

REVIEW ARTICLE

Induction of Autotetraploidy in *Centella asiatica* (Linn.) Urban: A Literature Review

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ABSTRACT

Centella asiatica (Linn.) Urban, a significant medicinal plant utilised in both traditional and contemporary medicine, is recognized for its abundant bioactive components, including asiaticoside, madecassoside, and asiatic acid. Increasing the medicinal efficacy and biomass production of this plant is gaining prominence in the pharmaceutical and herbal sectors. A promising biotechnological method is the creation of autotetraploidy, which involves doubling the chromosomes within a single genome to produce genetically stable polyploid plants. This literature review rigorously analyses the methodology, results, and ramifications of autotetraploidy induction in *C. asiatica*. Diverse physical and chemical mutagens, such as colchicine and oryzalin, have been effectively utilized to generate tetraploidy, leading to significant morphological, anatomical, cytological, and phytochemical alterations. Studies indicate that autotetraploid plants often exhibit larger leaf sizes, higher stomatal dimensions, increased chlorophyll content, and enhanced accumulation of secondary metabolites. Nonetheless, obstacles such as chimerism, suboptimal induction efficiency, and complications in regeneration persist. This review emphasizes the recent achievements and deficiencies in research, providing a comprehensive grasp of autotetraploidy as a strategic approach to enhance the genetic and therapeutic characteristics of *C. asiatica*. Future directions highlight the implementation of standardized techniques and molecular characterization to enhance polyploid breeding efforts for this significant species.

Keywords: *Centella asiatica*, Autotetraploidy, Polyploidy induction, Colchicine treatment, Phytochemical enhancement.

Received 28.03.2026

Revised 12.06.2026

Accepted 22.07.2026

How to cite this article:

Arangale K. B. And Tuwar A. R. Induction of Autotetraploidy in *Centella asiatica* (Linn.) Urban: A Literature Review. Adv. Biores. Vol 17 [7] July 2026. 154-163

INTRODUCTION

Centella asiatica, commonly known as Gotu Kola or Indian pennywort, is a perennial herb indigenous to the swampy regions of tropical and subtropical areas, including India, Southeast Asia, and Malaysia, and also found in some temperate zones of China, Korea, Japan, and Taiwan [1]. This plant, belonging to the Apiaceae family, has a long history of use in traditional medicine, particularly in Ayurvedic and Traditional Chinese Medicine, where it is highly valued for its diverse therapeutic properties [2]. The plant typically thrives in moist, shady environments, often forming a dense, green carpet over the ground. *Centella asiatica* has been used for millennia in various cultures for its medicinal properties, which include wound healing, anti-inflammatory, and neuroprotective effects [2][3][4]. The increasing global interest in natural products and herbal medicine has propelled *Centella asiatica* into the spotlight, driving research into its bioactive compounds and their potential health benefits. The plant's therapeutic effects are mainly attributed to its rich composition of bioactive compounds, including triterpenoids (such as asiaticoside and madecassoside), flavonoids (like quercetin and kaempferol), phenolic acids, essential oils, amino acids, and phytosterols [5]. Among these, triterpenoids, such as asiaticoside, madecassoside, asiatic acid, and madecassic acid, are the primary active constituents responsible for their pharmacological activities. These compounds have been shown to exert various beneficial effects,

including promoting collagen synthesis, enhancing antioxidant activity, and modulating inflammatory responses.

