

Colchicine-induced morphological and cytological changes in potato (*Solanum tuberosum* L.) root tips

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Abstract

Artificial polyploidization through colchicine treatment can enhance plant morphological traits. This study evaluated the effects of six colchicine concentrations (0, 0.5, 1, 2, 4, and 8 mg/mL) on root length, root base diameter, root surface area, cell size, nucleus size, and chromosome number in locally grown potato (*Solanum tuberosum* L.) collected from Laoag City, Ilocos Norte, Philippines. Root samples were analyzed through quantitative measurements and microscopic examination after fixation and staining, with statistical differences determined using one-way ANOVA. The 1 mg/mL treatment (T₂) consistently produced the highest values for all measured parameters and showed significant differences ($p < 0.05$) compared to other treatments and the control. Observed increases in cell and nucleus size suggest possible polyploidy induction, although chromosome counts could not be confirmed due to staining limitations. Overall, optimal colchicine concentrations improved both macroscopic and microscopic traits, whereas higher doses were detrimental. These results identify 1 mg/mL colchicine as a promising concentration for enhancing root traits in potato, warranting further whole-plant evaluation for varietal improvement.

Keywords cell size; crop varietal enhancement; microscopy; nucleus size; polyploidy.

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1 Introduction

Crops are a primary source of nutrients that sustain human health and wellness (Mohapatra, 2020). During production, they undergo germination, vegetative growth, and maturation (Acosta-Quezada et al., 2016), but at maturity, challenges such as inadequate size, low quality, and poor vigor may arise (Pereira et al., 2017). These issues, driven by biotic and abiotic stresses and socioeconomic factors, can compromise food quality and security (Longnecker, 2021; Wang and Frei, 2011). In response, agricultural sectors and food industries have turned to innovative methods for crop varietal improvement.

Potatoes (*Solanum tuberosum* L.), locally called patatas, are among the most valuable crops in the Philippines (Quilloy et al., 2019). However, rising market demand has outpaced supply, accelerating varietal degeneration due to diseases and threatening national food self-sufficiency (Gonzales et al., 2016). Varietal enhancement, achieved through plant tissue culture, transgenics, molecular breeding, or polyploidization, offers a pathway to improved yield and resilience (Adlak et al., 2019).

Polyploidization, the multiplication of complete chromosome sets (Zhang et al., 2019), often produces larger, more vigorous plants (Touchell et al., 2020) with greater market value. While it occurs naturally, unreduced gamete formation is rare, requiring synthetic induction using mitotic inhibitors (Manzoor et al., 2019). Colchicine, a mutagen that disrupts microtubule formation to double chromosome numbers (Noori et al., 2017), has been applied successfully in various crops, including onion (Ren et al., 2018), banana, cassava, and hot pepper (Tammu et al., 2021).

Potatoes, as naturally tetraploid plants, are suitable candidates for colchicine treatment (Watanabe, 2015). They rank as the third most important global food security crop according to the FAO, being safe, nutritious, and accessible (Devaux et al., 2014). Yet production is declining due to soil infertility, plant diseases, and seed degeneration from repeated recycling (Muthoni et al., 2013).

Applying colchicine to potato root tips could enhance quantitative and qualitative traits, benefitting farmers through improved vigor and sustainability, the food industry through higher-quality raw materials, and the academe through updated polyploidization data (Noori et al., 2017). However, incorrect concentrations of colchicine can induce structural abnormalities (Manzoor et al., 2019), and there is a lack of recent local studies on potatoes sourced from the Philippines.

Thus, this study aimed to evaluate the effects of varying colchicine concentrations on the morphological characteristics of *S. tuberosum* L. root tips. Specifically, the study assessed 0, 0.5, 1, 2, 4, and 8 mg/mL colchicine treatments for their effects on root length, base diameter, surface area, cell size, nucleus size, and chromosome number, and identified the optimal concentration using Analysis of Variance (ANOVA).

2 Materials and Methods

2.1 Acquisition of materials

The materials used in this study included a vernier caliper, blade, and medium-sized potato tubers (*Solanum tuberosum* L.). Potatoes were procured from a local marketplace in Laoag City, Ilocos Norte, Philippines. Colchicine tablets, 1 M hydrochloric acid (HCl), glacial acetic acid, 95% ethanol, and acetic orcein solution were obtained from the Philippine Science High School – Ilocos Region Campus (PSHS–IRC) Science Laboratory. Additional laboratory equipment, including a compound light microscope, watch glasses, glass slides with coverslips, beakers, capped test tubes, and an alcohol lamp, were also sourced from PSHS–IRC.

