1st International Conference on Multidisciplinary Engagement

Colchicine-Mediated Polyploidization for Improvement of French Marigold Traits

Aziz Nur Arifin[1*], Parjanto[2], Endang Yuniastuti[3]
[1]*Master of Agronomy Study Program, Faculty of Agriculture, Universitas Sebelas Maret (UNS), Jl. Ir. Sutami 36A, Kentingan, Surakarta, 57126, Central Java, Indonesia*
[2,3]*Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret (UNS), Jl. Ir. Sutami 36A, Kentingan, Surakarta, 57126, Central Java, Indonesia*

Corresponding Author.
Email: parjanto@staff.uns.ac.id .

Abstract: *This study evaluated the effects of colchicine concentration and soaking duration on vegetative morphology of French marigold (Tagetes patula L.) cv. Janie Spry, a naturally allotetraploid species (2n = 4x = 48). A 4×3 factorial completely randomized design was employed with four colchicine concentrations (0%, 0.1%, 0.2%, 0.3%) and three soaking durations (4, 8, 12 hours), totaling 12 treatments with three replications (n = 36). Germinated seeds at the radicle stage were soaked in colchicine solutions and grown in a greenhouse. Survival rate, plant height, stem diameter, and number of shoots were recorded during the vegetative phase. Two-way ANOVA revealed highly significant interaction effects ($p<0.001$) of concentration and duration on survival rate and shoot number, while plant height and stem diameter were not significantly affected. Survival declined sharply at higher concentrations with prolonged soaking, reaching only 30% at 0.3% colchicine for 12 hours. The 0.2% colchicine at 4-hour soaking treatment was identified as optimal, maintaining 90% survival while increasing shoot number by 37.7% relative to the control (30.7 vs. 22.3 shoots plant^{-1}). The highest shoot count (34.3 shoots plant^{-1}) occurred at 0.3% for 12 hours but was accompanied by unacceptable seedling mortality. These results indicate that moderate colchicine concentrations with short exposure offer an effective balance between morphological modification and seedling viability in T. patula. Cytological confirmation of ploidy levels through chromosome counting and flow cytometry is ongoing.*
Keywords: *Colchicine, Polyploidization, Tagetes Patula, French Marigold, Morphological Traits*

1. INTRODUCTION

French marigold (*Tagetes patula* L.) is an annual ornamental species belonging to the family Asteraceae, native to Mexico and Guatemala, and now cultivated worldwide for its vibrant flowers and adaptability to diverse agro-climatic conditions [1]. The genus *Tagetes* comprises approximately 55 species, of which *T. erecta* (African marigold) and *T. patula* are the two most commercially important [2]. Beyond its ornamental value, *T. patula* serves multiple purposes: its florets are used as natural colorants in poultry feed and food products, its essential oil finds application in perfumery, and the whole plant produces thiophene compounds with nematicidal properties against root-knot nematodes (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.) [3]. In Indonesia, marigold cultivation has not yet reached the scale of orchids, chrysanthemums, or roses despite its ease of production and broad utility [4]. A persistent limitation of French marigold in the market is its relatively small flower size and limited morphological diversity compared to African marigold, which reduces its competitiveness in the ornamental plant trade [5].

Polyploidization — the induction of additional chromosome sets — offers a well-established route for improving flower size, petal thickness, color intensity, and overall plant vigor in ornamental species [6]. In horticultural breeding, polyploidy has been induced successfully in chrysanthemum, gladiolus, crape myrtle, wallflower, gerbera, and several other crops using colchicine as a mitotic inhibitor [6], [7], [8], [9]. Colchicine binds to tubulin and prevents spindle fiber assembly during cell division, causing daughter cells to retain double the parental chromosome complement [10]. The efficiency of polyploidy induction depends on the interplay

between colchicine concentration, treatment duration, and the type and developmental stage of explant material [11]. Higher concentrations and longer exposure periods generally increase the probability of chromosome doubling but also raise the risk of cytotoxic damage, reduced survival, and chimeric tissue formation [11], [12]. Optimizing this trade-off between mutagenic effectiveness and seedling viability is therefore central to any polyploidy induction protocol.