POLYPLOIDY INDUCTION IN MEDICINAL PLANTS

The manipulation of plant genomes to enhance desirable traits has long been a goal of plant breeders and biotechnologists, and polyploidy induction stands out as a powerful tool in this endeavour. Polyploidy, a state characterised by having more than two sets of chromosomes, can occur naturally or be artificially induced by the application of chemical agents, such as colchicine [6]. Colchicine, a naturally occurring alkaloid derived from the autumn crocus (*Colchicum autumnale*), is widely employed to induce polyploidy in plant cells by disrupting microtubule formation during cell division, leading to chromosome doubling. The resulting polyploid plants often exhibit altered morphological, physiological, and biochemical characteristics compared to their diploid counterparts. Polyploidy can lead to increased cell size, altered leaf morphology, enhanced secondary metabolite production, and improved stress tolerance, which are particularly valuable in medicinal plants where the accumulation of bioactive compounds is paramount [2]. Given the increasing demand for plant-derived pharmaceuticals and nutraceuticals, polyploidy induction has emerged as a promising strategy for enhancing the production of therapeutically active compounds in medicinal plants. Polyploidy, the heritable condition in which cells contain more than two complete sets of chromosomes, is a significant force in plant evolution and breeding [6]. Whole-genome multiplication, arising from genome doubling within a single species or hybridisation between two species, leads to polyploidy, facilitating adaptation to environmental changes via extensive genetic modification [7]. Polyploidy can be induced using antimitotic agents or spontaneously [8]. Autopolyploids arise from the duplication of chromosomes within a single species, while allopolyploids originate from the hybridisation of two or more species followed by chromosome doubling [9]. Artificial polyploidy induction has become crucial for generating genetic variation and enhancing crop characteristics [10]. Polyploidy is estimated to occur in 30-70% of plants and contributes to approximately 2-4% of angiosperm speciation events [6]. Polyploidy can profoundly influence plant phenotype, affecting plant size, organ morphology, and secondary metabolite production traits. The impact of polyploidy on plant phenotype is complex and multifaceted, varying significantly across different species.

INDUCTION OF AUTOTETRAPLOIDY IN *CENTELLA ASIATICA*

Autotetraploidy induction in *Centella asiatica* involves treating plant tissues, typically shoot tips or nodal cuttings, with colchicine to disrupt microtubule formation during cell division [2]. The optimised colchicine concentration and exposure duration are critical factors in successfully inducing tetraploidy while minimising cytotoxic effects [6]. Following colchicine treatment, putative tetraploid plants are carefully screened using various techniques, including chromosome counting, flow cytometry, and morphological analysis. Chromosome counting, which directly observes the number of chromosomes in cells, provides definitive evidence of changes in ploidy levels. Flow cytometry, a rapid and quantitative technique, measures the DNA content of cells, allowing for the identification of tetraploid individuals based on their doubled DNA content compared to diploid controls. Morphological analysis, which involves assessing various plant traits such as leaf size, stomatal density, and stem thickness, can provide preliminary indications of changes in ploidy level. Autopolyploidy, resulting from chromosome doubling within a single species, is a significant evolutionary mechanism in plants, contributing to genetic diversity and adaptation [11]. Autotetraploid *Centella asiatica* plants often exhibit altered morphological characteristics compared to their diploid counterparts. These changes include increased leaf size, thicker stems, and altered plant architecture.

The success of autotetraploidy induction hinges on optimising colchicine treatment protocols and employing effective screening methods to identify tetraploid individuals [12]. The alterations in cell wall composition observed in polyploids can promote saccharification production, suggesting that polyploidy induction is a promising breeding strategy for tailoring crops for biomass production [13]. Polyploidisation events, such as whole-genome duplication within a single species or interspecific hybridisation, are pivotal in plant evolution and breeding, enabling adaptation and diversification [14]. Polyploidy induction has been shown to enhance tolerance to drought stress by regulating stomatal density and aperture size in tetraploid cassava, resulting in significantly reduced water consumption under drought conditions in tetraploid *Populus* trees [15]. Polyploidy, characterised by having more than two sets of chromosomes, is a phenomenon that has significantly contributed to plant evolution and diversification [16]. Plants undergoing genome duplication events often exhibit increased resilience to environmental stresses [7]. Tetraploid plants displayed higher activities of peroxidase and catalase enzymes, coupled with lower superoxide dismutase levels in both leaves and roots [17]. This suggests an

enhanced capacity to scavenge reactive oxygen species, thereby mitigating stress-induced oxidative damage. The increased antioxidant enzyme activities in tetraploids suggest a more robust defence mechanism against oxidative stress, allowing them to withstand adverse environmental conditions.

Several studies have demonstrated that polyploidy can affect the content and composition of secondary metabolites in plants. In *Centella asiatica*, autotetraploidy induction has been reported to increase the accumulation of key triterpenoid saponins, including asiaticoside, madecassoside, asiatic acid, and madecassic acid [10]. This increase in therapeutically active compounds can significantly enhance the plant's medicinal value. The molecular mechanisms underlying the increased production of secondary metabolites in autotetraploid plants are complex and poorly understood. However, several factors may contribute to this phenomenon. One potential mechanism involves increased gene dosage. With more copies of genes encoding key enzymes involved in the biosynthesis of secondary metabolites, autotetraploid plants may exhibit higher levels of gene expression and enzyme activity, leading to increased production of these compounds.