2.2 Procurement of potatoes

Medium-sized tubers (2 to 3 inches in diameter) were randomly selected from a single vendor in Laoag City, Ilocos Norte. Variety identification was based on vendor confirmation and regional availability, as Conchita was the only variety offered during procurement and is predominant in the locality. All tubers were propagated under five experimental colchicine treatments and one negative control, with five replicates per treatment.

2.3 Preparation of treatments

The preparation of dissolved colchicine solutions with various concentrations as experimental treatments with 100 mL of distilled water as the negative control treatment (T0). Various concentrations for experimental treatments were prepared by dissolving 1, 2, 4, 8, and 16 colchicine tablets, wherein each tablet contains 0.5 mg of colchicine, in 100mL distilled water, producing 0.5 mg/mL (T1), 1 mg/mL (T2), 2 mg/mL (T3), 4 mg/mL (T4), and 8 mg/mL (T5) treatments respectively (Noori et al., 2017). Each treatment was diluted in a

beaker by dropping the respective tablets in the 100 mL distilled water then placed into a magnetic spin for further dissolving.



Fig. 1 Potato Tubers (*Solanum tuberosum* L.).

2.4 Propagation of roots

Potato tubers were partially submerged, with the basal ends in solution, for seven days (Nefic et al., 2013). Control tubers were soaked in T_0 , and experimental groups in T_1 – T_5 . After propagation, roots were detached from the tubers using a sterile cutter and stored in labeled plastic vials for subsequent analysis.

2.5 Quantitative macroscopic testing

Root length and base diameter were measured using a vernier caliper. Length was recorded from base to tip, and base diameter was measured at the proximal end. Root surface area (mm^2) was calculated following Lisein et al. (2013) using the formula:

$$\text{Surface Area} = l \times d \times \pi \quad (1)$$

where l = root length (mm) and d = root base diameter (mm).

2.6 Fixation and staining

Root detachment was performed in the morning to minimize circadian effects. Freshly excised roots were immediately fixed in acetic ethanol (3:1 glacial acetic acid to absolute ethanol) (Singh et al., 2019) and refrigerated for 24 h (GTAC, n.d.). Roots were transferred into 1 M HCl (Nefic et al., 2013), heated for 5 s over an alcohol lamp, cooled, and incubated for 2 min. Samples were rinsed four times with distilled water (Manawadu et al., 2016).

Roots were stained with acetic orcein, heated for 5 s, and incubated for 10 min. A 1 mm transverse section of the root tip was excised and mounted on a slide with a drop of water. A coverslip was applied and gently tapped with blunt tweezers to squash the tissue. Properly prepared slides displayed a faint pink hue and were used for microscopic analysis (Çavuşoğlu et al., 2021).

2.7 Quantitative microscopic examination

For cell size, ten cells per replicate were randomly selected and measured (μm) using a calibrated microscope. For nucleus size, three cells per replicate were measured. Microscopy was performed using 10× (Low Power Objective), 40× (High Power Objective), and 100× (Oil Immersion) lenses (Zhang and Gross, 2019).

Images were captured using a mobile phone camera and MicroscopeVIS software connected to a laptop. ImageJ (Java version) was used for analysis. Images were calibrated by measuring a known reference, setting the scale via the “Set Scale” function, and applying this calibration to all images. Data from ImageJ were summarized for mean, standard deviation, minimum, and maximum values.

Chromosome counts were obtained from root tip cells at metaphase under 100× oil immersion, with each chromosome counted manually in clear photomicrographs.

2.8 Analysis of data

Normality of data distribution was tested using the Shapiro–Wilk test at $\alpha = 0.05$. Root length, base diameter, surface area, and cell and nucleus size were analyzed using one-way ANOVA (IBM SPSS Statistics 25) at $\alpha = 0.05$ (Alexander and Chong, 2014). Tukey’s Honest Significant Difference (HSD) post hoc test was used to determine significant pairwise differences (Kim, 2015). Pearson correlation analysis was conducted to assess relationships between parameters, with coefficients interpreted as: $r < 0.25$ = no relationship; $0.25 \leq r < 0.5$ = weak; $0.5 \leq r < 0.75$ = moderate; $r \geq 0.75$ = strong (Nettleton, 2014).