Within the genus *Tagetes*, most colchicine studies have focused on *T. erecta*, a diploid species (2n = 2x = 24), where in vitro treatment of nodal segments at 0.01–0.05% colchicine successfully induced tetraploids with larger stomata, reduced stomatal density, and enhanced floral characteristics [13], [14]. Yongyao et al. [15] reported that 0.15% colchicine applied to *T. erecta* seeds for 24 hours produced a 56% mutation rate, with confirmed tetraploid progeny displaying significantly greater stem diameter, palisade tissue thickness, and single flower weight. However, *T. patula* differs fundamentally from *T. erecta* in genomic constitution: cytogenetic evidence established that *T. patula* is an allotetraploid (2n = 4x = 48), likely originating from ancient hybridization between *T. erecta* and *T. tenuifolia* (2n = 24) or closely related diploid progenitors [16], [17]. This pre-existing polyploid status means that colchicine treatment of *T. patula* would aim to induce octoploidy (2n = 8x = 96) or at least generate mixoploid sectors with higher ploidy levels, rather than the tetraploidy targeted in diploid species. A recent study by Sari et al. [18] on *T. patula* cv. Sudamala Barak confirmed this possibility, reporting ploidy levels up to euploid-autoalooctaploidy (2n = 96) under chronic colchicine application. Despite these advances, studies that systematically evaluate factorial combinations of colchicine concentration and soaking duration on *T. patula* morphological responses remain scarce. Avdić et al. [19] examined concentration, exposure time, and application method effects on *T. patula* var. *nana*, but their work was limited to a single cultivar and did not employ a full factorial design with replication suitable for interaction analysis.

The present study addresses this gap by investigating the effects of four colchicine concentrations (0%, 0.1%, 0.2%, and 0.3%) and three soaking durations (4, 8, and 12 hours) on the vegetative morphological traits of *T. patula* cv. Janie Spry using a factorial completely randomized design. The specific objectives were to: (1) evaluate the main effects and interaction of colchicine concentration and soaking duration on seedling survival, plant height, stem diameter, and shoot number; and (2) identify the optimal treatment combination that maximizes desirable morphological changes while maintaining acceptable seedling viability. This paper presents partial results from an ongoing master's thesis research at Universitas Sebelas Maret, focusing on the vegetative growth phase. Cytological confirmation of ploidy levels and floral trait evaluation will be reported in a subsequent publication.

2. METHOD

This study was conducted from December 2025 to March 2026 at Palur, Jaten Sub-district, Karanganyar Regency, Central Java, Indonesia (200 m above sea level). The experiment used a completely randomized design (CRD) arranged as a 4×3 factorial with two treatment factors: colchicine concentration at four levels (M_1 = 0% as control, M_2 = 0.1%, M_3 = 0.2%, and M_4 = 0.3%) and soaking duration at three levels (J_1 = 4 hours, J_2 = 8 hours, and J_3 = 12 hours). The factorial combination yielded 12 treatments, each replicated three times, producing a total of 36 experimental units.

Seeds of French marigold (*Tagetes patula* L.) cv. Janie Spry were selected based on physical integrity and dark coloration. Viability was tested by flotation in water; only seeds that sank were used. Viable seeds were imbibed in distilled water for 6 hours and then germinated on moist tissue paper in Petri dishes until the radicle reached approximately 1 cm in length. At the radicle stage, germinated seeds were immersed in the respective colchicine solutions according to the assigned treatment combinations. This radicle-stage seed-soaking approach is recognized as one of the most effective methods for inducing polyploidy in species with actively dividing meristematic tissue [6], [11]. Colchicine stock solution (1%) was prepared by dissolving 1 g of colchicine in 5 mL ethanol and adding 95 mL distilled water; working concentrations of 0.1%, 0.2%, and 0.3% were obtained through serial dilution. Control seeds were soaked in distilled water for the corresponding durations. After treatment, all seeds were rinsed thoroughly under running water and sown in seedling trays filled with a growing medium consisting of soil, rice husk charcoal, and manure at a 1:1:1 ratio (v/v/v).

Seedlings were maintained under shade for approximately 14 days until the emergence of true leaves and then transplanted into 35×35 cm polybags containing the same medium. Plants were grown in a greenhouse under full sunlight. Irrigation was applied once daily or as needed, and fertilization was carried out twice weekly

using ABmix nutrient solution at 1000 ppm. Pest and disease management was performed using synthetic pesticides when necessary.

This paper reports preliminary results from the vegetative growth phase of the experiment. Four morphological parameters were recorded: survival rate (proportion of surviving plants per treatment), plant height (cm), stem diameter (mm), and number of shoots per plant. Measurements were taken after plants had established in the polybags and reached the active vegetative stage. Floral characteristics and cytological observations (chromosome analysis via the squash method) are part of the ongoing full study and will be reported separately.

Data were analyzed using two-way analysis of variance (ANOVA) at $\alpha = 0.05$ to test the main effects of colchicine concentration (M) and soaking duration (J), as well as their interaction (M×J). When significant differences were detected, Duncan's Multiple Range Test (DMRT) at the 5% significance level was applied for mean separation. This analytical framework follows the standard factorial approach commonly used in colchicine-mediated polyploidy studies [11], [18]. All statistical computations were performed using Python (statsmodels and scipy libraries). Interaction plots and bar charts were generated using matplotlib and seaborn to visualize treatment effects.

