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Review

In Vitro Synthetic Polyploidization in Medicinal and Aromatic Plants for Enhanced Phytochemical Efficacy—A Mini-Review

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Abstract: Medicinal and aromatic plants (MAPs) are well known for their valuable secondary metabolites and diverse phytochemicals responsible for a plethora of medicinal properties such as antimicrobial, antioxidant, anti-inflammatory, anticancerous, and analgesic activities, making them essential for various industries. Therefore, this significant market demand has led to the need to improve the quality and quantity of secondary metabolites and thus develop high-quality commercial products. In this context, polyploidization is considered a sound contemporary approach that produces new genotypes, leading to the overexpression of genes involved in biosynthesizing crucial metabolites. Enhanced natural metabolite production increases the biological activities of plant extracts along with enhanced tolerance against abiotic and biotic stresses to achieve homogeneity. This improvisation in the quality and quantity of plant secondary metabolites can maximize the medicinal value of the plants. Therefore, this mini-review aims to explore the importance of enhancing biological activity in medicinal plants, summarize the progress of synthetic polyploidization as a breeding tool in MAP species, and elucidate how this technique plays an important role in improving medicinal values. This breeding strategy could significantly advance future research and industrial applications by inducing superior genotypes with enhanced genomic complexity and improving traits like increased biomass, stress tolerance, and novel biochemical pathways. So, it can be concluded that in vitro synthetic polyploidization can be an effective tool for promoting the production of more distinctive genotypes with immense medicinal properties for a variety of commercial and pharmaceutical purposes.

Keywords: biological activity; epigenetic regulation; MAP species; polyploidization; secondary metabolites

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1. Introduction

Medicinal and aromatic plants (MAPs) have been extensively utilized throughout millennia to alleviate a wide range of diseases in both conventional and complementary medical systems, as well as in the food and cosmetic sectors for flavouring and fragrance [1]. The extracts and the essential oils from MAPs contain an array of phytochemicals such as alkaloids, flavonoids, terpenoids, and phenolic compounds, which are responsible for performing a plethora of biological activities such as antimicrobial, anti-viral, antioxidant, anti-inflammatory, and anticancer activities [2,3]. For instance, essential oils from *Melissa officinalis* L., *Sideritis cypria* Post, *Origanum dubium* Boiss., *Mentha piperita* L., *Thymus capitatus* (L.) Cav., and *Salvia fruticose* Mill. were found to exhibit antioxidant, antimicrobial, and cytotoxic properties [4]. By 2050, the value of the world's medicinal plant trade is estimated to exceed USD 5 trillion [5]. Industrialists are switching to plant-based products in cosmetics, pharmaceuticals, food packaging, aquaculture, fodder industries, etc. [6–8], as synthetic products lead to the emergence of multidrug resistance microbial strains, high toxicity, and environmental degradation [9,10]. Due to huge market and economic demand, there is an utmost need to improve the quality of plants by enhancing their phytochemical yield, which is naturally low, and their biological activities [11].

To enhance their yield and biological activities, various breeding approaches, such as backcross breeding, mass selection, pure-line selection, precursor feeding, in vitro culture, genome editing, and metabolic engineering, were introduced [12,13]. Then again, these techniques are quite time-consuming, labour-intensive, and expensive. Also, current methods often struggle with limited genetic diversity and stability, whereas synthetic polyploidization overcomes these limitations by expanding the genetic toolkit to create organisms with expanded genomic complexity and greater trait stability [13,14]. Synthetic polyploidization, however, is one of the safest and most ideal contemporary breeding approaches conducted under in vitro and in vivo conditions to induce polyploidy in organisms. This technique involves inducing chromosome doubling in cells or tissues, typically through treatment with antimitotic agents such as colchicine or oryzalin, which disrupt normal cell division. Alternatively, synthetic polyploidization can also be achieved through the fusion of cells from different species or individuals with varying chromosome numbers that generate hybrid cells with an increased number of chromosome sets [14]. Polyploidization in MAPs enhances their physiological, morphological, anatomical, and biochemical traits, etc. [15–18]. However, this is not always the case because gene duplication results are uncertain and can adversely influence plant traits [19]. Hence, selecting the appropriate genotypes and traits (which will be positively influenced) is necessary to overcome this problem [20].