3 Results and Discussion

The study determined the effects of varying colchicine treatments that induced significant macroscopic and microscopic developments in potato root tips, as well as identified the best concentrations of colchicine. To attain these outcomes, quantitative measurements and microscopic analyses were performed to test for the indicated parameters. All raw data were then subjected to various statistical tests and analyses. In this regard, the results of implemented methodologies and tests are discussed.

Table 1 Macroscopic and microscopic data of the control group and colchicine-induced treatments.

	T ₀ (untreated)	T ₁ (0.5 mg/mL)	T ₂ (1 mg/mL)	T ₃ (2 mg/mL)	T ₄ (4 mg/mL)	T ₅ (8 mg/mL)
Average Root Length	12.2 ^a	17.3 ^b	18.2 ^b	18.0 ^b	15.8 ^{bc}	14.6 ^c
Average Root Base Diameter	9.6 ^a	9.0 ^{ab}	10.7 ^{ac}	10.5 ^{acd}	9.4 ^{abcd}	9.3 ^{abd}
Average Root Surface Area	368.2 ^a	490.5 ^b	612.5 ^c	593.0 ^{bc}	466.3 ^{abd}	426.1 ^{abd}
Average Cell Size	9.6 ^a	14.0 ^b	22.2 ^c	19.7 ^d	12.4 ^{be}	11.0 ^{ae}
Average Nucleus Size	3.2 ^a	5.6 ^{ab}	9.3 ^c	7.1 ^{bcd}	4.9 ^{abde}	3.8 ^{abe}

Note: All means having the same letter attribution are not statistically different with each other at 0.05 level of significance in each parameter

Table 1 shows the mean values of each control and experimental treatment for the macroscopic (root length, root base diameter, root surface area), and microscopic (cell and nucleus size) parameters. It also presents the statistical difference between and among treatments as indicated by the letter attribution.

3.1 Root length

Quantitative measurements showed that the 1 mg/mL concentration (T₂) produced the greatest average root length, while the negative control (T₀) had the smallest. Treatments T_{1–4} did not differ significantly from each other ($p > 0.05$), indicating comparable effects within these concentrations. In contrast, 8 mg/mL (T₅) differed significantly from T_{0–T₃} ($p < 0.05$) but was statistically similar to 4 mg/mL (T₄). All experimental treatments (T_{1–T₅}) differed significantly from the negative control ($p < 0.05$), indicating a greater effect relative to T₀.

Colchicine concentration and water–colchicine ratio strongly influenced root length (Ayu et al., 2019). Although each treatment received 100 mL of distilled water, differences in colchicine content determined performance. The optimal concentration, 1 mg/mL (T_2), yielded the lowest p-value against the negative control and the highest positive effect. Lower concentrations such as 0.5 mg/mL (T_1) produced shorter roots due to insufficient colchicine to stimulate morphological enhancement. Higher concentrations: 2 mg/mL (T_3), 4 mg/mL (T_4), and 8 mg/mL (T_5), reduced root length, likely due to colchicine toxicity, which can impair plant growth and cause tissue decay (Soetopo and Hosnia, 2018). The overall ranking was $T_2 > T_3 > T_1 > T_4 > T_5 > T_0$. These results suggest that optimal root growth requires balancing colchicine dose and water volume, as water solubility influences colchicine's structural activity and effectiveness (Vicente - Blázquez et al., 2019).

3.2 Root base diameter

The largest average root base diameter was observed at 1 mg/mL (T_2), while the smallest occurred at 0.5 mg/mL (T_1), which was statistically similar to 4 mg/mL (T_4) and 8 mg/mL (T_5) but significantly smaller than T_2 and 2 mg/mL (T_3). T_3 was statistically similar to T_4 and T_5 but significantly larger than T_1 . None of the experimental treatments differed significantly from the negative control ($p > 0.05$), indicating that changes in macromorphology do not necessarily equate to treatment efficacy.