3. RESULTS AND DISCUSSION

3. RESULTS AND DISCUSSION

3.1. Survival Rate

Two-way ANOVA revealed that colchicine concentration, soaking duration, and their interaction all had highly significant effects ($p<0.001$) on the survival rate of *Tagetes patula* seedlings (Table 1). Control plants (M_1) maintained a survival rate between 0.60 and 1.00 across all soaking durations, whereas plants treated with 0.3% colchicine and soaked for 12 hours ($M_4 \times J_3$) exhibited the lowest mean survival at 0.30±0.00 (Table 2). A clear dose-dependent and duration-dependent decline in viability was observed: at the 4-hour soaking interval, all colchicine-treated groups retained survival rates of 0.90, comparable to the control (1.00). However, extending the soaking period to 12 hours sharply reduced survival across all treated groups, with the most drastic decline occurring in the 0.3% treatment, where survival dropped from 0.90 at 4 hours to 0.30 at 12 hours — a 66.7% reduction (Fig. 1A).

This pattern of declining viability at higher concentrations and longer exposure times is well documented in colchicine-mediated polyploidy induction. Colchicine disrupts spindle fiber assembly during mitosis, and prolonged cellular exposure exacerbates cytotoxic damage to meristematic tissues, eventually inhibiting cellular respiration and nutrient uptake [20], [21]. Eng and Ho [11] reported that the highest mutation rates during polyploid induction tend to occur near the lethal dose threshold, which creates an inherent trade-off between achieving chromosome doubling and preserving seedling viability. In *Tagetes erecta*, Sajjad et al. [22] similarly observed an inverse relationship between colchicine concentration and explant regeneration, with 0.05% colchicine reducing regeneration to 27.3% compared to 87.8% in untreated controls. The $M_3 \times J_1$ treatment (0.2% colchicine, 4 hours) in the present study achieved 90% survival while still being within the effective range for polyploidy induction, which aligns with reports on *Silene compacta* where 0.1% colchicine at 12 hours was identified as the optimal balance point [23]. The survival data suggest that 4-hour soaking preserves adequate viability regardless of colchicine concentration (all treatments ≥0.90), whereas extending treatment duration beyond 8 hours at concentrations above 0.2% pushes seedling mortality past acceptable thresholds.

