Vegetable Science (2025) 52(1): 122-127
doi: 10.61180/vegsci.2025.v52.i1.17
ISSN- 0970-6585 (Print), ISSN- 2455-7552 (Online)
www.isvsvegsci.in

RESEARCH ARTICLE

Development of autotetraploid garlic (A*llium sativum* L.) through *in-vitro* chromosome doubling

Vaishnavi Jadhao and Rushikesh Tahakik*

Abstract

Genetic variation is closely linked to crop improvement. Since garlic is primarily propagated vegetatively, it exhibits low genetic diversity. In this study, *in-vitro* colchicine treatment was used to induce polyploidy, aiming to enhance allelic variation. Shoot proliferation was optimized using different concentrations of BAP and TDZ, with 3 mg/L BAP and 0.25 mg/L TDZ showing the highest regeneration. Calli aged 21 days were subcultured and exposed to colchicine at varying levels. The results indicated that 0.05 mg/L colchicine promoted the highest survival, while higher concentrations (0.75 mg) reduced duplication due to toxicity. The LC_{50} for colchicine was determined to be between 0.03–0.05 mg/L. The induced autotetraploids showed potential for enhanced genetic diversity in garlic. This study highlights the optimal colchicine dose for ploidy induction with minimal mortality, offering a pathway to genetic improvement in vegetatively propagated crops like garlic.

Keywords: Colchicine, Garlic, Genetic variation, Polyploidy.

MGM College of Agricultural Biotechnology, Gandheli, Chhatrapati Sambhajinagar, Maharashtra, India.

***Corresponding author;** Email: rushi.mgmcabt@gmail.com

Citation: Jadhao, V., Tahakik, R. (2025) Development of autotetraploid garlic (*Allium sativum* L.) through in-vitro chromosome doubling Vegetable Science 52 (1): 122-127.

Source of support: Nil

Conflict of interest: None.

Received: 14/07/2024 **Revised:** 23/04/2025 **Accepted:** 10/05/2025

Introduction

Garlic (*Allium sativum*) belongs to the Alliaceae family and is native to Central Asia. It has been domesticated for thousands of years and is considered one of the oldest cultivated food crops, as evidenced by fossil records (Block, 2010). Garlic primarily consists of water (65%), followed by carbohydrates (26–30%), and contains other substances such as proteins, lipids, fiber, minerals, and saponins (Petropoulos, 2018). Additionally, garlic is rich in phenols, B-complex vitamins like thiamine, riboflavin, and niacin, as well as minerals including selenium, sulfur, zinc, magnesium, iron, sodium, and calcium (Gambelli, 2021). These nutrients contribute to its well-known antibacterial, antifungal, antiviral, antioxidant, and anticancer properties (Leyla Bayan, 2014). Molecular variation is a key driver of genetic improvement; however, in garlic, which is propagated vegetatively, natural variation is limited due to floral organ abortion and chromosomal abnormalities (Hailu, 2021). Furthermore, in-vitro propagated plants tend to become smaller over successive generations (Tahakik et al., 2024) . This reduction in plant vigor is attributed to in-vitro micropropagation proliferating older cells, forcing them to divide continuously, which reduces telomerase activity (Schrumpfová et al., 2014). Telomerase is a ribonucleoprotein complex responsible for maintaining telomere length. Reduced telomerase activity results in telomere shortening and may lead to the loss of

viable genes, ultimately increasing the plant's susceptibility to diseases (Procházková Schrumpfová, 2019). This, in turn, negatively affects molecular diversity and germplasm utility.

Biotechnological tools, such as the application of mutagens, offer effective approaches to enhance genetic diversity (Catalano et al., 2021). Among these, colchicine is widely used to induce polyploidy in garlic. Colchicine disrupts chromosomal doubling by interfering with spindle fiber formation during metaphase, binding to the plus end of microtubules and inhibiting microfilament polymerization (Caperta & Delgado, 2006). Polyploid plants, due to their higher cell-to-cytoplasm ratio, exhibit larger cloves and altered morphology compared to diploid plants. In-vitro colchicine-induced polyploids show increased DNA content and changes in epidermal surface cell morphology (Bernard, 2012). These plants typically have thicker cells but fewer cells per leaf blade compared to diploids (Manzoor, 2019). Although colchicine effectively induces polyploidy and promotes genetic variation, its success is highly dependent on concentration. The optimal concentration is influenced by factors such as bulb size, shape, plant age, passage, and growth stage (Roslim, 2015). For example, colchicine treatment at 0.5% for 12 and 24 hours resulted in negative effects, including fewer saplings, stunted root growth, thicker stems, and physiological changes such as enlarged stomata, reduced stomatal density, and increased chromosome numbers compared to the control (Raizer et al., 2019). The degree of plant sensitivity to colchicine is assessed by the lethal concentration (LC) value, with LC50 being the concentration that produces the highest mutation rate (Ayu, 2019).