Colchicine concentration and solution ratio again shaped results. T_2 produced the largest diameter, enhancing overall foraging capacity (Wu et al., 2016). Both lower (0.5 mg/mL) and higher (≥ 2 mg/mL) concentrations reduced diameter, with higher doses causing toxicity and decay (Soetopo and Hosnia, 2018).

3.3 Root surface area

The largest average root surface area occurred at 1 mg/mL (T_2), while the smallest occurred at 8 mg/mL (T_5), which was statistically similar to T_0 , T_1 , and T_4 . T_1 was comparable to T_3 – T_5 but significantly smaller than T_2 . Treatments T_1 – T_3 were significantly larger than the negative control ($p < 0.05$), while T_4 – T_5 did not differ significantly from T_0 (Gibbs, 2013).

Root surface area, the product of root length and base diameter, depends on both parameters (Lisein et al., 2013) and is influenced by colchicine concentration and solution ratio (Trojak-Goluch et al., 2021). T_2 's superiority may relate to colchicine-induced microtubule depolymerization, which promotes root hair growth (Terkeltaub, 2012) and thereby increases surface area. Lower concentrations provided insufficient colchicine to stimulate enlargement, while higher doses (≥ 2 mg/mL) reduced surface area due to toxicity (Soetopo & Hosnia, 2018).

3.4 Cell and nucleus size

Microscopic ImageJ analysis showed that T_2 (1 mg/mL) produced the largest average cell size, while T_0 had the smallest, statistically similar to T_5 . T_1 was similar to T_4 . Treatments T_1 – T_4 were significantly larger than T_0 ($p < 0.05$), whereas T_5 was not. Polyploidy often slows cell division, leading to larger cells (Manzoor et al., 2019), and polyploid cells typically exceed diploid progenitors in size (Ren et al., 2018). However, excessive colchicine reduces polyploid yields and cell size due to malformation and toxicity (Pirkoohi et al., 2011; Mo et al., 2020; Tank and Thaker, 2014). Larger cells correspond with phenotypic changes such as increased organ size, indicating successful polyploidization.

Nucleus size followed the same trend, with T_2 and T_3 being the largest, and T_0 the smallest. T_0 was statistically similar to T_1 , T_4 , and T_5 but significantly smaller than T_2 and T_3 . T_1 , T_3 , T_4 , and T_5 did not differ significantly from each other. Larger nuclei correlate with larger cells and higher DNA content (Frawley and Orr-Weaver, 2015), supporting the polyploidization hypothesis (Tank and Thaker, 2014).

3.5 Chromosome number

Chromosome structures in potato root tips were indistinct, likely due to poor aceto-orcein stain penetration despite its suitability for chromosome studies (Hartika, 2021). Potato starch content, which varies genetically (Robertson et al., 2018), can influence stainability; starch often remains invisible despite staining (Parker et al.,

2020). The thick root tissue may have further limited stain penetration. Methodological factors in slide preparation, fixation, and staining may also have contributed. Despite repeated optimization efforts, chromosomes were undetectable, and no counts were obtained.

3.6 Parameter relationships

Table 2 reveals the obtained Pearson correlation coefficient between macroscopic and microscopic parameters, which indicates the relationship strength between two (2) involved parameters. It also displays parameter pairs that exhibited a strong relationship.

Pearson correlation analysis revealed a strong positive association between root length, root base diameter, and root surface area ($r = 0.895$), except for a weaker relationship between root length and base diameter ($r = 0.361$). Larger root diameters combined with greater root length can enhance penetration into deeper soil strata (Narayanan et al., 2014). Such traits are consistent with polyploidization effects, which often produce larger, more extensive root systems with increased root exudation, enhancing competitiveness, adaptability, and disease resistance (Wu et al., 2016; Te Beest et al., 2012).

Table 2 Pearson correlation matrix of morphological traits of potato root tips.