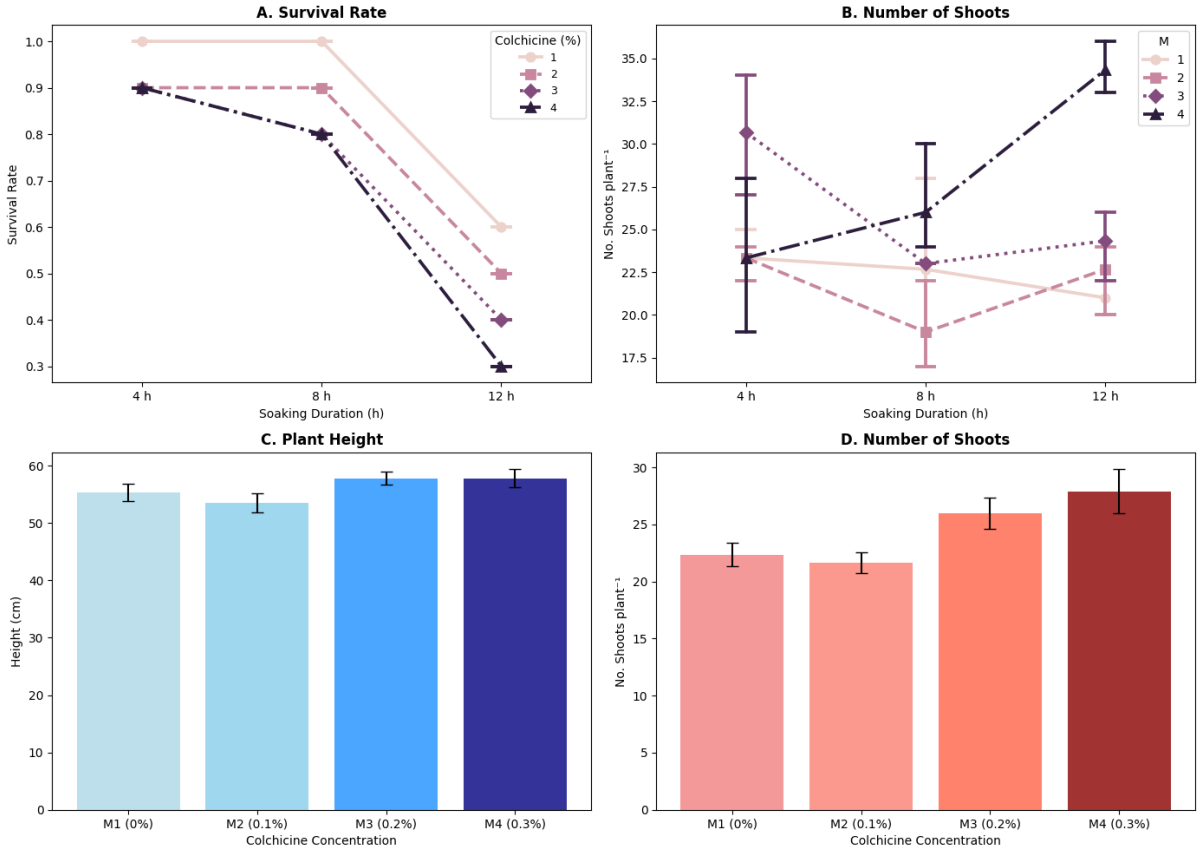

Figure 1. Interaction effects of colchicine concentration and soaking duration on morphological traits of colchicine-treated *Tagetes patula* L. seedlings. (A) Survival rate showing significant M×J interaction ($p<0.001$). (B) Number of shoots per plant with highly significant interaction ($p=0.0009$). (C-D) Main effects of colchicine concentration showing optimal vegetative growth at 0.2-0.3% (M3, M4).

3.2. Plant Height

Neither colchicine concentration ($F=2.19$, $p>0.05$), soaking duration ($F=2.60$, $p>0.05$), nor their interaction ($F=1.38$, $p>0.05$) significantly affected plant height (Table 1). Mean plant height ranged from 49.3±1.45 cm in $M_2 \times J_3$ to 60.3±2.96 cm in $M_3 \times J_1$ and 60.3±2.19 cm in $M_4 \times J_3$ (Table 2). When pooled by colchicine concentration, M_3 (0.2%) and M_4 (0.3%) both averaged 57.8 cm, representing only a 4.4% increase over the M_1 (control) average of 55.3 cm (Fig. 1C). This marginal and statistically non-significant difference indicates that colchicine treatment at the tested concentrations did not substantially alter the vertical growth pattern of *T. patula* seedlings during the vegetative phase.

Table 1. Two-way ANOVA of morphological traits in colchicine-treated Tagetes patula L.

Source	df	Survival Rate	Plant Height	Stem Diameter	Number of Shoots
M	3	1.55×10^{28} ***	2.19 NS	0.88 NS	9.65 **
J	2	1.81×10^{29} ***	2.60 NS	0.82 NS	3.63 *
M×J	6	1.66×10^{27} ***	1.38 NS	0.92 NS	5.67 ***
Error	24				

Note: NS = not significant ($p>0.05$); * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

The absence of a height response contrasts with findings in some other species. Manzoor et al. [21] observed colchicine-treated gladiolus plants showing reduced height and leaf abnormalities at 0.2–0.3% concentrations, and Ye et al. [24] reported distinctly shorter and more compact tetraploid crape myrtle lines. Conversely, some researchers have noted height increases in colchicine-treated populations of parsley and sage [25]. These contradictory reports across species point to a genotype-dependent response. *Tagetes patula*, as an allotetraploid (2n=48) with an already duplicated genome, may exhibit greater buffering capacity against the morphological disruptions that colchicine typically induces in diploid species. Alternatively, the measurement was taken during the vegetative phase, and height differences may become apparent later during the reproductive stage when polyploid plants often display more compact architecture [26]. The relatively high within-treatment variability (standard errors reaching 4.0 cm in $M_2 \times J_1$) may also have masked subtle treatment effects. Supporting this interpretation, Sari et al. [18] similarly found no significant height response during the vegetative phase of colchicine-treated T. patula, suggesting that ploidy-related morphological changes in this species may be more evident during reproductive development than during vegetative growth.

3.3. Stem Diameter

Stem diameter was similarly unaffected by colchicine concentration ($F=0.88$, $p>0.05$), soaking duration ($F=0.82$, $p>0.05$), and their interaction ($F=0.92$, $p>0.05$), as shown in Table 1. Treatment means ranged narrowly from 11.3±0.82 mm in $M_1 \times J_2$ to 13.0±0.64 mm in $M_4 \times J_3$ (Table 2). Across colchicine levels, the pooled averages were 12.1 mm for M_1, 11.8 mm for M_2, 12.1 mm for M_3, and 12.6 mm for M_4, showing minimal variation. The non-significant result for stem diameter is noteworthy because successful polyploidization often manifests as thicker, more robust stems with enlarged cell sizes. In *Tagetes erecta*, polyploid individuals induced by 0.15% colchicine for 24 hours showed significantly greater stem diameter than their diploid counterparts [15]. The lack of a measurable stem diameter response in the current study could indicate that true polyploid conversion was limited to a subset of cells or that the measurement timing preceded the developmental stage at which polyploidy-related stem thickening becomes detectable. Chimeric tissue composition, where only some cell layers undergo chromosome doubling while others remain at the original ploidy, is a commonly reported outcome of colchicine seed soaking treatments [10]. Such chimeras may not show the uniform organ-level enlargement expected in fully converted polyploids.

