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COLCHICINE AS A CHROMOSOMAL MODIFIER: A STUDY ON *VIGNA RADIATA*

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ABSTRACT
The present study investigates the effect of different concentrations of colchicine on mitotic cell division and the induction of polyploidy in plant cells. Colchicine, a well-known spindle inhibitor, was applied at four concentrations (0.05%, 0.1%, 0.15%, and 0.2%) using the seed soaking method for a duration of 12 hours. The study aimed to evaluate the impact of colchicine on mitotic chromosomal behavior and to identify the occurrence of polyploid cells. Cytological analysis revealed significant mitotic aberrations, including lagging chromosomes, chromosomal bridges, and micronuclei. The frequency and severity of these aberrations increased with higher colchicine concentrations. Notably, polyploid cells were observed in mitotic preparations, indicating colchicine's ability to induce polyploidy through mitotic arrest. The highest concentration (0.2%) exhibited the maximum number of polyploid cells and chromosomal abnormalities. Root tips were processed for cytological examination using standard protocols, including pre-treatment with Carnoy's fixative, hydrolysis in hydrochloric acid, and staining with acetocarmine or Feulgen stain. Chromosome numbers and arrangements were analyzed via squash preparation and light microscopy. Chromosomal structures in colchicine-treated samples were compared against untreated controls to assess polyploidy-induced variations. These findings underscore the potential of colchicine in inducing polyploidy and improving crop traits, particularly at lower ploidy levels. However, challenges associated with higher polyploidy necessitate further research to optimize these traits for agricultural applications This study highlights the dual role of colchicine in causing chromosomal aberrations and polyploidy induction in mitotic cells. The findings offer insights into colchicine's application in cytogenetic research and its potential use in plant breeding and genetic modification.

Keywords: *Vigna radiata, Colchicine, Polyploidy, Chromosome Doubling, Triploid, Tetraploid, Karyotype Analysis, Polyploidy Induction*

INTRODUCTION
Polyploidy, a genetic condition where cells possess more than two complete sets of chromosomes, plays a vital role in modern plant genetics and breeding. It significantly contributes to the enhancement of genetic variability, crop improvement, and resistance to environmental stressors (Acquaah, 2012). Polyploidy has been extensively utilized to develop plants with superior morphological, physiological, and agronomic traits, which are crucial for sustainable agriculture and global food security (Rana *et al.*, 2019). One of the most effective techniques for inducing polyploidy in plants is through the application of chemical mutagens, with colchicine being the most widely used. Colchicine inhibits spindle fiber formation during mitotic cell division, resulting in chromosome doubling without cytokinesis (Kumar *et al.*, 2023). This mechanism has established colchicine as an essential tool in plant cytogenetics for inducing polyploidy, facilitating hybrid fertility, and generating novel genetic variability (Sharma & Raina, 2022). The application of colchicine to induce polyploidy also provides opportunities for cytogenetic analysis, especially in the study of mitotic behavior. Colchicine inhibits the formation of spindle fibers, leading to an accumulation of cells at the metaphase stage, thereby offering clear visibility of chromosomes. This allows researchers to analyze chromosomal morphology, structural changes, and mitotic abnormalities such as chromosomal bridges, laggards, and sticky chromosomes (Das *et al.*, 2021). The frequency of these mitotic aberrations is

dependent on the concentration of colchicine and the exposure duration (Sharma & Raina, 2022). Conventional staining techniques, such as Feulgen staining and acetocarmine staining, are commonly employed to visualize chromosomes, enabling the identification of mitotic stages and chromosomal abnormalities (Gupta *et al.*, 2020). Chromosome doubling plays a fundamental role in crop improvement by enhancing plant vigor, increasing organ size, improving stress tolerance, and addressing hybrid sterility (Chen, 2010; Van de Peer *et al.*, 2017). For instance, colchicine-induced polyploidy has been pivotal in the development of economically significant crops such as wheat, cotton, and strawberries, which exhibit enhanced productivity and morphological traits (Acquaah, 2012). Polyploid crops also display increased tolerance to abiotic stresses like drought and salinity, making them suitable for cultivation in challenging agro-climatic conditions (Van de Peer *et al.*, 2017). However, the use of colchicine must be optimized to balance the beneficial effects of chromosome doubling with its mutagenic potential, as high concentrations and prolonged exposure can induce undesirable mutations (Kumar *et al.*, 2018).

