



Induced tetraploid in Sunnhemp (*Crotalaria juncea* L.) and Dhaincha (*Sesbania bispinosa* (Jacq.) W. Wight).

PARMESHWAR DEORAM KARALE* and RAMDAS DAGDU BORSE

Department of Botany and Research Centre, Padmashri Vikhe Patil College of Arts, Science and Commerce Pravaranagar (Loni), Tal Rahata Dist. Ahmednagar Maharashtra, India.

Abstract

A mutation technique provides good exciting opportunities for modern plant breeding. Induced autotetraploidy has potential for genetic improvement of various crops species. The crops like Sunnhemp ($2n=16$) and Dhaincha ($2n=24$) are diploid species, which are included in family Fabaceae and mostly cultivated as yielding of bast fibre green and manure purpose in all over the India. The M2 generation in these plants showed mitotic chimera changes due to colchicine treatment. The chimeric mutants mitotic cell division were showed irregular chromosome number and resulting a mixoploid composition of both plants.



Article History

Received: 22 November 2024

Accepted: 24 February 2025

Keywords

Autotetraploidy; Chimera; Colchicine; Mitosis; Mixoploid; Mutants.

Introduction

Sunnhemp (*Crotalaria juncea* L.) and Dhaincha [*Sesbania bispinosa* (Jaque. W. Wight)] both are tropical Asian plants included in Legume family. In India, cultivation of these plants for green manure purpose in kharif as well as rabbi seasons for improvement of soil fertility. The plants show fast growth which increases nitrogen and high biomass in soil within a short time according to researcher Kumar and Dwivedi.¹ Polyploidy or whole genome duplication (WGD) may be used to refer to new lineages or experimental hybrid.²

Colchicine used as a mutagenic agent, causing the multiplication of sets of plant chromosomes which

has a best choice for inducing genetic mutations. Colchicine treatment inhibits the formation of microtubules and halts the cell division and cell wall formation at the early anaphase stage induce chromosome doubling forms polyploids.³ After the colchicine treatment the physiological disturbances results in slower of growth and development as well as rates of cell division.⁴ The increase in thickness and greenness of the leave were uniform, thereby, the existence of sectorial chimera is ruled out in this mutant. Stomatal density, size, and chloroplast number were different in mutants of polyploids than the normal plants. Increase in stomatal length and frequency was the accurate indicator of the polyploidy level in many plants.^{5,6}

CONTACT Parmeshwar Deoram Karale ✉ karaleparmeshwar@gmail.com 📍 Department of Botany and Research Centre, Padmashri Vikhe Patil College of Arts, Science and Commerce Pravaranagar (Loni), Tal Rahata Dist. Ahmednagar Maharashtra, India.



© 2025 The Author(s). Published by Enviro Research Publishers.

This is an  Open Access article licensed under a Creative Commons license: Attribution 4.0 International (CC-BY).

Doi: <https://dx.doi.org/10.12944/CARJ.13.1.29>

Plant mutation naturally takes place and results the formation of new species through increase in the number of chromosomes.⁷ The increase in the number of chromosomes using colchicine was reported to result showing enhanced sizes of morphological characters, such as larger leaves, flowers, and seeds in plants. In legumes, evaluation of nodule number, nodule size, terminal bacteroid differentiation, and symbionts quality (nodule development, partner choice, host sanctions, etc.) revealed that plant polyploidy enhanced some key aspects of legume-rhizobia mutualism hence that helps to improve green manure crops like Sunnhemp and Dhaincha. The aim of this research was to assess the mutagenic effects of Colchicine on the morphology and growth performance of Sunnhemp (*Crotalaria juncea* L.) and Dhaincha [*Sesbania bispinosa* (Jacq.) W. Wight].

Material and Methods

The present study was carried out during 2021-22 at the Department of Botany and research centre of Padmashree Vikhe Patil College of Arts, Science and Commerce Pravaranagar Taluka Rahata District Ahmednagar Maharashtra. For the present experiment, Seeds of Sunnhemp and Dhaincha of Mahabeej Seed Industries were used that were purchased from local market.

Colchicine Treatment

Dried seeds of uniform size were selected for each treatment. The seeds were then sterilized with 70% ethanol for 1 minute and washed 2 to 3 times in distilled water to remove ethanol. The seeds of Sunnhemp and Dhaincha were soaked in distilled water for 6hrs then well soaked 100 seeds for each treatment treated with (Control) 0.0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, and 0.7% concentration of colchicine.⁸ After 3hrs treatment seeds were rinsed with distilled water and the seeds were sown in well prepared field in 3cm ×4cm distance in plot size 4.5m ×5m size, with regular watering in 15 to 20 days interval for 90 days. The seeds were harvested after 140 days when pods were completely dried. These Seeds were used for mitotic study by seed germination techniques in Petri plates.

Cytological Study

The root tips of control and colchicine treated plants were pre-fixed at 7.00 AM to 8.00 AM in 1,3 dichlorobenzene for 2 hrs at 25°C. The tips were

washed 2-3 times with distilled water and then used for temporary slide preparation immediately. The root tips were taken into small Petri plate with 3ml 1N HCl and heated up to 60°C for hydrolysis. Then root tips were transferred into distilled water for to remove excess HCl. The whitish root tip squash taken on slide with 1 to 2 drops of 2% aceto-orcein stain, cover the coverslip and then spread the squash with the help of needle. Then cover the filter paper and press the thumb on it and the slide was observed under Leica DM 1000 microscope with 100% magnification of 100X oil immersion according to Levan et.al.1964.⁹

Result

The study of colchicine induced cytochimeral plant in M_2 generation of *Crotalaria juncea* and *Sesbania bispinosa* (Jacq.) Wight showed two different set of chromosome number (2x and 4x). The observations showed that in Sunnhemp and Dhaincha, colchicine treatment increase the number of chromosomes, but the multiple rates of chromosome set number were different (aneuploid). It is not an exact multiple of the haploid number as well as gains or losses of chromosomes and shows irregularities in chromosomes arrangement. We observed the chromosome numbers of the diploid control plants were $2n=2x=16$ and $2n=2x=24$ and the chromosome numbers of high concentration treatments colchiploid plants were varied from 16 to 32 and 24 to 48 i.e. irregular and showed morphological abnormalities in both the plants.