Table 2. Treatment means ± standard error for morphological traits of Tagetes patula L.

Treatment	Colchicine×Time	Survival Rate	Plant Height (cm)	Stem Diameter (mm)	No. Shoots
1	M1×J1	1.00±0.00 a	59.7±2.19 ab	12.4±0.46 ab	23.3±0.88 bc
2	M1×J2	1.00±0.00 a	54.0±2.52 bcd	11.3±0.82 bcd	22.7±3.18 bc
3	M1×J3	0.60±0.00 bc	52.3±1.76 cd	12.5±0.12 ab	21.0±0.58 c
4	M2×J1	0.90±0.00 ab	56.0±4.00 abcd	12.0±0.27 abc	23.3±0.67 bc
5	M2×J2	0.90±0.00 ab	55.3±1.45 abcd	11.4±0.59 bcd	19.0±1.53 c
6	M2×J3	0.50±0.00 cd	49.3±1.45 d	12.0±0.95 abc	22.7±1.33 bc
7	M3×J1	0.90±0.00 ab	60.3±2.96 a	11.5±0.55 cd	30.7±2.03 a

8	M3×J2	0.80±0.00 abc	56.0±1.00 abcd	12.6±0.30 a	23.0±0.00 bc
9	M3×J3	0.40±0.00 d	57.0±1.00 abc	12.3±0.50 ab	24.3±1.20 bc
10	M4×J1	0.90±0.00 ab	57.3±2.33 abc	11.9±0.77 abcd	23.3±2.60 bc
11	M4×J2	0.80±0.00 abc	55.7±3.76 abcd	12.8±0.59 a	26.0±2.00 ab
12	M4×J3	0.30±0.00 d	60.3±2.19 a	13.0±0.64 a	34.3±0.88 a

Note: M1 = 0% (control); M2 = 0.1%; M3 = 0.2%; M4 = 0.3%. J1 = 4 h; J2 = 8 h; J3 = 12 h. Means within columns followed by different letters differ significantly by DMRT (α=0.05).

3.4. Number of Shoots

The number of shoots per plant was significantly affected by colchicine concentration ($F=9.65$, $p<0.01$), soaking duration ($F=3.63$, $p<0.05$), and — most notably — their interaction ($F=5.67$, $p<0.001$) (Table 1). This was the most responsive trait among the four morphological parameters evaluated. The $M_3 \times J_1$ treatment (0.2%, 4 hours) produced 30.7±2.03 shoots plant^{-1}, a 37.7% increase over the control mean of 22.3 shoots plant^{-1}. The highest absolute shoot number was recorded in $M_4 \times J_3$ (0.3%, 12 hours) at 34.3±0.88 shoots plant^{-1}, representing a 53.8% increase over the control (Table 2, Fig. 1D). Both treatments were classified into the same DMRT grouping (group "a"), significantly exceeding most other treatment combinations.

The highly significant M×J interaction for shoot number (Fig. 1B) reveals divergent response trajectories depending on colchicine concentration. In the M_3 group (0.2%), shoot production peaked at the 4-hour soaking interval (30.7) and then declined with longer exposure (23.0 at 8 hours, 24.3 at 12 hours). By contrast, the M_4 group (0.3%) showed a continuously increasing trend from 23.3 shoots at 4 hours to 26.0 at 8 hours and 34.3 at 12 hours. This divergence suggests that the underlying mechanism of shoot proliferation differs between the two concentrations. At 0.2%, brief exposure may be sufficient to induce chromosomal changes in meristematic tissue that promote axillary bud activation, while longer exposure at this concentration becomes cytotoxic to the same tissues without additional mutagenic benefit. At 0.3%, the higher colchicine dose appears to require extended contact time to penetrate and affect enough meristematic cells, with the resulting chromosomal disruption — whether full polyploidy, mixoploidy, or aneuploid sectors — stimulating compensatory shoot branching in surviving cells.

The strong shoot proliferation response observed here is consistent with reports in other ornamental species. In *Tagetes erecta*, colchicine-treated plants exhibited increased branching, which has been attributed to the disruption of apical dominance and subsequent release of axillary buds [22], [27]. Manzoor et al. [21] documented similar branching responses in colchicine-treated gladiolus, where polyploid plants produced more lateral shoots than diploid controls. Chromosome doubling and the associated changes in endogenous hormone balance — particularly shifts in the auxin-to-cytokinin ratio — are thought to mediate this response [28]. In *Cyclocarya paliurus*, induced polyploid plants showed significant reductions in indole-3-acetic acid (IAA) and increases in abscisic acid (ABA), altering growth allocation patterns [28]. Notably, a comparable increase in branching was reported by Sari et al. [18] in T. patula cv. Sudamala Barak treated with colchicine, further corroborating that shoot proliferation is a consistent early morphological indicator of colchicine activity in this species.