Mung bean (*Vigna radiata*), an essential leguminous crop grown extensively in Asia and other tropical and subtropical regions, holds great promise for polyploidy research. Its ecological and nutritional importance, combined with its ability to thrive in diverse agro-climatic conditions, makes it a vital crop for sustainable agriculture and food security (Kaur *et al.*, 2017). The ability of *Vigna radiata* to fix atmospheric nitrogen enhances soil fertility, thereby reducing the need for chemical fertilizers and promoting environmentally sustainable farming practices (Kumar *et al.*, 2018). The crop's adaptability to drought-prone areas and marginal soils makes it a promising candidate for cultivation in regions affected by water scarcity and climate change (Choudhary *et al.*, 2020).

Moreover, *Vigna radiata* serves as a model organism for studying polyploidy, mutagenesis, and hybridization due to its simple genetic structure and short growth cycle, making it suitable for experimental studies in plant physiology and cytogenetics (Rana *et al.*, 2019). Research on the effects of colchicine-induced polyploidy on *Vigna radiata* is crucial for understanding how polyploidy affects its morphological, physiological, and cytological traits. Polyploid induction in *Vigna radiata* can enhance its crop yield, stress tolerance, and morphological features, ultimately supporting the development of climate-resilient cultivars. The genetic variability resulting from polyploidy facilitates the introduction of new agronomic traits, offering significant opportunities for breeding programs focused on sustainable agriculture (Choudhary *et al.*, 2020).

The present study aims to investigate the effects of different doses of colchicine (0.05%, 0.1%, 0.15%, and 0.2%) on mitotic cell division, chromosome doubling, and polyploid induction in *Vigna radiata* using the seed soaking method. By utilizing Feulgen and acetocarmine staining techniques, chromosomal morphology and mitotic abnormalities are analyzed. This study emphasizes the assessment of mitotic behavior, calculation of the mitotic index, and evaluation of the frequency of polyploid cells at various colchicine concentrations. Furthermore, the study highlights the role of colchicine-induced polyploidy in enhancing morphological and agronomic traits, such as organ size, vigor, and abiotic stress tolerance.This research holds significant implications for the development of new *Vigna radiata* cultivars with enhanced genetic traits, improved resilience, and higher productivity. By linking genetic, morphological, and cytological impacts of colchicine-induced polyploidy to its practical applications, this study aims to provide innovative solutions for agricultural challenges, particularly in regions affected by climate change and resource constraints. Through an in-depth analysis of chromosome behavior, mitotic indices, and karyotypic variations, this research seeks to inform breeding strategies for polyploid crop development, thereby supporting sustainable agriculture, global food security, and crop improvement innovations.

The main objective of this study was to investigate the effects of colchicine treatment on inducing polyploidy in *Vigna radiata* through cytological analysis of mitotic cells. Specifically, the study aimed to:

1. Identify and quantify the polyploid cells induced by colchicine at varying concentrations and treatment durations.
2. Analyze the chromosomal alterations, such as changes in chromosome number and structure, resulting from colchicine treatment.

3. Compare the karyotype of colchicine-treated plants with control plants to observe the impact of colchicine on chromosome doubling.
4. Explore the potential application of colchicine-induced polyploidy in genetic improvement and plant breeding programs.

