase in chromosome number was positively correlated with an increase in flower diameter and the number of chloroplasts. Plant height showed a negative correlation with chromosome number. Higher ploidy levels in plants lead to gigas traits, such as larger flowers than diploid plants (Kruthika et al. 2023). Cells with more chromosomes and DNA enlarge to maintain a constant relationship between the cytoplasmic volume and this increase, which can be observed in organ and plant size (Iannicelli et al. 2020). According to Manzoor et al. (2019), cell size increase is the most crucial result of polyploidization due to extra gene copies. Along with the increased size of various vegetative and reproductive parts in tetraploid plants, chromosome duplication can alter growth habits, sexual patterns, and sterility and sometimes enhance cold resistance. The increased sterility of induced tetraploids makes vegetative propagation the primary method for ornamental plants. Delayed flowering, increased flower diameter, and flowers with different colors on the same branch are common characteristics of colchicine-treated plants.

The application of colchicine on *Tagetes patula* Sudamala Barak cuttings affect various morphological and genetic aspects of the plant. Based on the quadratic fit curve equation, the LC_{50} value was obtained at a concentration of 0.346%, which represents the critical colchicine concentration that potentially causes cutting death. The colchicine treatment influenced plant height, canopy width, number of primary branches, stem diameter, flowering time, and flower diameter. Additionally, the colchicine treatment affected the stomatal characteristics and chloroplast count. The colchicine treatment resulted in variations in ploidy levels, ranging from euploid to aneuploid. Generally, the chronic method resulted in more variation than the acute method. In the acute method, the highest ploidy level, euploid-pentaploid (2n = 60), was found at concentrations of 0.05%; 0.1%; 0.15%; 0.25%; and 0.3%. Meanwhile, in the chronic method, the highest ploidy level, euploid-autoalooktaploid (2n = 96), was found at 10× and 20× LC_{50} dilution concentrations. The increased variation resulting from the chronic treatment highlights its potential as a more efficient method for generating advantageous mutations in marigold breeding program. The ploidy level variations induced by colchicine treatments could act as a genetic resource for the development of superior marigold cultivars with enhanced ornamental characteristics and increased adaptability.

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