3.5. Optimal Treatment Protocol

Evaluating the combined performance across all measured traits, the $M_3 \times J_1$ treatment (0.2% colchicine, 4-hour soaking) emerges as the most promising protocol for polyploidy induction in *T. patula* cv. Janie Spry

under the conditions tested. This treatment maintained high survival (90%), produced tall plants (60.3±2.96 cm), and yielded the second-highest shoot count (30.7±2.03 shoots plant^{-1}) among all combinations. Although $M_4 \times J_3$ produced more shoots (34.3), its survival rate of only 30% makes it impractical for any breeding program that requires viable plant material for subsequent evaluation and selection. From a practical standpoint, a treatment that retains 90% of treated seedlings while increasing branching by nearly 38% relative to the control provides a workable starting population for further cytological screening and polyploid confirmation via flow cytometry or chromosome counting.

This finding is broadly compatible with the general principle that moderate colchicine concentrations combined with short exposure periods yield the best trade-off between induction efficiency and plant survival [11], [29]. In *Kaempferia parviflora*, 0.4% colchicine for 12 hours was identified as optimal, producing morphological indicators of polyploidy with acceptable survival [30]. In *Lilium regale*, 0.01% for 24 hours gave the highest polyploidy induction rate with 72% survival [31]. The optimal protocol is clearly species-specific and depends on tissue permeability, cell cycle duration, and inherent tolerance to colchicine toxicity. For *T. patula*, an allotetraploid species, the effective concentration range (0.2–0.3%) is higher than what is typically used in diploid ornamentals, which may reflect reduced sensitivity to colchicine-mediated spindle disruption in polyploid cells.

The present study provides preliminary morphological evidence supporting the potential for polyploid induction in *T. patula* using 0.2% colchicine at 4 hours soaking. Cytological confirmation through chromosome squash and flow cytometry analysis is required to verify whether the observed morphological changes — particularly in shoot proliferation — correspond to actual ploidy-level changes. Subsequent evaluation of floral traits (diameter, petal number, color intensity) and reproductive parameters will be necessary to determine the ornamental value of the putative polyploid lines.

4. CONCLUSION

Two-way ANOVA of the factorial experiment confirmed that colchicine concentration and soaking duration interact to shape both seedling survival and shoot proliferation in *Tagetes patula* cv. Janie Spry, while plant height and stem diameter remained unaffected during the vegetative growth phase. Survival rate decreased progressively with increasing colchicine concentration and prolonged soaking, following a dose- and duration-dependent pattern consistent with the known cytotoxic behavior of colchicine on meristematic tissues. Among the 12 treatment combinations tested, the 0.2% colchicine at 4-hour soaking protocol emerged as the most practical option for polyploidy induction efforts, yielding 90% seedling survival alongside a 37.7% increase in shoot number relative to the untreated control. Although the 0.3% colchicine at 12-hour treatment produced the highest absolute shoot count (34.3 shoots plant^{-1}), its 30% survival rate renders it unsuitable for breeding programs that require viable populations for downstream selection. The significant interaction between concentration and soaking duration underscores the importance of factorial optimization rather than single-factor screening when developing colchicine treatment protocols for allotetraploid species such as *T. patula*. These findings provide a practical starting point for polyploidy-based breeding in French marigold. Confirmation of actual ploidy-level changes through chromosome counting and flow cytometry, together with evaluation of floral traits including flower diameter, petal number, and color intensity, will be necessary to determine whether the observed vegetative responses translate into ornamentally valuable polyploid lines.

ACKNOWLEDGEMENTS

Author would like to express their sincere gratitude to the academic supervisors for their invaluable guidance, constructive feedback, and continuous support throughout the research process. Their insights and scholarly direction have significantly contributed to the completion of this study.

Authors also extend their appreciation to LPTP (Lembaga Pengembangan Teknologi Pedesaan) for providing institutional support, research facilities, and a conducive environment that enabled the implementation

of this study. The technical assistance and field support provided by the institution were essential in ensuring the successful execution of the experimental work.

Finally, Author acknowledge all parties who have directly or indirectly contributed to this research.