Several studies have been conducted on the morphological, biochemical, and anatomical features of polyploids in MAP species [16,21,22]; however, studies on their enhanced biological activities and elucidating insights are scarce. Hence, it is important to understand the mechanism of polyploidization involved in increasing the production of major plant metabolites and biological activities. This study aims to highlight and explore the immense potential and current status of synthetic polyploidization in MAP species for enhancing plant secondary metabolites and their biological activities, emphasizing their insights for upregulating natural metabolites solely responsible for higher biological activities. Furthermore, it would illustrate the importance of the improvisation of MAP species and endorse breeders to accept artificial polyploidization with an integrated omics-based selection approach as one of the sound breeding techniques facilitating economically important MAP species in the current as well as future pharmaceutical, food, and cosmetic industries.

2. In Vitro Synthetic Polyploidization in MAP Species Improvement: Current Status Focusing on Enhancement of Natural Metabolite Production and Biological Activity

Given the high demand for valuable plant secondary metabolites across industries, synthetic polyploidization emerges as a promising breeding method to boost the phytochemical efficacy of MAPs [23], while aligning with consumer preferences for natural genetic profiles and avoiding concerns about genetically modified organisms [24]. New polyploid lineages in plants emerge through chromosomal doubling in somatic cells and the reunion of unreduced gametes [25]. This can be achieved via in vitro, ex vitro, and in vivo systems. In vitro polyploidization is the most common and preferred method in research and commercial breeding due to its controlled environment, enabling precise application of growth regulators, leading to high chromosome doubling efficiency, low mortality, and minimal mixoploidy [26,27]. However, in vivo polyploidization applies antimitotic agents to intact plants, often resulting in variable outcomes due to less precise control, lower induction rates, and higher mixoploidy. In contrast, ex vitro polyploidization, using methods like foliar spraying, is even less efficient due to chemical evaporation, reduced absorption, and further increased mixoploidy [28,29]. In specific contexts where high consistency and uniformity are critical, in vitro polyploidization is favoured for its superior efficiency in producing stable polyploids with precise, desired phenotypic traits. For instance, a study by Navratilova et al. (2022) [30] reported that the tetraploid genotype of *Ajuga reptans* induced by oryzalin treatment showed increased levels of trans-teupolioside, trans-verbascoside, and 20-hydroxecdysone content than the diploid genotype. These results indicated that synthetic polyploidization can induce better genotypes to enhance substances with potential pharmaceutical and economic applications. Similarly, another study by Priya and Pillai (2023) [21] reported a 160-fold increase in the production of major compound andrographolide in colchiploid calluses than the diploid, which has high economic values due to its pharmacological properties including anticancer, antimicrobial, antiparasitic, choleretic, hypocholesterolemia, anti-inflammatory, antidiabetic, hepatoprotective, immunomodulatory, and cardiovascular activity [31]. Artemisinin, a valuable plant secondary metabolite used as the most effective antimalarial drug, was reported to increase from 39% to 56% in induced tetraploid *Artemisia annua* plants using colchicine. This study also reported the upregulation of FPS, HMGR, and artemisinin metabolite-specific Aldh1 genes related to the artemisinin biosynthetic pathway. These results suggested that synthetic polyploidization positively influenced the key enzymes for the biosynthesis of artemisinin, which resulted in the increased production of this valuable metabolite. The successful induction of polyploid medicinal plants and enhancing plant secondary metabolites through colchicine has been reported in several plants such as *Anoectochilus formosanus* [32], *Cichorium intybus* [33], *Datura stramonium* [34], *Papaver bracteatum* [35], *Salvia miltiorrhiza* [36], *Stachys byzantine* [37], and *Trachyspermum ammi* [38]. Similarly, Bharati et al. (2023) [16] reported the successful induction of polyploid *Mentha spicata* by synthetic polyploidization using oryzalin as an anti-mitotic agent with an increased amount of valuable major compounds such as carvone and limonene compared to their diploid control. Another study by Bharati also reported an increased amount of geranial and neral in oryzalin-induced tetraploid *Melissa officinalis* [17].