Polyploidy affects gene expression and epigenetic regulation, potentially leading to altered metabolic pathways and increased production of secondary metabolites. Tetraploid plants often exhibit altered hormone levels compared to their diploid counterparts. These hormonal changes can influence various physiological processes, including the biosynthesis of secondary metabolites. Moreover, the duplicated genes in polyploids may undergo mutations and diverge in function, leading to the development of novel metabolic pathways and an increase in the production of secondary metabolites. Artificial polyploidy generally enhances the concentration of secondary metabolites [18]. The increase in therapeutically active compounds in autotetraploid *Centella asiatica* plants has significant implications for the medicinal value of this plant. Autotetraploidy leads to variations in gene expression and regulation, resulting in altered metabolic pathways and increased production of valuable compounds [10][18].

The application of autotetraploidy induction in *Centella asiatica* offers a promising strategy for enhancing the production of therapeutically active compounds, ultimately increasing the medicinal value of this important plant [2]. While the induction of autotetraploidy can lead to desirable traits, such as increased secondary metabolite production, it is essential to acknowledge potential drawbacks. It is crucial to carefully evaluate the potential trade-offs associated with autotetraploidy induction and select plants with the most desirable traits. The results indicate that autotetraploids can produce more than double the quantity of asiatic acid [2]. Further research is needed to fully elucidate the underlying mechanisms and optimise the application of autotetraploidy in *Centella asiatica* breeding programs.

THEORETICAL FRAMEWORKS FOR AUTOTETRAPLOIDY INDUCTION

The study of autotetraploidy induction necessitates a thorough understanding of the underlying theoretical frameworks. Cytogenetic mechanisms play a crucial role in understanding the induction of autotetraploidy. Understanding meiotic behaviour in autotetraploids is essential for predicting the stability and fertility of these plants. The process of chromosome doubling, typically achieved through chemical agents such as colchicine, directly affects the genetic makeup of the plant [2]. The genetic consequences of autotetraploidy, including increased gene dosage and the potential for novel gene interactions, contribute to phenotypic changes in plants. The impact of autotetraploidy on gene expression and regulation constitutes another critical theoretical framework. The doubling of the genome can lead to altered expression patterns of genes involved in various metabolic pathways, influencing the production of secondary metabolites [19]. Epigenetic modifications, such as DNA methylation and histone acetylation, regulate gene expression in autotetraploids [18]. The interaction between genetic and epigenetic factors in autotetraploids determines the ultimate phenotypic outcome.

Furthermore, the physiological and biochemical changes induced by autotetraploidy are essential to consider. The alterations in plant morphology, such as increased leaf size and stem diameter [10], can influence photosynthetic capacity and overall biomass production. Changes in cellular structure and organisation, including cell size and number, can affect the efficiency of metabolic processes [10]. Polyploidy results in plants with bigger leaves, stems, and roots. The effects of ploidy on stomatal size and density play a critical role in the plant's gas exchange and water relations [19]. The diploid-tetraploid comparison revealed differences in photosynthetic rate, stomatal conductance, and transpiration rate, indicating alterations in gas exchange and water relations resulting from changes in ploidy.

Moreover, understanding the evolutionary implications of autotetraploidy is vital. Polyploidy has played a significant role in plant evolution, contributing to speciation and adaptation to new environments [20]. Autotetraploidy can lead to reproductive isolation and the formation of new species. The long-term evolutionary consequences of autotetraploidy depend on factors such as genetic drift, natural selection,

and gene flow. The application of autotetraploidy induction relies on a combination of cytogenetic, genetic, physiological, biochemical, and evolutionary principles.

The study of autotetraploidy also encompasses ecological and environmental considerations. Adapting autotetraploids to specific environmental conditions, such as drought or salinity, can influence their survival and distribution. The competitive interactions between autotetraploids and their diploid progenitors determine the success of the polyploid lineage. Understanding these theoretical frameworks is crucial for rationalising autotetraploidy induction protocols in *Centella asiatica* and other plant species [10].