Materials and Methods

Plant material and explant preparation

Garlic cloves from 20 accessions of the variety 'Godavari' (G1), a diploid and late-maturing cultivar developed by the National Horticultural Research and Development Foundation (NHRDF), were selected as explants for micropropagation studies (Bridge, J. A. 2008); Lawande et al., 2009; Trojak-Goluch & Skomra, 2013). To eliminate fungal contamination, cloves were initially pre-treated with 1% Bavistin for 30 minutes, as described by Eed et al. (2010). Surface sterilization was then performed using 1% sodium hypochlorite and 2% hydrogen peroxide for 10 minutes each (Eed et al., 2011; Lahoty et al., 2013). The sterilized cloves were rinsed with 70% ethanol, followed by three washes with double-distilled water (ddH_2O), and were then inoculated onto Murashige and Skoog (MS) medium.

Culture medium and conditions

The MS medium was supplemented with 2% (w/v) sucrose, 1 mg L^{-1} pyridoxine-HCl, MS vitamins, and varying concentrations of the plant growth regulators 6-benzylaminopurine (BAP) and thidiazuron (TDZ) as previously established (Lahoty et al., 2013). The pH of the medium was adjusted to 5.8 before autoclaving. Cultures were incubated at a temperature range of 24–26 °C under a 16/8 h photoperiod with fluorescent light at an intensity of 45 µmol m^{-2} s^{-1} (Eed et al., 2011; Ghaderi & Jafari, 2014). Subculturing was performed every three weeks. The establishment time of explants was defined as the period required to reach a height of 4 cm. The cultures were initially incubated in the dark to enhance shoot initiation and then maintained at 25 ± 2 °C with 70% relative humidity under a 16/8 h light/dark photoperiod and 2500 lux light intensity (Gantait et al., 2011).

Establishment and Shoot Induction

The establishment of explants was confirmed when they reached a height of 4 cm. To evaluate the effect of growth regulators on shoot induction, different concentrations and combinations of BAP and TDZ were tested. The number of shoots per explant was recorded every 30 days up to the third subculture, and the rooting percentage was assessed at 90 days.

In-vitro polyploidy induction with colchicine

Polyploidy was induced *in-vitro* using colchicine treatments in a completely randomized design, comprising six treatment levels (0.00, 0.02, 0.04, 0.06, 0.08, and 0.10 mg L^{-1}) and five replications per treatment (Liu et al., 2007). About 21-day-old seedlings were subcultured, and second-generation calli were exposed to colchicine along with 3 mg L^{-1} BAP, 0.5 mg L^{-1} TDZ, 3% sucrose, 0.8% agar, and activated charcoal as per the protocol of Rêgo et al. (2011). Each replication bottle contained approximately four calli, totaling 140 explants.

Molecular and cytological confirmation of polyploidy

Cytological confirmation of polyploidy was conducted using root tips (1–2 cm) from four-day-old plantlets. The root tips were soaked in 1% sucrose for one hour, washed thoroughly, and fixed in absolute methanol following the method of Chiareli et al. (1972). Fixed samples were stained with a 1:3 diluted Giemsa solution for one hour (Remya et al., 2019), washed three times, and dissected to obtain 2 mm segments. These were mounted on slides with a drop of cedarwood oil to enhance the refractive index and observed under a light microscope at 100× magnification (Gantait et al., 2011; Chen et al., 1994). For molecular characterization, genomic DNA was isolated from 0.2 g of young leaves using a CTAB extraction buffer. Samples were incubated at 60°C for 40 minutes as per the protocol of (Yi et al. 2018), followed by centrifugation at 14,000 rpm for 10 minutes. The supernatant was extracted using a chloroform:isoamyl alcohol mixture (24:1), re-centrifuged, and the aqueous phase was transferred to a new tube. DNA was precipitated using chilled absolute ethanol, centrifuged

at 10,000 rpm for 5 minutes at 10°C, and the resulting pellet was air-dried and resuspended in 50 µL of TE buffer (Shukre et al., 2024). The DNA concentration and purity (A260/280 ratio) were assessed using a NanoDrop spectrophotometer (Winnepenninckx et al., 1993; Robin et al., 2016).