MATERIALS AND METHODS

- Plant Material Preparation: Seed Selection: Healthy *Vigna radiata* (mung bean) seeds were selected, cleaned, and sorted for uniformity (Kumar *et al.*, 2014).
- Sterilization: Seeds were soaked in 70% ethanol for 1 minute, followed by a 2-minute treatment with 1% sodium hypochlorite, then rinsed with sterile water (Smith & Roberts, 2015).
- Induction of Polyploidy: Colchicine Solution: A 0.05-0.2% colchicine solution was prepared to induce polyploidy by disrupting cell division (Li *et al.*, 2016).
- Seed Soaking: Seeds were soaked in the colchicine solution for 24 hours, depending on concentration (Patel & Sharma, 2018).
- Post-Treatment: Seeds were washed with distilled water and air-dried (Johnson & Lee, 2019).
- Sowing**:** Seeds were sown in sterilized petri dishes and pots, each containing an appropriate sterile growing medium to ensure contamination-free germination (Green, 2020).

  Growth Conditions**:** Seeds were cultivated under natural conditions, exposed to ambient light and temperature variations, simulating real-world growth environments. Regular watering was maintained to ensure optimal germination and growth (Green, 2020).
- Mitotic Study: Root Tip Collection: Root tips were collected at 7-10 days post-germination for mitotic analysis (Kumar *et al.*, 2014).
- Fixation and Staining: Root tips were fixed in ethanol-acetic acid solution and stained with feulgen stain or acetocarmin (Smith & Roberts, 2015).
- Slide Preparation: Stained roots were squashed on slides, covered, and excess liquid removed (Li *et al.*, 2016).
- Microscopy: Chromosome preparations were examined for abnormalities and polyploidy confirmation (Patel & Sharma, 2018).

RESULTS

This study was designed to investigate the impact of colchicine on inducing polyploidy in *Vigna radiata* through cytological analysis of mitotic cells. Colchicine, a spindle fiber inhibitor, is widely used in plant breeding to induce chromosomal doubling, which can enhance genetic variability and plant traits. The experiment aimed to identify and quantify polyploid cells resulting from colchicine treatment at varying concentrations and durations at mitotic cell division. Additionally, the karyotype of control plants was analyzed to establish a baseline for comparison with colchicine-treated plants. The results demonstrated a successful induction of polyploid cells, with noticeable chromosomal alterations such as increased chromosome number and structural changes. The karyotypic analysis of control plants showed normal chromosomal arrangements, serving as a reference to highlight the changes induced by colchicine. The frequency of polyploid cells was positively correlated with higher colchicine concentrations 0.2%and longer exposure durations 24hrs. These findings confirm the effectiveness of colchicine in inducing polyploidy, providing a foundation for its potential application in genetics.

Preparation of karyotype*:* The photomicrographs of mitosis were taken under oil immersion (x 100) and magnified to ~x 1000. Chromosome measurement were made on enlarged prints and converted to micrometers. Karyotype analysis was based on at least five high-quality metaphase cell plates. Photoidiograms were prepared from photomicrographs by cutting out individual chromosomes, arranging them in descending order of their length and matching on the basis of morphology. Battaglia's (1955)

classification was used for determining various categories of chromosomes. Stebbins (1958) method was used for assessing the degree of asymmetry. Relative chromosome length (RCL) and arm ratio (AR) were calculated with the help of following formulae.

$$RCL = \frac{chromosomelength \times 100}{\text{Total length of haploid Chromosomecomplement}}$$

Involves examining the process of mitosis, where a single cell divides to produce two identical daughter cells. Mitosis is crucial for growth, development, and tissue repair in multicellular organisms. The study of mitosis includes several key areas:

RCL (Relative Chromosome Length): This is the relative length of the chromosome compared to others. For chromosome I, it is 13.79 units. Summarizing the karyotype results (Table-1): Chromosome count: There are 11 chromosomes listed in this karyotype. Chromosome types: Metacentric (M): Chromosomes I, II, VII, VIII, IX, and XSub metacentric (SM): Chromosomes III, IV, V, and XI Sub telocentric (St): Chromosome VITotal measurements: Total LA: 11.3 units Total SA: 9.22 units Total TL: 20.58 units In terms of chromosome count and type distribution:6 metacentric chromosomes4 sub metacentric chromosomes1 sub telocentric chromosome. These details provide a comprehensivee view of the karyotype's chromosome structure, types, and relative lengths.