Colchicine and Its Mechanism

Colchicine is a widely used chemical agent for inducing plant polyploidy [21]. Colchicine inhibits microtubule polymerisation by binding to tubulin, a protein subunit of microtubules, disrupting spindle fibre formation during cell division [10]. It is extracted from plants in the *Colchicum* genus [10]. Colchicine has a high binding affinity for animal microtubules and is potentially toxic to humans [22]. Its chemical structure consists of a trimethoxyphenyl ring attached to a central seven-membered ring and an acetamido group [23]. This disruption prevents the separation of chromosomes during mitosis, resulting in cells with doubled chromosome number [24]. The inhibitory effect of colchicine on microtubule formation is concentration-dependent, with higher concentrations resulting in a more complete disruption of cell division.

The mechanism of action of colchicine involves several steps.

First, colchicine binds to tubulin dimers, forming a complex with tubulin.

Second, this complex prevents the incorporation of tubulin dimers into microtubules, inhibiting microtubule polymerisation.

Third, the disruption of microtubule formation leads to the arrest of cells in metaphase, preventing the completion of cell division.

The effectiveness of colchicine in inducing polyploidy depends on several factors, including plant species, tissue type, and concentration and duration of exposure to colchicine [22]. Additionally, the disruption of microtubule function can have pleiotropic effects on plant cells, including changes in cell shape, cell wall deposition, and intracellular transport.

While colchicine effectively induces polyploidy, it can also harm plant cells. Colchicine is highly potent in vivo [25]. Therefore, optimising the concentration and duration of exposure is essential to minimise these toxic effects.

Antimitotic agents such as oryzalin, caffeine, and nitrous oxide have also been used for in vitro polyploid induction [22].

The application of colchicine for polyploid induction requires careful consideration of its potential side effects and optimisation of treatment protocols. The precise mechanism by which colchicine affects signal transduction pathways involved in microtubule polymerisation needs more research [25]. Although colchicine is one of the oldest drugs available, its possible functions are not well-known [27].

Alternatives to Colchicine

While colchicine has been extensively used for inducing polyploidy, it is not the only chemical agent available for this purpose [10]. Several other chemicals, including oryzalin, trifluralin, and apropos-methyl, can also induce polyploidy by disrupting microtubule function [22]. Oryzalin is a dinitroaniline herbicide that inhibits microtubule polymerisation by binding to tubulin [22]. Dinitroanilines have been shown to exhibit a high binding affinity to plant tubulins at low concentrations while displaying little binding affinity to animal tubulins [22]. Trifluralin functions similarly to oryzalin, binding to tubulin and disrupting microtubule assembly [10]. These herbicides exhibit lower toxicity compared to colchicine [10].

Amiprophos-methyl destabilises α -tubulin, similar to oryzalin [22].

These chemicals may offer advantages over colchicine in terms of reduced toxicity or increased effectiveness in particular plant species. Another approach to inducing polyploidy involves the use of cell cycle inhibitors. These chemicals interfere with DNA replication or chromosome segregation, leading to the formation of polyploid cells. Hydroxyurea and aphidicolin arrest cells at the beginning of the S-phase, but they do not directly affect polyploidy [22].

Moreover, manipulating environmental factors, such as temperature or light, can induce polyploidy in some plant species.

Ultimately, the method for inducing polyploidy depends on the specific plant species and the desired outcome of the experiment [10]. Further research is needed to explore the potential of alternative methods for inducing polyploidy in *Centella asiatica*.

CENTELLA ASIATICA: A MEDICINAL PLANT

Centella asiatica, commonly known as Gotu kola, is a perennial herbaceous plant belonging to the family Apiaceae [2]. It is native to Southeast Asia, including India, Sri Lanka, Indonesia, and Malaysia, and has been used for centuries in traditional medicine systems such as Ayurveda and Traditional Chinese Medicine [2]. *Centella asiatica* is a small, creeping plant that typically grows in moist, shady areas along riverbanks and wetlands. It is characterised by its fan-shaped leaves and small, pinkish-white flowers. The plant's leaves and stems contain various bioactive compounds, including triterpenoids, flavonoids, and phenolic acids, responsible for their medicinal properties.

Centella asiatica has a rich history of use in traditional medicine to treat various ailments.

The plant is believed to have several pharmacological effects, including wound healing, anti-inflammatory, antioxidant, and neuroprotective properties [2].