Morphological Traits	Root Length (in mm)	Root Base Diameter (in mm)	Root Surface Area (in sq mm)	Cell Size (in μm)	Nucleus Size (in μm)
Root Length (in mm)	1				
Root Base Diameter (in mm)	0.361	1			
Root Surface Area (in sq mm)	0.895*	0.737	1		
Cell Size (in μm)	0.729	0.552	0.786*	1	
Nucleus Size (in μm)	0.701	0.441	0.713	0.885*	1

Note: * - strong relationship

Cell size and nucleus size also showed a strong positive relationship ($r = 0.885$), supporting the nucleotypic effect in which increased DNA content enlarges both cells and nuclei (Frawley and Orr-Weaver, 2015; Robinson et al., 2018). These traits are considered phenotypic consequences of polyploidy rather than direct gene effects (Slabodnick et al., 2017). The resulting reduction in surface area-to-volume ratio is a hallmark of the “gigas” effect (Doyle and Coate, 2019). Moderate correlations between cell size and macroscopic traits ($r = 0.729, 0.552, 0.786$) further suggest that microscopic enlargement can scale to organ-level growth, as seen in improved root, leaf, and shoot dimensions (Trojak-Goluch et al., 2021).

Although chromosomes were not successfully visualized, likely due to staining or sample limitations, differences in repetitive DNA content can have a greater impact on cell and nucleus size than chromosome number itself (Dodsworth et al., 2015). High chromosome counts do not always correspond to large cell sizes, indicating that factors beyond chromosome number influence morphology (Bowers and Paterson, 2021).

Across both macroscopic and microscopic parameters, statistical analysis indicated that polyploidy-related traits were more pronounced in colchicine-treated groups than in the negative control (T_0) (Gibbs, 2013). The 1 mg/mL colchicine treatment (T_2) consistently produced the highest values, with significant improvements in most parameters compared to T_0 . Significant differences were observed for all treatments in root length; for root surface area in all treatments except T_4 – T_5 ; for cell size in all treatments except T_5 ; and for nucleus size in all treatments except T_3 – T_5 . Root base diameter showed no significant difference compared to T_0 .

4 Conclusions

Quantitative analysis revealed that colchicine concentration significantly influenced root length, root base diameter, root surface area, cell size, and nucleus size in potato root tips. These morphological and cytological changes are consistent with polyploidization effects, as increased cell and nucleus dimensions may indicate elevated DNA content. The most pronounced positive effects were observed at 1 mg/mL colchicine (T_2), which produced the highest values in both macroscopic and microscopic parameters and yielded the most significant statistical differences ($p < 0.05$). In contrast, higher concentrations reduced growth, suggesting potential toxicity. Although chromosome counts could not be obtained due to inadequate staining, the observed trends support the hypothesis that lower colchicine doses can enhance growth-related traits, while excessive levels are detrimental. These findings highlight 1 mg/mL colchicine as a promising concentration for inducing beneficial morphological traits in potatoes, warranting further investigation at the whole-plant level to confirm its potential for varietal improvement.

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References

- Berner EK, Berner RA. 1996. Global Environment: Water, Air and Geochemical Cycles. Prentice Hall, New Jersey, USA
- Acosta-Quezada P, Riofrío-Cuenca T, Rojas J, Vilanova S, Plazas M, Prohens J. 2016. Phenological growth stages of tree tomato *Solanum betaceum* Cav., an emerging fruit crop, according to the basic and extended BBCH scales. *Scientia Horticulturae*, 199: 216-223. 10.1016/j.scienta.2015.12.045
- Adlak T, Tiwari S, Tripathi M, Gupta N, Sahu V, Bhawar P, Kandalkar V. 2019. Biotechnology: An Advanced Tool for Crop Improvement. *Current Journal of Applied Science and Technology*, 33: 1-11. 10.9734/cjast/2019/v33i130081
- Alexander A, Chong K. 2014. Combination of biological agents in suppressing colonization of *Ganoderma boninense* of basal stem rot. *American-Eurasian Journal of Sustainable Agriculture*, 8: 1-7
- Ayu, G. M., Elimasni, Nurwahyuni, I. 2019. Effect of concentration and duration of colchicine treatment to garlic *Allium sativum* L. Cv. Doulu. *International Journal of Scientific & Technology Research*, 8. <https://www.ijstr.org/final-print/june2019/Effect-Of-Concentration-And-Duration-Of-Colchicine-Treatment-To-Garlic-allium-Sativum-L-Cv-Doulu.pdf>
- Bowers JE, Paterson AH. 2021. Chromosome number is key to longevity of polyploid lineages. *New Phytologist*, 231: 19-28.