Even though bioactivity analysis of polyploid medicinal plants is still in the nascent stage, some studies have characterized induced polyploid medicinal plants focusing on metabolite enhancement and biological activity. A study by Gupta et al. (2024) [3] elucidated that oryzalin-induced *Thymus vulgaris* essential oil showed higher antibacterial, antioxidant, and anti-inflammatory activities along with higher concentrations of thymol and γ-terpinene than diploid essential oil. This study also indicated that a high concentration of thymol content in the tetraploid genotype is mainly responsible for its enhanced biological activity. Similarly, another study on induced tetraploid *Thymus vulgaris* exhibited higher insecticidal activity along with an increased amount of bio-active compounds such as carvacrol, thymol, trans-caryophyllene, γ-terpinene, and 4-cymene than the diploid genotype [39]. Bhuvaneswari et al. (2019) [40] and Mei et al. (2020) [41] also exhibited higher antioxidant activity in colchicine-induced *Citrus limon* and *Echinacea purpurea* with increased secondary metabolites. Additionally, Pansuksan et al. (2014) [42] reported higher antibacterial activity along with 40 unique bio-active compounds in tetraploid *Mitracarpus hirtus* compared to its diploid progenitor. However, elevated concentrations of specific bioactive components in secondary metabolites do not automatically translate to improved biological activity. The effectiveness is primarily driven by the synergistic interactions among these components.

In vitro polyploidization facilitated by synthetic antimitotic agents presents a potentially effective approach for generating polyploid plants with augmented biological traits. However, it is not applicable every time as the outcomes of gene duplication remain skeptical. For that, a genome selection-based predictive accuracy model can be employed to accomplish desirable genotypes through artificial polyploidization. However, the overall findings suggest that artificial polyploidization could be a sustainable approach for improving MAP species by focusing on the enhancement of metabolite production and biological activity. Table 1 summarizes the major attempts of in vitro synthetic polyploidization conducted in MAP species focusing on the enhancement of their secondary metabolites and biological activity.

Table 1. List of major attempts of in vitro synthetic polyploidization in MAP species focusing on enhanced secondary metabolite production and biological activity.

Species Name	Family Name	Antimitotic Agent	Short Description	References
Ajuga reptans	Lamiaceae	Oryzalin	Tetraploid variants showed increased content of 20-hydroxyecdysone along with an increased amount of trans-verbascoside and trans-teupolioside compared to diploids.	[30]
Allium sativum L.	Alliaceae	Colchicine	A 30.7% increase in the pharmaceutically active metabolite allium, which exhibits anti-bacterial, anti-fungal, and anti-atherosclerotic activities, was observed in the polyploids	[43]
Andrographis paniculata (Burm.f.) Wall. ex. Nees.	Acanthaceae	Colchicine	Andrographolide was observed to be increased 160-fold compared to diploids.	[21]
Anoectochilus formosanus Hayata	Orchidaceae	Colchicine	A higher content of total flavonoid and gastrodin was observed in the leaves, stem, and whole plant of the polyploid when compared to diploid.	[32]
Artemisia annua	Asteraceae	Colchicine	Tetraploids produced an increase in levels of artemisinin content from 39% to 56% compared to diploids.	[44]
Bacopa monnieri	Plantaginaceae	Colchicine	Tetraploids exhibited a 2.3-fold increase in total bacoside content compared to diploids.	[45]
Cannabis sativa L.	Cannabaceae	Oryzalin	A 71.5% increase in terpene content was observed in tetraploid along with other compounds like α-humulene, which doubled, and cis-nerolidol. The content of cannabidiol increased by 8.9%, guaiol by 60%, and cannabidivarinic acid by 15.2% in tetraploids. α-bisabolol was also found in teraploid leaves which was absent in diploid.	[46]
Cichorium intybus L.	Asteraceae	Colchicine	Tetraploids showed a 1.9-fold increase in total phenolics content and a 10-fold increase in chlorogenic acid in the leaves.	[33]
Citrus limon (L.) Osbeck	Rutaceae	Colchicine	Limonene and lanceol content increased drastically in tetraploids compared with that in diploids and showed the presence of β-bisabolene that was absent in diploids. The increase in the antioxidant activity of tetraploids was also observed due to the increased limonene and lanceol content.	[40]
Datura stramonium L.	Solanaceae	Colchicine	A 7.25% increase in total alkaloid content was observed. For instance, Hyoscyamine and scopolamine content increased 2.6 and 3.0 times, respectively, in contrast to diploid control.	[34]
Dracocephalum kotschyi Boiss	Lamiaceae	Colchicine	Tetraploid genotypes displayed a 19.37% increase in the production of flavonoids compared to diploids.	[47]
Echinacea purpurea (L.) Moench	Asteraceae	Colchicine	Increased production of cichoric acid, caffeic acid derivatives, and alkamides in polyploids.	[48]
Echinacea purpurea (L.) Moench	Asteraceae	Colchicine	Higher contents of cichoric acid, caffeic acid, chlorogenic acid, caftaric acid, and 1,5-dicaffeoyl quinic acid were observed in tetraploids compared to diploids. An increase in antioxidant activity and total phenolic content was also observed in tetraploids.	[41]
Melissa officinalis L.	Lamiaceae	Oryzalin	A 75% increase in essential oil content was reported, along with an increase in geranial and neral of 11.06% and 9.49%, respectively, in tetraploids compared to diploid.	[17]