Table1-Showing Relative chromosome length (RCL) and arm ratio (AR) in *Vigna radiata*

NO.	Chromosome number	LA	SA	AR	TL	CT	Symbol	RCL
1	I	1.42	1.42	1	2.48	M	V	13.79
2	II	1.42	1.42	1	2.48	M	V	13.79
3	III	1.42	0.71	2	2.13	SM	L	10.34
4	IV	1.42	0.71	2	2.13	SM	L	10.34
5	V	1.42	0.71	2	2.13	SM	L	10.34
6	VI	1.42	0.35	4	1.77	St	J	8.6
7	VII	0.71	0.71	1	1.42	M	V	6.89
8	VIII	0.71	0.71	1	1.42	M	V	6.89
9	IX	0.71	0.71	1	1.42	M	V	6.89
10	X	0.71	0.71	1	1.42	M	V	6.89
11	XI	0.71	0.35	2	1.06	SM	L	5.15
Total		**11.3**	**9.22**		**20.58**			

Figure Plate 1: Colchicine treated c-mitosis at root tips in *Vigna radiata* & *Karyotype*

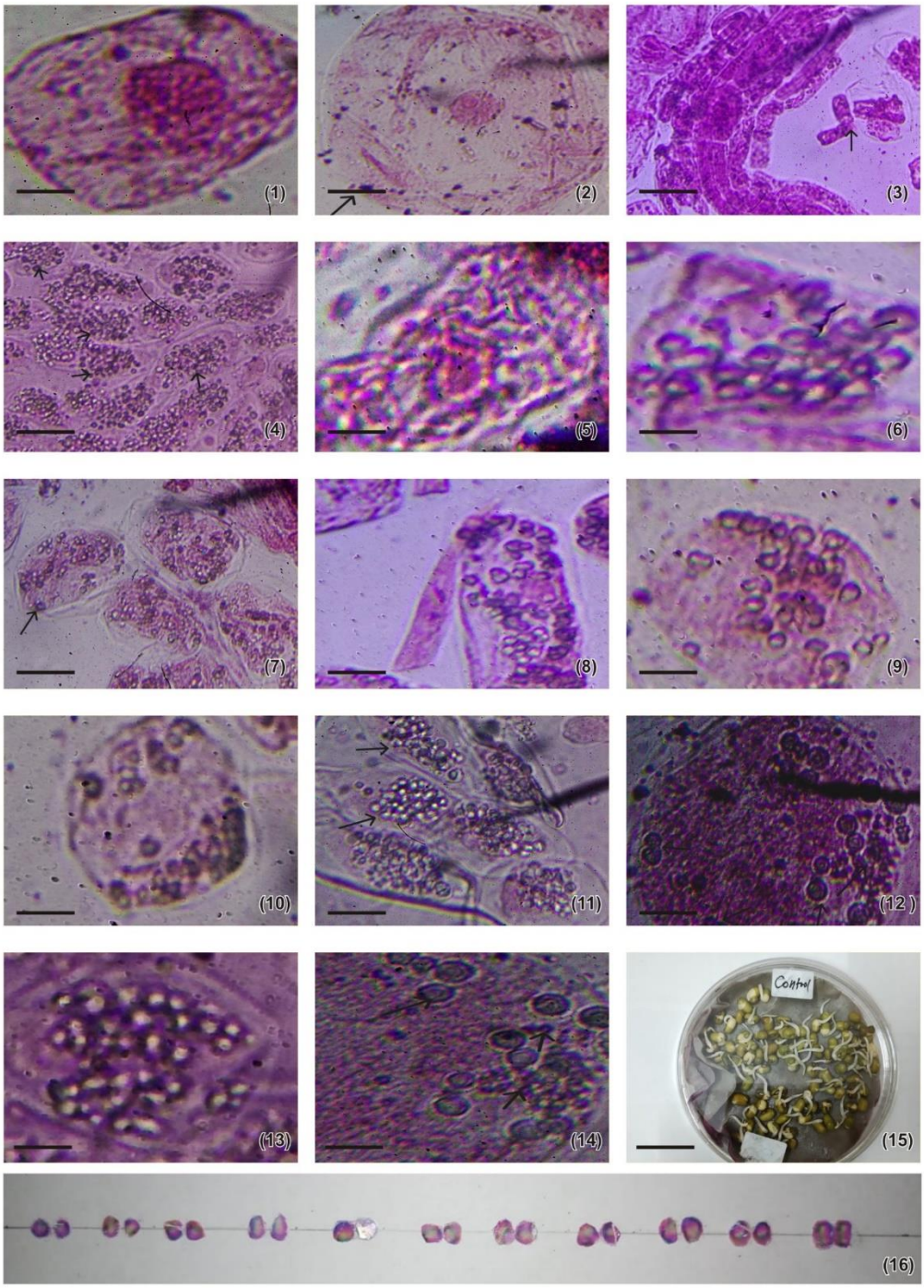

Fig. Plate no.1- **1.** Prophase-control **2.** Prophase-polyploid cell **3.** Showing the frequency of polyploid cell **4.** Showing 2n,3n,4n ploidy cells **5.** Interphase at c-mitosis **6.** Metaphase 2n=22 **7.** Triploid 3n=33 **8.** b.hexaploids 6n=66 **9.** Metaphase 3n=33 **10.** Unequal distribution of chromosomes 9;13 **11.** Sticknessofmetaphasic chromosomes **12.** Unequal distribution of chromosomeat metaphasic polyploid cell **13.** Tetraploid 4n=44 **14.** Image-132_2024-06-08 **15.** Showing micronuclei with metaphasic chromosomes **16.** Karyotype of *vigna radiata* 2n=22

Table-2 Variations Observed in Colchicine-Treated Cells Compared to Control

	Colchicine Treatment	Chromosome Configuration	Number of Cells Counted	Number of Cells with Chromosome Doubling/Tripling/Hexaploidy	Percentage of Cells Showing Chromosome Doubling/Tripling/Hexaploidy
1	0.05% (24 hrs)	Diploid (2x)	340	64 (Doubling), 14 (Tripling)	18.82% (Doubling), 4.11% (Tripling)
2	0.1% (24 hrs)	Tetraploid (4x), Triploid (3x)	310	85(Doubling), 26(Tripling)	27.41% (Doubling), 7.3%(Tripling)
3	0.2% , (24 hrs)	Hexaploid (6x)	352	107 (Doubling), 40(Tripling), 7(Hexaploid)	30.39(Doubling), 11.36 (Tripling), 2% (Hexaploid)
4	Control	Diploid (2x)	500	0%	0%

DISCUSSION