- Çavuşoğlu D, Macar T, Macar O, Yalçın E, Çavuşoğlu K. 2021. Extenuating role of lycopene against 254-nm UV-C radiation-mediated damages in *Allium cepa* L. roots. Environmental Science and Pollution Research, 1-10. 10.1007/s11356-021-14047-6
- Charles. 2012. What it means when “no significant differences were found”. Purpose Research. <http://purposeresearch.com/wdprs/2012/04/what-it-means-when-no-significant-differences-were-found/>
- Chen X, Kou M, Tang Z, Li H, Wei M. 2017. Responses of root physiological characteristics and yield of sweet potato to humic acid urea fertilizer. PLoS One, 12, e0189715. 10.1371/journal.pone.0189715
- Devaux A, Kromann P, Ortiz O. 2014. Potatoes for sustainable global food security. Potato Research, 57, 185-199. 10.1007/s11540-014-9265-1
- Dewitte A., Van Laere K, Van Huylenbroeck J. 2012. Use of 2n gametes in plant breeding. Plant Breeding, 59-86
- Doyle JJ, Coate JE. 2019. Polyploidy, the nucleotype, and novelty: The impact of genome doubling on the biology of the cell. International Journal of Plant Sciences, 180. 10.1086/700636
- Frawley L, Orr-Weaver T. 2015. Polyploidy. Current Biology, 25, 353-358. <https://doi.org/10.1016/j.cub.2015.03.037>
- Gene Technology Access Centre. n.d. Mitosis in growing root tips. https://gtac.edu.au/wp-content/uploads/2016/01/Mitosis_Roottips_LabPreparation.pdf
- Gibbs NM. 2013. Errors in the interpretation of ‘no statistically significant difference. Journal Sage Pub. 10.1177/0310057X1304100203
- Gonzales IC, Kiswa CG, Bautista AB. 2016. Sustainable potato production in the Philippine Cordillera Region. International Journal of Engineering and Applied Sciences, 3, 257649.
- Gupta, P. 2018. Chapter 11 - Poisonous plants. Illustrated toxicology: with study questions, 309-329. 10.1016/B978-0-12-813213-5.00011-0
- Hartika G, Zulharmita Z, Asra R. 2021. Utilization of natural dyes substances for histological staining: A Review. Asian Journal of Pharmaceutical Research and Development, 9: 149-158. 10.22270/ajprd.v9i1.925
- Kim H. 2015. Statistical notes for clinical researchers: Post-hoc multiple comparisons. Restorative Dentistry & Endodontics, 40: 172-176. 10.5395/rde.2015.40.2.172
- Lisein J, Linchant J, Lejeune P, Bouché P, Vermeulen C. 2013. Aerial surveys using an unmanned aerial system UAS: Comparison of different methods for estimating the surface area of sampling strips. Tropical Conservation Science, 6: 506-520. 10.1177/194008291300600405
- Longnecker N. 2021. Nutrient deficiencies and vegetative growth. Mechanisms of Plant Growth and Improved Productivity, 137-172. 10.1201/9781003210252-5
- Hossain M, Ghosh D, Molla MMH, Rahman MM, Kabir MH, Rahman M. 2020. In vitro induction of polyploidy in *Chrysanthemum morifolium* Ramat. using colchicine and oryzalin. Plant Tissue Culture and Biotechnology, 30(1): 1-11. <https://doi.org/10.3329/ptcb.v30i1.47543>
- Hussain A, Qarshi IA, Nazir H, Ullah I. 2012. Plant tissue culture: Current status and opportunities. In: Recent Advances in Plant in vitro Culture (Leva A, Rinaldi LMR, eds). 1-28, IntechOpen, Rijeka, Croatia. <https://doi.org/10.5772/50568>
- Iqbal M, Khan IA, Azhar FM, Khan AA, Yasmin T. 2019. Effect of colchicine on morphological and cytological characteristics of diploid cotton (*Gossypium arboreum* L.). Pakistan Journal of Botany, 51(2): 513-519. [https://doi.org/10.30848/PJB2019-2\(35\)](https://doi.org/10.30848/PJB2019-2(35))
- Javadian N, Karimzadeh G, Sharifnia F. 2017. Polyploidy induction of *Echinacea purpurea* (L.) Moench using colchicine: Morphological, anatomical, and phytochemical changes. Plant Cell, Tissue and Organ Culture, 131(3): 543-551. <https://doi.org/10.1007/s11240-017-1300-1>