Table 1. *Cont.*

Species Name	Family Name	Antimitotic Agent	Short Description	References
Mentha spicata L.	Lamiaceae	Oryzalin	Higher essential oil yield is observed in polyploids, along with a higher concentration of carvone and limonene.	[16]
Mitracarpus hirtus L.	Rubiaceae	Colchicine	Increased antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* with the detection of 9-Octadecyne (2); Stigmast-5-en-3-ol, oleate; and 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z), which were absent in diploids.	[42]
Nigella sativa L.	Ranunculaceae	Colchicine	An increase of 46.3% in thymoquinone was observed in the polyploid variants.	[49]
Papaver bracteatum Lindl	Papaveraceae	Colchicine	Tetraploids exhibited increased benzylisoquinoline alkaloid content. Increased production of noscapine (30.55-fold increase) and thebaine (5.86-fold increase) with increased antioxidant activity in polyploids.	[35]
Salvia miltiorrhiza Bunge	Lamiaceae	Colchicine	Enhanced production of dihydrotanshinone I and total tanshinones in polyploids compared to diploid.	[36]
Stachys byzantina L.	Lamiaceae	Colchicine	An increase in linalool, α-cadinol, cubenol, α-terpineol, and menthone content was reported in polyploids.	[37]
Stevia rebaudiana (Bertoni)	Asteraceae	Colchicine	An approximate 2.4% increase in stevioside production was observed in the polyploid variants.	[50]
Taxus baccata L.	Taxaceae	Colchicine	Tetraploid variety exhibited a 10.8-fold higher taxane content compared to diploids, indicating increased production of the chemotherapeutic drug.	[51]
Tetradenia riparia (Hochst.) Codd	Lamiaceae	Colchicine	An increase in the percentage of fenchone from 25.42% in diploids to 48.4% in tetraploids was observed. Additionally, major bioactive compounds with antifungal properties, such as γ-terpineol (10.16%) and viridiflorol (5.58%), were found exclusively in tetraploids and not in diploids.	[52]
Thymus persicus L.	Lamiaceae	Colchicine	Increased production of betulinic acid (69.73%), oleanolic acid (42.76%), and ursolic acid (140.6%) in their polyploids.	[53]
Thymus vulgaris L.	Lamiaceae	Oryzalin	With an increase of 41.11% in essential oil yield, an enhanced production of thymol and γ-terpinene was observed in polyploids. Similarly, an increase in antibacterial, antioxidant, and anti-inflammatory activities was observed in the polyploid compared to the diploid.	[3]
Thymus vulgaris L.	Lamiaceae	Oryzalin	An increase in the contents of carvacrol, thymol, trans-caryophyllene, γ-terpinene, and 4-cymene was observed in the polyploid. Increased insecticidal activity was observed in polyploids rather than diploids.	[39]
Trachyspermum ammi L.	Apiaceae	Colchicine	Thymol was reported to increase from 49.67% in diploids to 69.2% in tetraploids. The essential oil yield was 2.5 times more in tetraploids than in diploids.	[38]