Results and Discussion

Establishment of garlic explants

Garlic explants were cultured on MS medium supplemented with 2% (w/v) sucrose, MS vitamins, and varying concentrations of BAP and TDZ to evaluate the effect of these growth regulators on explant establishment and shoot induction. Culture conditions were maintained at 24–26 °C under a 16/8 h photoperiod with 45 µmol m^{-2} s^{-1} light intensity, and subculturing was carried out every three weeks. The establishment phase was considered successful when explants attained a height of 4 cm. The percentage of explants established (≥4 cm height) under different growth regulator treatments is summarized in Table 1. The results showed that the percentage of established explants increased steadily with the increase in BAP concentration up to 3.0 mg/L (T6), with T5 (2.5 mg/L BAP + 0.25 mg/L TDZ) and T6 yielding the highest establishment rates (73.25% and 73.50%, respectively). A slight decline was noted at the highest concentration of BAP (T7), suggesting that excessive cytokinin levels may negatively impact early explant development (Tahakik et al., 2022). This observation aligns with findings by Ghaderi & Jafari (2014) and Erland et al. (2020), who emphasized the importance of using optimal concentrations of BAP in combination with auxins or other regulators for promoting efficient regeneration.

Effect of growth regulators on multiple shoot induction

The influence of different BAP and TDZ treatments on multiple shoot induction was further evaluated across three subculture cycles. Observations were recorded every 30 days, and rooting percentages were assessed at 90 days. The data are presented in Table 2. T6 and T7 treatments, both containing higher levels of BAP (3.0 and 3.5 mg/L respectively), showed the highest shoot induction and rooting percentages. The average number of shoots per explant increased progressively from T1 to T6 and plateaued at T7. This suggests a saturation point beyond which increasing cytokinin levels does not significantly improve morphogenesis. This finding is corroborated by Erland et al. (2020), who reported that TDZ, at low concentrations, facilitates de novo shoot organogenesis and somatic embryogenesis. Moreover, the literature indicates that auxin and cytokinin interactions play a crucial role in organogenesis. According to Ghaderi & Jafari (2014) and Amelia et al. (2020), cytokinins like BAP regulate shoot apical meristem (SAM) activity, influencing shoot and leaf development through enhanced cell division and chloroplast differentiation. The presence of TDZ in all treatments possibly acted synergistically to stimulate shoot organogenesis, even though its concentration remained constant at 0.25 mg/L. Among all treatments, T6 (3 mg/L BAP + 0.25 mg/L TDZ) emerged as the most effective for shoot induction and rooting, followed closely by T7. These findings served as a basis for selecting T6 for subsequent experiments involving colchicine treatment, targeting genetic variability and polyploidy induction as described by Gantait et al. (2011), (Tahakik et al., 2023) and Lahoty et al. (2013).

Table 1: Establishment percentage of garlic explants under different growth regulator treatments

Treatment	*BAP (mg/L)*	*TDZ (mg/L)*	*No. of Explants Inoculated*	*Established explants (%, ≥4 cm height)*
T1	0.5	0.25	45	41.00
T2	1	0.25	45	42.75
T3	1.5	0.25	45	52.25
T4	2	0.25	45	64.50
T5	2.5	0.25	45	73.25
T6	3	0.25	45	73.50
T7	3.5	0.25	45	69.75
SE				2.00
CD (5 %)				5.95
CV (%)				6.72

Effect of colchicine treatment on polyploidy induction, growth, and morphological characteristics in garlic plantlets

The effects of colchicine treatments on garlic plantlets, as shown in Table 3 and LD50 analysis in Table 4, reveal a clear dose-dependent relationship between colchicine concentration and various plant parameters, including polyploidy induction, growth, and survival. At the control level (0 mg/L), no polyploidy was observed, with all plantlets remaining diploid (2n). However, as colchicine concentration increased, there was a corresponding increase in polyploidy, with the highest polyploidy percentage observed at 0.10 mg/L (87%), resulting in a mixploid population. This finding is consistent with previous studies (Eed et al., 2010; Suwal et al., 2020), which reported that colchicine effectively induces polyploidy in various plants, including garlic, and is particularly effective at moderate concentrations. The DNA content increased significantly with colchicine treatment, reaching a peak of 9897.5 ng/ml at 0.08 mg/L, which correlates with the higher polyploidy observed at these concentrations. The A260/A280 ratio, a measure of DNA purity, ranged from 1.69 to 1.90, indicating that colchicine treatment did not adversely affect DNA quality, with the highest purity at 0.04 mg/L.