- Kondeti S, Padmanabhan S, Sairam R, Singh S. 2018. Effects of colchicine on microtubules and mitosis in plants. *Journal of Plant Biology*, 61(1): 1-9. <https://doi.org/10.1007/s12374-017-0268-8>
- Kumar N, Bhardwaj N, Sharma R, Sharma M, Sharma AK. 2016. Colchicine-induced polyploidy in *Andrographis paniculata* (Burm.f.) Nees. *Brazilian Journal of Botany*, 39(2): 587-594. <https://doi.org/10.1007/s40415-015-0249-1>
- Li W, Chen J, Du J, Zhang L. 2018. Colchicine-induced polyploidy in *Arachis hypogaea* and its effect on morphology, anatomy, and yield. *Plant Cell Reports*, 37(7): 1039-1049. <https://doi.org/10.1007/s00299-018-2292-3>
- Liu C, Yang Y, Liu Y, Ma J, Xie Y. 2019. Polyploid induction and morphological analysis of *Salvia miltiorrhiza* Bunge by colchicine treatment. *Industrial Crops and Products*, 133: 289-297. <https://doi.org/10.1016/j.indcrop.2019.03.029>
- Maheshwari P, Malaviya DR. 2018. Polyploidy and colchicine application in plant breeding. *Indian Journal of Genetics and Plant Breeding*, 78(1): 1-12. <https://doi.org/10.5958/0975-6906.2018.00001.4>
- Manzoor A, Ahmad T, Bashir MA, Baig MMQ, Quresh AA, Hafiz IA. 2019. Induction and identification of colchicine induced polyploidy in *Gladiolus grandiflorus* "White Prosperity". *Folia Horticulturae*, 31(1): 103-111. <https://doi.org/10.2478/fhort-2019-0010>
- Modgil M, Sharma DR, Bhardwaj SV. 2019. Colchicine induced polyploidy in apple rootstock MM106. *Scientia Horticulturae*, 246: 918-924. <https://doi.org/10.1016/j.scienta.2018.11.056>
- Niu L, Tao Y, Chen M, Fu Q, Li W. 2016. Induction of polyploidy in *Lilium davidii* var. unicolor by colchicine in vitro. *Euphytica*, 209(2): 313-323. <https://doi.org/10.1007/s10681-016-1652-0>
- Patel M, Patel S, Patel R. 2018. In vitro polyploidy induction in banana using colchicine. *International Journal of Current Microbiology and Applied Sciences*, 7(3): 1254-1263. <https://doi.org/10.20546/ijcmas.2018.703.150>
- Rao S, Kumari P, Reddy V. 2017. Morphological and cytological studies on colchicine-induced polyploidy in *Cymbopogon martinii*. *Cytologia*, 82(3): 301-307. <https://doi.org/10.1508/cytologia.82.301>
- Sajjad Y, Jaskani MJ, Qasim M, Mehmood A, Ahmad R. 2013. Effect of colchicine on in vitro polyploidy induction in *Ficus carica* L. *Pakistan Journal of Botany*, 45(3): 911-916.
- Shao J, Chen J, Shi X, Wang X, Wang J. 2016. Induction of polyploidy in *Echinacea purpurea* by colchicine in vitro. *Horticultural Plant Journal*, 2(6): 338-344. <https://doi.org/10.1016/j.hpj.2016.10.005>
- Singh SK, Sehgal D, Kumar S, et al. 2014. Colchicine induced polyploidy in *Stevia rebaudiana* Bertoni. *Journal of Plant Biochemistry and Biotechnology*, 23(2): 135-142. <https://doi.org/10.1007/s13562-013-0200-5>
- Wu J, Yang C, Yang Y, Guo J, Li H. 2020. Colchicine-induced polyploidy in *Dendranthema nankingense* (Nakai) Tzvel. *Plant Cell, Tissue and Organ Culture*, 142(2): 369-377. <https://doi.org/10.1007/s11240-020-01850-w>
- Zhang WJ, Zhong XQ, Liu GH. 2008. Recognizing spatial distribution patterns of grassland insects: neural network approaches. *Stochastic Environmental Research and Risk Assessment*, 22(2): 207-216