3. Unveiling the Mechanisms: Insights into In Vitro Artificially Induced Polyploid Plants for Enhanced Phytochemicals and Biological Activities

Often, it is postulated that synthetic polyploidization augments both primary and secondary metabolite production by inducing chromosome doubling, thereby influencing the biological activities of polyploid plants [54]. However, one such report contradicted this notion, revealing that the diploid plants had higher flavonoid and phenolic content, including increased rutin and quercetin levels, which enhanced their anti-proliferative and anti-inflammatory effects compared to tetraploids [19]. Polyploidization impacts genetic composition and gene expression, facilitating the emergence of new regulatory pathways [55]. This leads to enhanced adaptability, expanded geographical niches, and altered community structures in various plant species [56]. Molecular mechanisms involved include transcriptome changes [57], microRNAs [58], alternative splicing [59], histone modifications [60], chromatin remodelling, RNA-binding proteins [61], DNA methylation [62], and N6-methyladenosine RNA methylation [63] contributing to the evolutionary process of polyploids by which polyploidy reshapes gene expression, expands proteome diversity, and alters epigenetic landscapes, leading to the differential regulation of duplicated genes. These modifications enhance genetic and epigenetic plasticity, driving the adaptation, stability, and evolutionary diversification of polyploids. Antimitotic agents involved in synthetic polyploidization like colchicine, oryzalin, trifluralin, and amiprophosmethyl disrupt spindle formation during cell division by binding with tubulin dimers, preventing microtubule formation and chromatid migration leading to chromosome doubling and conversion to higher ploidy levels such as triploids, tetraploids, hexaploids, and octaploids [64].

Gene duplication leads to DNA amplification, increasing the copy number of individual genes. This amplification enhances mRNA expression, leading to the overproduction of key biosynthetic enzymes involved in secondary metabolite synthesis. As a result, enzyme activity is elevated, and metabolic pathways are upregulated, thereby influencing the quantity, composition, and proportions of secondary metabolites [65,66] (Figure 1). According to Lavania (2005) [67], genome duplication causes a decrease in the ratio of the nuclear membrane to chromatins so that more chromatins come into contact with the nuclear membrane, which enhances the genetic activity of the cell and influences the production of secondary metabolites. The duplicated gene copy leads to the production of more RNA molecules and was found to be dominant in the whole genome duplication, which is retained in the polyploids [68]. Comai (2005) [69] also stated that an increase in gene number increased the overall gene expression. Gene duplication leading to increased protein expression, which could ultimately increase the production of target enzymes or metabolites, has also been reported by Dar and Rehman (2017) [70].

Simultaneously, polyploidy can influence different mechanisms that can increase, decrease, or even silence the gene expression that influences plants' physiological and biochemical traits [15]. A study by Hassanzadeh et al. (2020) [71] described that physiological functions or gene expressions were greatly influenced by polyploid induction. Javadian et al. (2017) [72] reported that the expression levels of some key genes encoding specific enzymes involved in the podophyllotoxin biosynthetic pathway were enhanced by increasing the plant ploidy, including phenylalanine ammonia-lyase (PAL), cinnamoyl-CoA reductase (CCR), cinnamyl-alcohol dehydrogenase (CAD), and pinoresinol-lariciresinol reductase (PLR).

As the chromosome number increased, DNA content and enzyme activity per cell were also increased [73]. Talebi et al. (2017) [74] reported increased enzyme activity (CAT and POD) and protein content in the tetraploid *Agastache foeniculum* plants. Enhancement in enzyme activity with increased ploidy level has also been confirmed by some other researchers [15,75]. The emergence of novel compounds in the polyploid genotype is attributed to the derepression of previously silenced or weakly expressed genes, while the absence of specific compounds results from the epigenetic modification of gene expression, leading to the inhibition of expression of previously active genes (Figure 1). A study by Parsons et al. (2019) [46] reported the presence of α-bisabolol in oryzalin-induced *Cannabis sativa*, which was absent in the diploid genotype.