REFERENCES

[1] D. K. Mishra, S. Singh, and P. Singh, "Therapeutic benefits and processing of marigold (Tagetes species): A review," *Indian J. Health Care Med. Pharm. Pract.*, vol. 5, no. 1, pp. 148–166, 2024. doi: 10.59551/IJHMP/25832069/2024.5.1.190

[2] G. S. Abrol, P. Sharma, A. Kumar, and G. Sharma, "Different Preharvest Factors and Their Role in the Floriculture," in *Ornamental Horticulture: Latest Cultivation Practices and Breeding Technologies*, B. Bhargava, P. Kumar, and V. Verma, Eds., Singapore: Springer Nature Singapore, 2024, pp. 227–242. doi: 10.1007/978-981-97-4028-4_12. doi: 10.1007/978-981-97-4028-4.

[3] A. Uniyal and A. Kumar, "Minor flowers of European and American countries," in *Edible flowers*, Elsevier, 2024, pp. 385–430. Accessed: Mar. 18, 2026. 10.1016/B978-0-443-13769-3.00020-0

[4] F. Kurniati, "Potensi Bunga Marigold (Tagetes erecta L.) Sebagai Salah Satu Komponen Pendukung Pengembangan Pertanian," *Media Pertan.*, vol. 6, no. 1, May 2021, doi: 10.37058/mp.v6i1.3010.

[5] A. Buchori *et al.*, "assessment of tagetes patula mutants and its wild type for flower morphology, polyphenol contents, and antioxidant activity," *sabrao j. Breed. Genet.*, vol. 56, pp. 1147–1158, Jun. 2024. 10.54910/sabrao2024.56.3.21

[6] A. Manzoor, T. Ahmad, M. A. Bashir, I. A. Hafiz, and C. Silvestri, "Studies on Colchicine Induced Chromosome Doubling for Enhancement of Quality Traits in Ornamental Plants," *Plants*, vol. 8, no. 7, Jun. 2019, doi: 10.3390/plants8070194.

[7] Y. M. Ye, J. Tong, X. P. Shi, W. Yuan, and G. R. Li, "Morphological and cytological studies of diploid and colchicine-induced tetraploid lines of crape myrtle (Lagerstroemia indica L.)," *Sci. Hortic.*, vol. 124, no. 1, pp. 95–101, 2010. 10.1016/j.scienta.2009.12.016

[8] F. Fakhrzad, A. Jowkar, A. Shekafandeh, and M. J. Kermani, "Tetraploidy induction enhances morphological, physiological and biochemical characteristics of wallflower (Erysimum cheiri (L.) Crantz)," *Sci. Hortic.*, vol. 308, p. 111596, 2023. 10.1016/j.scienta.2022.111596

[9] K. Bhattarai, A. Kareem, and Z. Deng, "In vivo induction and characterization of polyploids in gerbera daisy," *Sci. Hortic.*, vol. 282, p. 110054, 2021. 10.1016/j.scienta.2021.110054

[10] M. C. Sattler, C. R. Carvalho, and W. R. Clarindo, "The polyploidy and its key role in plant breeding," *Planta*, vol. 243, no. 2, pp. 281–296, Feb. 2016, doi: 10.1007/s00425-015-2450-x.

[11] W.-H. Eng and W.-S. Ho, "Polyploidization using colchicine in horticultural plants: A review," *Sci. Hortic.*, vol. 246, pp. 604–617, 2019. 10.1016/j.scienta.2018.11.010

[12] N. Ermawati, N. Sjamsijah, R. A. Syaban, H. Prasetyo, and M. Safi'i, "Induksi Poliploidi Menggunakan Perlakuan Kolkisin pada Anggrek Bulan (Phalaenopsis sp.) secara In Vivo," *Agriprima J. Appl. Agric. Sci.*, vol. 8, no. 1, pp. 61–70, 2024. 10.25047/agriprima.v8i1.657

[13] Y. Sajjad, M. J. Jaskani, A. Mehmood, I. Ahmad, and H. Abbas, "Effect of colchicine on in vitro polyploidy induction in African marigold (Tagetes erecta)," *Pak J Bot*, vol. 45, no. 3, pp. 1255–1258, 2013.

[14] R. S. Raghunath Sadhukhan, A. G. Arghadeep Ganguly, P. K. Singh, and H. K. Sarkar, "Study of induced polyploidy in African marigold (Tagetes erecta L.).," 2014, Accessed: Mar. 18, 2026. 10.5555/20143382196

[15] F. U. Yongyao, Y. Liping, Z. Kaimin, and X. U. Wenji, "Induction and Characterization of Medicinal Polyploid Tagetes erecta," *Chin. J. Trop. Crops*, vol. 42, no. 5, pp. 1318–1325, May 2021, doi: 10.3969/j.issn.1000-2561.2021.05.017.

[16] J. W. Towner, "cytogenetic studies on the origin of tagetes patula. I. Meiosis and morphology of diploid and allotetraploid t. Erecta × t. Tenuifolia," *Am. J. Bot.*, vol. 48, no. 9, pp. 743–751, Oct. 1961, doi: 10.1002/j.1537-2197.1961.tb11706.x.