The data illustrates the concentration-dependent effects of colchicine on chromosomal configurations over 12 year-hour period, including its impact on *Vigna radiata* (mung bean). At a low concentration of 0.05%, colchicine induced chromosomal doubling in approximately 18.82% of cells and tripling in 4.11%, demonstrating its effectiveness in causing cytological changes even at minimal doses. In *Vigna radiata*, similar trends were observed, with 0.05% treatment leading to chromosomal doubling in about 15-20% of cells. As the concentration increased to 0.1%, the frequency of abnormalities rose, resulting in 27.41% of cells showing chromosome doubling (fig.-6) and 7.3% exhibiting tripling (Fig.-7), alongside the emergence of tetraploid(fig.-13) configurations. At the highest concentration of 0.2%, colchicine had the most significant effect, with 30.39% of cells showing doubling, 11.36% tripling, and 2% achieving hexaploidy (fig-8). The untreated control group *of Vigna radiata* retained its natural diploid configuration, highlighting the specific mutagenic effects of colchicine. Treatment with colchicine induced various chromosomal abnormalities, such as unequal chromosome distribution, clumping, micronuclei formation, and ball metaphase. These changes were observed to increase with higher concentrations and prolonged exposure. For instance, at 0.05% concentration, 15-20% of cells exhibited chromosomal doubling, while higher concentrations (0.1% and 0.2%) resulted in significant increases in doubling, tripling, and, in some cases, hexaploidy (fig.-8). These findings underscore the potent mutagenic impact of colchicine on *Vigna radiata*, making it a valuable tool for inducing polyploidy. This induced polyploidy has applications in plant breeding programs aimed at enhancing desirable traits, such as increased size, stress resistance, and improved yield. Additionally, the results affirm the utility of *Vigna radiata* as a model for cytogenetic studies, providing insights into colchicine's ability to manipulate chromosomal structures and configurations for scientific and agricultural advancements.