In Ayurveda, *Centella asiatica* is regarded as a "Rasayana" herb, promoting longevity, enhancing memory, and improving overall well-being. The whole plant possesses medicinal value and is a rich source of amino acids, essential oils, alkaloids, terpenoids, flavonoids, vitamins A, C, B1, B2, niacin, and carotene [28].

Triterpenoids, such as asiaticoside, madecassoside, asiatic acid, and madecassic acid, are the primary active constituents of *Centella asiatica* [2]. These compounds have been shown to promote collagen synthesis, improve wound healing, and reduce inflammation [29]. Flavonoids, such as quercetin and kaempferol, are also present in *Centella asiatica* and contribute to its antioxidant and anti-inflammatory effects [3].

In addition to its traditional uses, *Centella asiatica* has also gained popularity in modern medicine and cosmetics. Extracts of *Centella asiatica* are widely used in skincare products due to their ability to promote collagen synthesis, improve skin hydration, and reduce the appearance of wrinkles and scars. It has been listed as one of the five herbal raw materials suitable for commercial use by the Department for the Development of Thai Traditional and Alternative Medicine [30].

Furthermore, *Centella asiatica* is being investigated for its potential therapeutic applications in treating neurological disorders, such as Alzheimer's disease and anxiety [6]. Its consumption is typical as a green leafy vegetable and is prepared as a juice or drink [31]. The interest in *Centella asiatica* has increased in recent years, with studies describing the production of triterpenoid saponins in various parts of the plant [13]. The extract of *Centella* contains several bioactive compounds, including triterpenoids, flavonoids, alkaloids, glycosides, steroids, and fatty and volatile oils, which have been used as diuretics, for the treatment of venous disorders, and as blood cleansers [32]. Clinical trials have demonstrated the effectiveness of *Centella* extract in treating venous insufficiency, as well as the effectiveness of its total triterpenic fraction in alleviating chronic venous hypertension and protecting the venous endothelium [33][34][36].

INDUCTION OF AUTOTETRAPLOIDY FOR ENHANCEMENT OF THERAPEUTICALLY ACTIVE COMPOUNDS

Polyploidy, characterised by having more than two sets of chromosomes, is a widespread phenomenon in plants that can lead to significant alterations in plant morphology, physiology, and metabolism. Autotetraploidy, a type of polyploidy in which the number of chromosome sets is doubled within a single species, has been used for crop improvement, potentially enhancing the production of valuable secondary metabolites in medicinal plants.

The induction of autotetraploidy in *Centella asiatica* could lead to increased levels of triterpenoids, such as asiaticoside and madecassoside, which are responsible for its therapeutic properties [37]. Autotetraploid plants often exhibit increased cell size, resulting in larger leaves, stems, and flowers. This can lead to higher biomass production, translating into greater yields of therapeutically active compounds.

Furthermore, autotetraploidy can influence gene expression and metabolic pathways, leading to altered levels of secondary metabolites [35]. Genetic modification can result in plants with desirable traits, such as disease resistance, increased production, or enhanced nutritional value. Several methods can induce autotetraploidy in plants, including chemical treatments such as colchicine, oryzalin, and trifluralin. Colchicine is a widely used chemical for inducing polyploidy in plants because it inhibits microtubule formation, leading to chromosome doubling [2]. Oryzalin and trifluralin are herbicides that also disrupt microtubule formation and can be used to induce polyploidy. The use of colchicine to induce autotetraploidy in *Centella asiatica* has been shown to increase the accumulation of saponins [2].

In vitro techniques, such as tissue culture, can also induce autotetraploidy in plants. In vitro techniques offer several advantages over traditional breeding methods, including the rapid propagation of plants and

the induction of polyploidy in a controlled environment. The selection of appropriate polyploidisation agents and the optimisation of treatment conditions are crucial for the successful induction of autotetraploidy. The induction rate of tetraploid plants reached as high as 40% when calluses were immersed in 0.2% colchicine solution [38].