[17] R. Cicevan *et al.*, "Biological Traits and Genetic Relationships Amongst Cultivars of Three Species of Tagetes (Asteraceae)," *Plants*, vol. 11, no. 6, p. 760, Jan. 2022, doi: 10.3390/plants11060760.

[18] Y. N. SARI, S. I. AISYAH, D. SUKMA, and M. SYUKUR, "Increasing ploidy level of marigold (Tagetes patula Sudamala Barak) using colchicine with acute and chronic methods.," *Biodiversitas J. Biol. Divers.*, vol. 26, no. 6, 2025, Accessed: Mar. 18, 2026. 10.13057/biodiv/d260617

[19] J. Avdić, N. Sarajlić, B. Bečić, S. Murtić, and A. Turalija, "The effect of concentration, exposure time and type of colchicine treatment on the morphological characteristics of French marigold (Tagetes patula L. var. nana).," 2013, Accessed: Mar. 18, 2026. 10.5555/20153005572

[20] A. Manzoor, T. Ahmad, M. A. Bashir, I. A. Hafiz, and C. Silvestri, "Studies on colchicine induced chromosome doubling for enhancement of quality traits in ornamental plants," *Plants*, vol. 8, no. 7, p. 194, 2019. 10.3390/plants8070194

[21] A. Manzoor *et al.*, "Assessment of biological damage and toxic potency of colchicine in gladiolus (Gladiolus grandiflorus) plants.," 2023, Accessed: Mar. 18, 2026. 10.5555/20231604874

[22] Y. Sajjad, M. J. Jaskani, A. Mehmood, I. Ahmad, and H. Abbas, "Effect of colchicine on in vitro polyploidy induction in African marigold (Tagetes erecta)," *Pak J Bot*, vol. 45, no. 3, pp. 1255–1258, 2013.

[23] F. P. KARAGÖZ, A. Dursun, B. Dumlu, M. Karaşal, and H. Karagöz, "Colchicine-induced polyploidy and morphological changes in wild Silene compacta Fischer: Potential as an ornamental plant in Türkiye," *J. Biometry Stud.*, vol. 4, no. 2, pp. 79–90, 2024. 10.61326/jofbs.v4i2.04

[24] Y. M. Ye, J. Tong, X. P. Shi, W. Yuan, and G. R. Li, "Morphological and cytological studies of diploid and colchicine-induced tetraploid lines of crape myrtle (Lagerstroemia indica L.)," *Sci. Hortic.*, vol. 124, no. 1, pp. 95–101, 2010.

[25] S. Nasirvand, R. A. Zakaria, N. Zare, and B. Esmaeilpoor, "Polyploidy induction in parsley (Petroselinum crispum L.) by colchicine treatment," *Cytologia (Tokyo)*, vol. 83, no. 4, pp. 393–396, 2018. 10.1508/cytologia.83.393

[26] W. H. Eng, W. S. Ho, and K. H. Ling, "In vitro induction and identification of polyploid Neolamarckia cadamba plants by colchicine treatment," *PeerJ*, vol. 9, p. e12399, 2021. 10.7717/peerj.12399

[27] R. Sadhukhan, A. Ganguly, P. K. Singh, and H. K. Sarkar, "Study of Induced polyploidy in African marigold (Tagetes ecrecta L.)," *Env. Ecol*, vol. 32, no. 4, pp. 1219–1222, 2014. 10.5555/20143382196
[28] G. Bian *et al.*, "Colchicine-induced polyploidization influences the morphological, physiological, and biochemical characteristics of Cyclocarya paliurus," *Plants*, vol. 14, no. 17, p. 2778, 2025. 10.3390/plants14172778
[29] K. Bhattarai, A. Kareem, and Z. Deng, "In vivo induction and characterization of polyploids in gerbera daisy," *Sci. Hortic.*, vol. 282, p. 110054, 2021. 10.1016/j.scienta.2021.110054
[30] K. S. Koh, S. Gantait, M. F. Ismail, N. S. Naharudin, and U. R. Sinniah, "Induction of in vitro Tetraploids in Kaempferia parviflora Wall. ex Baker and its Characterization," *Vitro Cell. Dev. Biol. - Plant*, vol. 61, no. 2, pp. 268–281, Apr. 2025, doi: 10.1007/s11627-024-10483-4.
[31] N. I. Jeloudar, E. Chamani, A. Shokouhian, and R. A. Zakaria, "Induction and identification of polyploidy by colchicine treatment in Lilium regale," *Cytologia (Tokyo)*, vol. 84, no. 3, pp. 271–276, 2019. 10.59551/IJHMP/25832069/2024.5.1.190