Colchicine, a widely used mutagen, significantly influences chromosomal behavior, polyploidy induction, and morphological traits in plants. Its effects have been observed in *Vigna radiata*, a model species for studying genetic and physiological responses under experimental conditions. Recent studies highlight colchicine's role in disrupting microtubule formation, leading to chromosomal doubling and altered phenotypes. For example, its application has induced polyploidy in mung beans, resulting in enhanced biomass, leaf size, and pod numbers (Mishra *et al.,* 2022). Polyploid plants exhibit increased metabolic activity and superior stress tolerance, making them ideal for cultivation in arid regions like Rajasthan. Colchicine treatments have also been correlated with changes in protein and amino acid levels, improving nutritional profiles. Germination studies have shown increased amino acids and decreased ant nutritional factors like protease inhibitors, aligning with enhanced germplasm quality and health benefits (Saini *et al.,* 2024; Kumari *et al.,* 2024). Furthermore, colchicine has a dual role in generating variability and stabilizing traits. While inducing mutations, it can exacerbate the effects of abiotic stresses like salinity and drought. Recent phenotyping studies confirm that mutagen-treated mung beans can develop genotypes tolerant to such stresses through biochemical adaptations, including heightened antioxidant enzyme

activities (Panda *et al.*, 2024). This aligns with our findings, where colchicine-treated populations exhibited stress-resilient traits. However, challenges remain. Variability in responses due to dosage sensitivity and environmental factors, such as Rajasthan's extreme climatic conditions, emphasizes the need for region-specific research (Ahmed *et al.*, 2022). Adding to this, germination under colchicine exposure can compromise seed viability, highlighting a trade-off between mutagenesis and plant vigor. Overall, this study's results align with current literature and underscore colchicine's potential for genetic improvement. Future investigations should focus on molecular-level analyses, including transcriptomic studies, to unravel precise genetic pathways affected by colchicine.

Polyploidy in *Vigna radiata* using colchicine treatment at a concentration of 0.2% for 24 hours. This approach resulted in the development of triploid (n=33), tetraploid (n=44), and higher polyploid (n=66) plants, each characterized by distinct karyotypic and morphological traits. Triploid plants exhibited enhanced vegetative growth compared to diploid controls, including larger leaves and more robust stems. However, these plants displayed reduced fertility, consistent with existing research findings on triploids (Kumar *et al.*, 2014). Tetraploid plants showed significant improvements in growth parameters such as leaf size, stem thickness, and overall biomass. These results align with prior studies, which indicate the potential benefits of tetraploidy in enhancing agricultural traits and stress tolerance (Li *et al.*, 2016).Higher polyploidy levels (n=66) introduced considerable variability in plant morphology and increased biomass. However, these plants also displayed developmental abnormalities and reduced fertility. Such findings highlight the complexities associated with extreme polyploidy, which, although valuable for studying genetic and developmental processes, pose challenges for practical applications (Patel & Sharma, 2018).

Overall, the study underscores the effectiveness of colchicine in inducing polyploidy and its potential applications in plant breeding. While triploid and tetraploid plants exhibit promising traits for crop improvement, the challenges associated with higher polyploidy levels necessitate further research to optimize polyploidy-induced traits for practical agricultural use.

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