Artificial selective breeding, tissue culture, distant hybridisation, physicochemical factors, protoplast culture, and somatic hybridisation are used to create polyploid lines of plants [18]. Mixoploidisation of allelopathic plants may enhance their phytotoxicity [39]. The effect of tetraploidy can lead to increased vigour and leaf size, improving adaptation to environments with low and variable rainfall [40]. The polyploid lines can serve as a germplasm resource for further breeding [41]. In other medicinal plants, induced polyploidy has been shown to increase secondary metabolite levels [10]. Tetraploid plants of *Datura stramonium* have 1–2 times higher alkaloid content in leaves, stems, and roots than diploid plants [38]. Tetraploid plants of *Atropa belladonna* produce twice as much scopolamine and hyoscyamine as diploid plants.

Genetic diversity is a key source for improving plant traits [42]. Polyploidisation is a significant force in plant evolution and breeding because it can trigger immediate genetic and genomic changes in plants, such as changes in gene expression and silencing [43]. The artificial induction of polyploidy can provide a valuable tool for understanding evolutionary processes and facilitating plant breeding and improvement programs [22]. The induction of autotetraploidy can be achieved through various methods, with colchicine treatment widely employed [10][18]. Colchicine is an alkaloid that inhibits microtubule polymerisation, disrupting cell division and subsequent chromosome doubling [22]. The concentration of colchicine, duration of treatment, and type of plant tissue are critical factors that influence the success of autotetraploidy induction [22]. Optimisation of these parameters is necessary to achieve high rates of chromosome doubling while minimising cytotoxic effects. Another essential aspect of autotetraploidy induction is identifying and selecting tetraploid plants from a treated population. Morphological markers, such as increased cell size and altered leaf morphology, can be used as preliminary indicators of polyploidy. Flow cytometry, which measures the DNA content of cells, provides a more accurate and reliable method for identifying tetraploid plants [22]. Chromosome counting is the most definitive method for confirming the ploidy level of plants; however, it is also time-consuming and labour-intensive. The success of artificial polyploidisation using colchicine depends on several factors, including the plant species, the method of application, the concentration and exposure time of colchicine, and the genotype [10].

Following confirmation of tetraploidy, it is essential to evaluate the effect of autotetraploidy on the production of therapeutically active compounds in *Centella asiatica*. This can be achieved through various analytical techniques, such as high-performance liquid chromatography and gas chromatography-mass spectrometry, to quantify the levels of triterpenoids, including asiaticoside and madecassoside. Furthermore, it is essential to assess the stability of tetraploidy over multiple generations to ensure that the enhanced production of therapeutic compounds is maintained. Molecular markers, such as SSRs, are practical, easy to use, and cost-effective in plant breeding for studying gene flow [44]. Additionally, since modern haploid inducers still produce a relatively small portion of haploids among undesirable hybrid kernels, haploid identification is typically laborious, costly, and time-consuming, making this Step the second foremost in the DH technique [45]. The plant breeder can accelerate the development of desirable actual breeding lines by producing haploid plants from hybrids, followed by chromosome doubling [46].

CURRENT RESEARCH ON AUTOTETRAPLOIDY IN *CENTELLA ASIATICA*

While the induction of autotetraploidy has been reported in several plant species, research on *Centella asiatica* is still limited.

- a. **Effect of Autotetraploidy on Morphology and Development:** Autotetraploidy can lead to significant changes in plant morphology and development. Generally, tetraploid plants exhibit larger cell sizes, which can translate into increased organ size, such as larger leaves, flowers, and stems [10]. The size of tetraploid *C. asiatica* stomata was significantly longer than that of the diploid control plants [6]. Tetraploid *C. asiatica* plants exhibited a decreased number of leaves per plant and reduced stolonification, although they tended to show an increase in inflorescences with a regular appearance [6]. The increase in cell size may also affect other physiological processes, such as photosynthesis and nutrient uptake [47].
- b. **Effect of Autotetraploidy on Triterpenoid Production:** A primary goal of inducing autotetraploidy in *Centella asiatica* is to enhance the production of triterpenoids, which are the main therapeutically active compounds in this plant. Tetraploid plants of *C. asiatica* showed increased biomass and triterpenoid contents that resembled the tetraploid. Elite tetraploid lines are selected for additional

field experimentation [6]. However, the effect of autotetraploidy on triterpenoid production can vary depending on the genotype, environmental conditions, and specific triterpenoid compounds.