Regarding plant growth, plantlet height and weight exhibited an inverse relationship with colchicine

Table 2: Effect of growth regulator treatments on multiple shoot induction in garlic

		Subculture 1		*Subculture 2*		*Subculture 3*		
Treatment	*Callus (g)*	*No of leaves / Explant*	*No of Shoots / Explant*	*No of leaves / Explant*	*No of Shoots / Explant*	*No of leaves / Explant*	*No of Shoots / Explant*	*Rooting % (at 90 Days)*
T1	2.71	13.18	2.71	13.80	2.67	14.82	2.92	42.50
T2	2.64	12.92	2.64	13.73	2.78	14.06	3.20	56.25
T3	3.39	14.13	3.39	13.78	3.18	15.50	3.29	58.75
T4	4.46	14.87	4.46	14.97	4.56	15.17	4.39	60.00
T5	5.19	15.05	5.19	15.01	5.32	15.25	5.08	60.00
T6	5.85	15.04	5.85	15.01	5.85	15.95	5.41	61.25
T7	5.70	17.09	5.70	16.52	5.85	17.13	5.84	61.17
SE	0.17	0.37	0.27	0.49	0.18	0.41	0.23	1.56
CD (5 %)	0.50	1.09	0.84	1.47	0.54	1.21	0.69	4.62
CV (%)	7.88	5.00	10.87	6.72	8.48	5.41	10.81	5.44

Table 3: Effects of colchicine treatments on garlic plantlets

Colchicine (mg/L)	*Polyploidy (%)*	*Diploid/ Tetraploid*	*A260/ A280*	*DNA Content (ng/ml)*	*Plantlet Height (cm)*	*Total Plantlet Weight (g)*	*Bulblet Weight (g)*	*Chlorophyll (mg/g FW)*	*Stomata Width (µm)*	*Survival Rate (%)*	*Morphological Character*
0	0	2n	1.77	4586.46	9.79	4.1	3.42	1.84	12.34	100	NIL
0.02	31.25	2n/4n	1.86	4569.56	5.85	5.35	3.14	2.87	22.42	65	DG/TL
0.04	41.25	2n/4n	1.9	7548.58	7.78	5.92	3.62	3.49	25.1	44	DG/TL
0.06	59	2n/4n	1.69	7549.58	7.12	6.65	4.41	4.24	24.46	36	RD/CL/DG
0.08	75.5	Mixploid	1.77	9897.5	6.48	8.17	4.01	3.73	21.99	26	RD/CL/TL
0.1	87	Mixploid	1.76	7956.44	5.99	7.72	4.4	3.89	23.08	18	RD/CL/TL
SE	2.2				0.28	0.18	0.21	0.13	0.6		
CD	6.53				0.85	0.54	0.22	0.39	1.75		
CV	8.18				8.14	5.54	10.42	7.84	5.44		

DG: Dark Green Leaves,, TL: Thick Leaves, RD: Reduced Growth,, CL: Curved Leave

concentration (Rego M., 2011). At the control level, plantlets reached a height of 9.79 cm, but this decreased significantly to 5.99 cm at 0.10 mg/L, indicating that higher colchicine concentrations can hinder overall plant development. This is in line with findings by Gabriella Marry Ayu (2019), who noted that colchicine concentrations above a certain threshold could induce toxicity, leading to reduced growth and survival. Bulblet weight followed a similar pattern, with a peak at 0.06 mg/L (4.41 g) before declining at higher concentrations. These results suggest that moderate colchicine concentrations promote bulblet development, which is critical for garlic yield enhancement. The increase in chlorophyll content at intermediate concentrations (4.41 mg/g FW at 0.06 mg/L) suggests improved photosynthetic capacity, which could potentially offset the negative effects of colchicine on plant growth (Gantait et al., 2011). Stomata width also increased at higher colchicine concentrations, peaking at 4.24 µm at 0.06 mg/L, which may indicate enhanced gas exchange and water regulation, important for the overall health and growth of polyploid plants (Ayesha Manzoor et al., 2019). However, higher colchicine concentrations led to a significant decrease in survival rate, from 100% in the control group to 18% at 0.10 mg/L, reflecting the toxicity of colchicine at higher concentrations, which is supported by studies indicating that excessive colchicine exposure can reduce plant survival (Liu et al., 2007; Gabriella Marry Ayu, 2019). The morphological traits of the garlic plantlets also varied with colchicine concentration,