The stomatal density of colchiploids of Sunnhemp plants were varied from 155 to 280 stomata per mm^2 , while the control was 270 to 300 stomata per mm^2 and in Dhaincha 160 to 220 stomata per mm^2 , while the control was 300 to 400 stomata per mm^2 . The mean of chloroplast number in guard cells of colchiploid plants was varied from 08 to 20 which were less than control.

Discussion

The M_2 phenotypical population of Sunnhemp and Dhaincha plant showed number of variations in plant height, leaf size and colour and seeds shape, colour and number of seeds in pod. Mitotic study in the $2n=16$ in Sunnhemp and $2n=24$ bivalents in Dhaincha diploids plants but the cytochimeral mutant were characterized by the presence of both 2x and 4x chromosome numbers in the same mutant plants.

This abnormality indicates considerable sterility in M2 mutants. In the tetraploids, $4n=32$ bivalents in Sunnhemp and $4n=48$ in Dhaincha were present but exact 32 and 48 bivalents chromosomes were noticed in very less percentage in mitotic Index observed values. According to Riley and Chapman,¹⁰ in autotetraploid high frequency of bivalents have been occurred but it results the short size of chromosomes than the diploid. Colchicine treatments revealed the induction of various chromosomal abnormalities in somatic cells which includes chromatin stickiness, unoriented chromosomes, univalent, laggards, bridges, and micronuclei.^{11,12} These abnormalities increased along with increased colchicine concentrations.

Conclusion

Here we reported a colchicine induced cytochimeral plant in M2 generation of *Crotalaria juncea* L. and *Sesbania bispinosa* (Jacq.) W. Wight. Induced tetraploid plants grow slowly and showed growth abnormalities due to colchicine treatment. The uniform distribution of diploid and tetraploid cells is that at the time of colchicine treatment those cells which were at metaphase stages may have under gone polyploidisation while others remained unaffected. After resumption of growth both diploid and tetraploid cells were intermixed resulting a mixoploid composition. Polyploidy in both the plants characterized by a stomatal density reduction, increase in stomata size, and increase in the number of guard cells chloroplasts. The results suggested that colchiploid plants in this study have varied stomatal density, larger stomatal size and larger chloroplasts than control.

Acknowledgement

We would like to thank Savitribai Phule Pune University for granting the Ph.D. research work. The Department of Botany, Padmashri Vikhe Patil College, Pravaranagar, is highly appreciated for allowing the cytological laboratory work for my research in plant breeding.

Funding Sources

The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Authors contributions

- **Parmeshwar Deoram Karale:** Conceptualization, Methodology, Writing – Original Draft.
- **Ramdas Dagdu Borse:** Data Collection, Analysis, Writing – Review & Editing.

References

1. Kumar S. and Dwivedi S. K. Identification of drought tolerant rice genotypes by analysing drought tolerance indices and morpho-physiological traits. *SABRAO Journal of Breeding and Genetics*. 2014; 46 (2):217-230.
2. Mattingly K. Z. and Hovick S. M. Autopolyploids of *Arabidopsis thaliana* is more phenotypically plastic than their diploid progenitors. *Annals of Botany*. 2023;131: 45–57.
3. Dhooche E., K. Van L. Mitotic chromosome doubling of plant tissues in vitro. *Springer Nature*. 2011;104 359–373.
4. Gunckel J.E. and Sparrow A.H. Ionizing radiation: Biochemical physiological and morphological aspects of their effects on plants. *Encyclopaedia of Plant Physiology*. 1967; 16: 556-611.
5. Speckman G.J., Post J. and Dijkstra M. The length of stomata as an indicator for polyploidy in rye-grass. *Euphytica* 1965; 14:225-230.
6. Vijayan K., Chakraborti S.P., Roy B.N., Setua M. and Qadri S.M.H. Cytomorphological

- characteristics of colchicine induced chimeric mutant in Mulberry. *Cytologia* 1998; 63:27-31.
7. Storme N. and Masom A. Plant speciation through chromosome instability and ploidy changes: Cellular mechanisms, Molecular factors and evolutionary relevance. *Current Plant Biology* I. 2014; 10-33.
 8. Borkar A. T. and More A. D. Induced flower colour mutations in *Phaseolus vulgaris* Linn. Through physical and chemical mutagens. *Advances in Bioresearch*. 2010; 1 (1) 22-28.
 9. Levan A., Fredga K. and Sandberg A. Nomenclature for Centromeric Position on Chromosomes. *Hereditas* 1964; 52(2): 201-220.
 10. Riley R. and Chapman V. Genetic Control of the Cytologically diploid behaviour of Hexaploid Wheat. *Nature* 1958; 182: 713-715.
 11. Dnyansagar V.R. and Nadkarni R.S. Induced Tetraploidy in *Crotalaria juncea* Linn. *Cytologia* 1982; 48: 483-489.
 12. Verma R.C. and Raina S.N. Cytogenetics of *Crotalaria*. VII. Induced cytochimeral and Asynaptic mutants of *Crotalaria juncea* (Sunnhemp). *Indian J. Genet*, 1990; 51 (3): 315-319.