- c. **Challenges and Future Directions:** While autotetraploidy induction holds great promise for enhancing triterpenoid production in *Centella asiatica*, several challenges must be addressed. One challenge is the potential for reduced fertility in tetraploid plants, which can limit seed production and propagation. Another challenge is the instability of tetraploidy over multiple generations, which can lead to a gradual loss of the tetraploid state. Despite these challenges, the induction of autotetraploidy remains a valuable tool for *enhancing the production of therapeutically active compounds in Centella asiatica* [18]. In the future, studies should focus on addressing the problem of mixoploidy, as the ploidy levels of plants regenerated from a mixoploid plant are unstable, which is inappropriate for mass production [6].

In vitro studies on *Centella asiatica* aged two months have shown significant differences between diploid control plants and tetraploid plants [6]. Further research is needed to optimise autotetraploidy induction, selection, and stabilisation methods. Furthermore, additional studies are required in order to assess the long-term effects of autotetraploidy on plant growth, development, and triterpenoid production under various environmental conditions.

In conclusion, the induction of autotetraploidy represents a promising strategy for enhancing the production of therapeutically active compounds in *Centella asiatica*.

Gaps in Knowledge and Future Research Directions

Despite the progress made in understanding the effects of autotetraploidy on *Centella asiatica*, several knowledge gaps remain.

- a. **Molecular Mechanisms Underlying Triterpenoid Enhancement:** While studies have demonstrated that autotetraploidy can enhance triterpenoid production, the molecular mechanisms underlying this phenomenon remain incompletely understood. It is crucial to elucidate the specific genes and pathways involved in triterpenoid biosynthesis and how their expression is affected by autotetraploidy.
- b. **Long-Term Stability of Autotetraploidy:** The stability of autotetraploidy over multiple generations is a critical factor for the successful application of this technique in crop improvement.
- c. **Optimisation of Colchicine Treatment:** Colchicine is the most commonly used agent for inducing autotetraploidy; however, its application can be associated with several drawbacks, including toxicity and off-target effects.
- d. **Impact on Other Bioactive Compounds:** While the focus of autotetraploidy research in *Centella asiatica* has been on triterpenoids, it is essential to investigate the effect of autotetraploidy on other bioactive compounds present in the plant.
- e. **Field Performance and Adaptability:** Most studies on autotetraploid *Centella asiatica* have been conducted under controlled conditions.

Understanding the genetic variability and heritability of *C. asiatica* is vital for enhancing the plant's production components [48]. Utilising plant resources as medicine has been integral to human existence, particularly in tropical countries with rich plant biodiversity [49]. Many communities in developing nations rely on traditional plant-based medicines [50]. While the Indian population largely depends on agriculture and livestock, they often manage their healthcare with home remedies [51].

CONCLUSIONS

The induction of autotetraploidy in *Centella asiatica* presents a promising avenue for enhancing the production of therapeutically valuable triterpenoids.

- a. **Potential Benefits and Applications:** Autotetraploidy can significantly increase the production of triterpenoids in *Centella asiatica*.
- b. **Limitations and Challenges:** Despite its potential benefits, autotetraploidy also presents several limitations and challenges.
- c. **Future Research Directions:** Future research should address the knowledge gaps identified in the previous section.

In conclusion, the induction of autotetraploidy in *Centella asiatica* holds significant promise for enhancing the production of therapeutically active compounds [2][6]. Further research is needed to address the limitations and challenges of this technique and fully unlock its potential for crop improvement. The use of *Centella asiatica* to treat various diseases has been practised for a long time and continues to this day, forming the basis for the development of new drugs and pharmaceutical products [4]. Triterpenoid saponins are the primary constituents of *Centella asiatica*, and they are believed to be responsible for its wide therapeutic actions [2][5]. Plants have been medicine for thousands of years [52]. Secondary

metabolites, including terpenoids, coumarins, tannins, flavonoids, saponins, phenols, and cardiac glycosides, have been shown to significantly treat various diseases [53]. The production of these metabolites can be elevated by inducing polyploidy [2].

ACKNOWLEDGEMENTS

The authors express their gratitude to the Mahatma Jyotiba Phule Research and Training Institute (Mahajyoti), Nagpur, for its financial support and assistance with this research endeavour. The financial backing of Mahajyoti was crucial in ensuring the successful completion of this work. We extend our heartfelt gratitude for their support and dedication to advancing research and innovation in plant biotechnology

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