Table 4: LC_{50} analysis of colchicine on garlic tissue

Lethal concentration	*Model*	*Coefficient*	*Concentration*
0.10	$Y = -18.4851 + \frac{8.9598 + 18.4851}{1 + \left(\frac{X}{0.4241}\right)^{0.6142}}$	A= 8.9598 B=18.4851 C=0.6142	0.4241

with dark green (DG) and thick leaves (TL) observed at lower concentrations, and reduced growth (RD) and curved leaves (CL) at higher concentrations. These morphological changes align with previous research suggesting that colchicine-induced polyploidy often results in altered plant morphology, especially at higher doses (Eed et al., 2010; Remya et al., 2019).

Conclusion

Colchicine has proven effective for inducing polyploidy in garlic, with optimal concentrations between 0.04–0.06 mg/L balancing high induction rates and plantlet viability. Concentrations beyond this range significantly increased mortality and morphological abnormalities, underscoring the need for precise dosage control. The LC_{50} was estimated between 0.03–0.05 mg/L. In this study, 0.05 mg/L colchicine yielded the highest survival among treated calli. Shoot regeneration was most successful with 3 mg/L BAP and 0.25 mg/L TDZ, supporting efficient *in-vitro* multiplication. The resulting autotetraploid lines demonstrated potential for enhanced genetic diversity in garlic—a crop traditionally limited by vegetative propagation and low allelic variation. These findings contribute to the development of genetically improved garlic cultivars through targeted chromosome engineering.

References

Amelia, Z. R., Supriyanto, & Wulandari, A. S. (2020). Effect of 6-BAP application on shoot production of Melaleuca alternifolia seedlings. IOP Conference Series: Earth and Environmental Science, 528(1). https://doi.org/10.1088/1755-1315/528/1/012063

Ayesha Manzoor, T. A. (2019). Studies on Colchicine Induced Chromosome Doubling for Enhancement of Quality Traits in Ornamental Plants. Plants (Basel), *8*(7), 194.

Ayu, G. M. (2019). Effect of concentration and duration of colchicine treatment to garlic (Allium sativum L.) cv. Doulu. International Journal of Scientific & Technology Research, 8(6), 173–175.

Bernard, F. (2012). Treatment of licorice seeds with colchicine: Changes in seedling DNA levels and anthocyanin and glycyrrhizic acid contents. Natural Product Communications, 7(11), 1457–1460.

Block, E. (2010). Garlic and other Alliums: The lore and the science. Royal Society of Chemistry. https://doi.org/10.1039/9781849732129

Bridge, J. A. (2008). Advantages and limitations of cytogenetic, molecular cytogenetic, and molecular diagnostic testing in mesenchymal neoplasms. Journal of Orthopaedic Science, 13(3), 273–282. https://doi.org/10.1007/s00776-007-1215-1

Caperta M Delgado, F. R. (2006). Colchicine-induced polyploidization depends on tubulin polymerization in c-metaphase cells. Protoplasma, *227*(2-4), 147-53.

Chen, R., Song, W., Li, X., & An, Z. (1994). Chromosome G-banding in plants by inducing with trypsin and urea. *Cell Research*, *4*(1), 79–87. https://doi.org/10.1038/cr.1994.8

Chiareli, A. B., Shafer, D., & Sarti, M. (1972). Chromosome banding with trypsin. Bolletino Di Zoologia, *39*(1), 89–91. https://doi.org/10.1080/11250007209429179

Catalano, C., Di Guardo, M., Distefano, G., Caruso, M., Nicolosi, E., Deng, Z., . . . La Malfa, S. (2021). Biotechnological Approaches for Genetic Improvement of Lemon (Citrus limon (L.) Burm. f.) against Mal Secco Disease. Plants, *10*(2), 1002. doi:https://doi.org/10.3390/plants10051002

Gabriella Marry Ayu, E. I. (2019). Effect Of Concentration And Duration Of Colchicine Treatment To Garlic (Allium SativumL.) Cv. Doulu. International Journal of Scientific & Technology Research, *8*(6), 173-75.

Eed, A. M., Begum, H., & Sivaramakrishnan, S. (2010). Rapid Protocol for *in-vitro* Multiplication of Citrus limonia Osbeck Rootstock, IJPDB,Golbal science book, 5-1, 78-82

Erland, L. A. E., Giebelhaus, R. T., Victor, J. M. R., Murch, S. J., & Saxena, P. K. (2020). The Morphoregulatory Role of Thidiazuron : Metabolomics-Guided Hypothesis Generation for Mechanisms of Activity. *Ix*.

Gantait, S., Mandal, N., Bhattacharyya, S., & Das, P. K. (2011). Induction and identification of tetraploids using in-vitro colchicine treatment of Gerbera jamesonii Bolus cv. Sciella. Plant Cell, Tissue and Organ Culture, 106(3), 485–493. https://doi.org/10.1007/s11240-011-9947-1

Ghaderi, N., & Jafari, M. (2014). Efficient plant regeneration, genetic fidelity and high-level accumulation of two pharmaceutical compounds in regenerated plants of Valeriana officinalis L. South African Journal of Botany, *92*, 19–27. https://doi.org/10.1016/j.sajb.2014.01.010

Leyla Bayan, P. H. (2014). Garlic: a review of potential therapeutic effects. *Avicenna J Phytomed, 4*(1), 50-24..

Lawande, K. E., Khar, A., Mahajan, V., Srinivas, P. S., Sankar, V., & Singh, R. P. (2009). Onion and garlic research in India Onion and garlic research in India, J. Hortl. Sci.Vol. 4 (2): 91-119

Lahoty, B. R., Patel, D. K., Reddy, M. P., & Maurya, K. R. (2013). Micropropagation and field evaluation of elite sugarcane genotypes. *Sugar Tech*, 15(1), 15–20. https://doi.org/10.1007/s12355-012-0183-4

Liu, G., Li, Z., & Bao, M. (2007). Colchicine-induced chromosome doubling in Platanus acerifolia and its effect on plant morphology. *Euphytica*, 157(1–2), 145–154. https://doi.org/10.1007/s10681-007-9406-6

Raizer, M. D. M., Quisen, R. C., Valente, M. S. F., Lopes, R., & Lopes, M. T. G. (2019). Morphological and stomatal characterization of Heliconia Chartacea var. sexy pink induced polyploidy. *Bioscience Journal*, *35*(1), 222–235. https://doi.org/10.14393/BJ-v35n1a2019-41748

Rego, M. E. R., Bruckner, C. H., Finger, F. L., & Otoni, W. C. (2011). *In-vitro* induction of autotetraploids from diploid yellow passion fruit mediated by colchicine and oryzalin. *Plant Cell, Tissue and Organ Culture*, 107(3), 451–459. https://doi.org/10.1007/s11240-011-9995-6

Remya, R. S., Hariharan, S., Keerthi, V., & Gopakumar, C. (2019). Preprocessing G-banded metaphase: towards the design of automated karyotyping. *SN Applied Sciences*, *1*(12), 1–8. https://doi.org/10.1007/s42452-019-1754-z

Robin, J. D., Ludlow, A. T., La Ranger, R., Wright, W. E., & Shay, J. W. (2016). Comparison of DNA quantification methods for next generation sequencing. *Scientific Reports*, *6*, 1–10. https://doi.org/10.1038/srep24067

Roslim, D. I. (2015). Lethal Dose 50 (LD 50) of mungbean (Vigna radiata L. wilczek)cultivar Kampar. *Sabrao Journal Of Breeding and Geentics, 47*(04), 510-516.

Shukre, V.M., Tahakik, R., Kumar, K.G. *et al.* (2024) *In-vitro* Screening of Molecular Diversity Among Sorghums (*Sorghum bicolor* (L.) Landraces in Marathwada Region by Molecular Markers. *Appl Biochem Biotechnol* **196**, 6585–6594. https://doi.org/10.1007/s12010-023-04724-2

Suwal, M. M., Lamichhane, J., & Gauchan, D. P. (2020). Regeneration Technique of Bamboo Species through Nodal Segments: A Review. *Nepal Journal of Biotechnology*, *8*(1), 54–68. https://doi.org/10.3126/njb.v8i1.30209

Tahakik, R. R., Deshmukh, A. G., Moharil, M. P., & Shukre, V. M. (2024). Transitioning from the Green Revolution to the Gene Revolution: Strengthening nutritional security using climate-resilient traditional crops. *Bulletin of the National Research Centre, 48*, 123. https://doi.org/10.1186/s42269-024-01281-4

Tahakik, R. R., Shukre, V., Giram, P., & Jadhao, V. (2023). Colchicine-induced polyploidy induction in garlic (*Allium sativum* L.) and its effect on the mortality of *in-vitro* propagated plants. *Annals of Experimental Biology*, 11(3), 75–83.

Tahakik, R. R., Nilesh, M., & Arsode, P. (2022). Effect of different concentrations of BAP and constant NAA on tissue-cultured regeneration of manga bamboo (*Dendrocalamus stocksii*). *Journal of Biotechnology and Crop Science, 10*(15), 176-182.

Trojak-Goluch, A., & Skomra, U. (2013). Artificially induced polyploidization in Humulus lupulus L. and its effect on morphological and chemical traits. *Breeding Science*, *63*(4), 393–399. https://doi.org/10.1270/jsbbs.63.393

Winnepenninckx, B., Backeljau, T., & De Wachter, R. (1993). Extraction of high molecular weight DNA from molluscs. *Trends in Genetics*, *9*(12), 407. https://doi.org/10.1016/0168-9525(93)90102-N

Yi, S., Jin, W., Yuan, Y., & Fang, Y. (2018). An Optimized CTAB Method for Genomic DNA Extraction from Freshly-picked Pinnae of Fern, Adiantum capillus-veneris L. *Bio-Protocol*, *8*(13), 8–13. https://doi.org/10.21769/bioprotoc.2906.

सारांश

जैव विविधता फसल सुधार का आधार है, क्योंकि इससे रोग-प्रतिरोधक क्षमता बढ़ती है, उत्पादन में स्थिरता आती है और नई–नई वांछित विशेषताएँ विकसित होती हैं। चूँकि लहसुन मुख्यतः अलैंगिक प्रजनन के माध्यम से विकसित होती है, इसमें आनुवंशिक विविधता कम होती है, जिसके कारण यह रोगों और कीटों के प्रति संवेदनशील हो जाती है, वांछित लक्षणों की कमी रहती है और पर्यावरणीय अनुकूलन क्षमता सीमित होती है। इस अध्ययन में आनुवंशिक विविधता बढ़ाने के लिए ऊतक-संस्कृति द्वारा गुणसूत्र अभियांत्रिकी के माध्यम से कोल्चिसिन उपचार द्वारा पॉलीप्लोइडी प्रेरित की गई। विभिन्न सांद्रता पर बीएपी (3 मि.ग्रा./ली.) एवं टीडीजेड (0.25 मि.ग्रा./ली.) के प्रयोग से शूट पुनरुत्पादन को अनुकूलित किया गया, जहाँ इन स्तरों पर सर्वाधिक वृद्धि देखी गई। 21 दिनों के कॉलस को उपसंस्कृत करके दूसरी पीढ़ी में विभिन्न कोल्चिसिन सांद्रताओं (0.03–0.75 मि.ग्रा./ली.) पर परीक्षण किया गया। परिणामों से ज्ञात हुआ कि 0.05 मि.ग्रा./ली. कोल्चिसिन पर सर्वाधिक जीवितता प्राप्त हुई, जबकि उच्चतम 0.75 मि.ग्रा./ली. पर विषाक्तता के कारण डुप्लिकेशन दर न्यूनतम रही। कोल्चिसिन का LC_{50} 0.03–0.05 मि.ग्रा./ली. के बीच पाया गया। प्रेरित ऑटोटेट्राप्लॉइड पौधों ने आनुवंशिक विविधता में वृद्धि की स्पष्ट संभावनाएँ दिखाईं। इस अध्ययन ने कोल्चिसिन-प्रेरित प्लॉयडी के लिए न्यूनतम मृत्युदर के साथ आदर्श खुराक निर्धारित की, जिससे लहसुन में उच्च आनुवंशिक सुधार की राह खुलेगी।