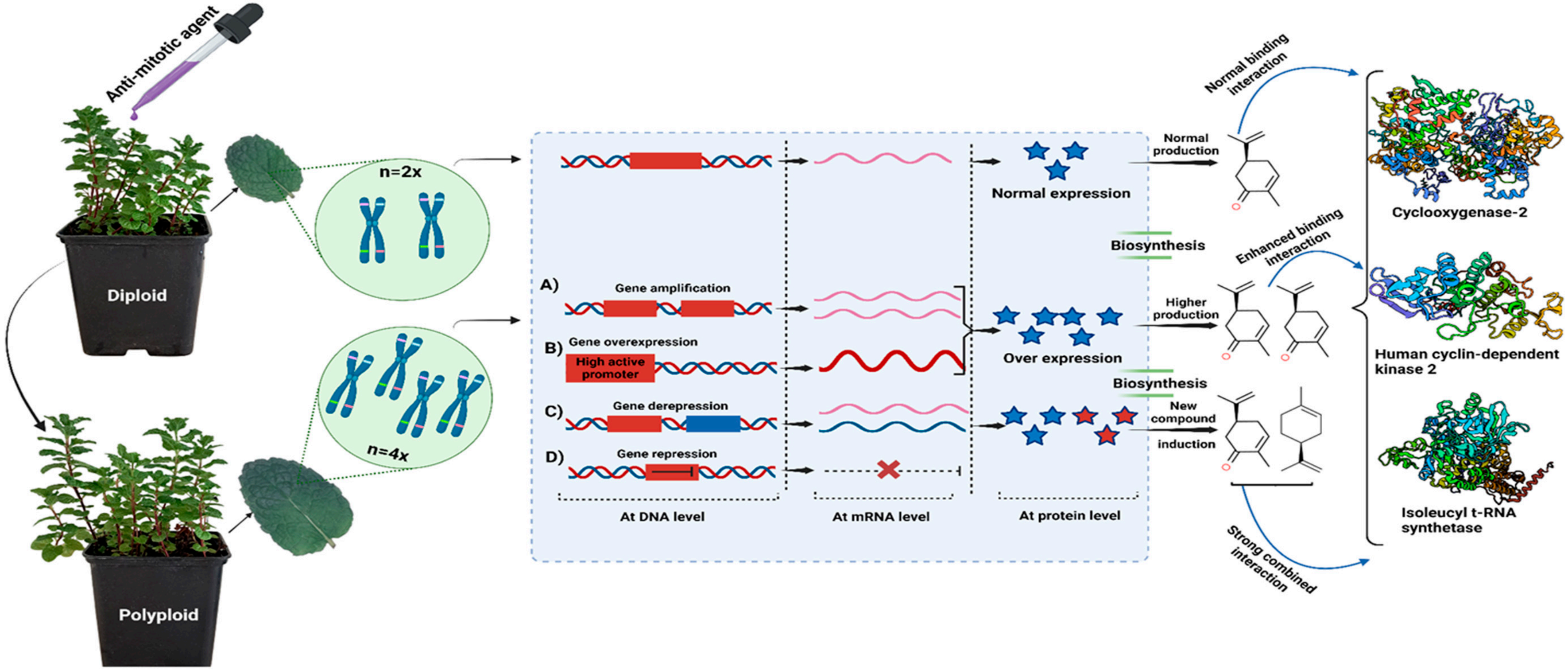

Figure 1. A schematic diagram showing the mechanism of synthetic polyploidization for enhancing major phytochemicals and bio-activity of MAPs. The treatment with antimitotic agents such as oryzalin or colchicine leads to chromosome doubling that results in amplification, overexpression, depression, or repression of genes encoding key biosynthetic enzymes of the plant's major metabolites due to the epigenetic and transcriptional regulation. A. Gene duplication leads to DNA amplification, boosting expression by replicating the coding region of the target gene, resulting in additional copies within the genome. This results in enhanced expression at the mRNA level and contributes to the overexpression of key biosynthetic enzymes. B. Overexpression of the coding region of the desired gene resulting in the over-production of the key enzyme. C. Derepression of previously silenced or weakly expressed gene that encodes another key enzyme for new metabolites. D. Repression or inhibition of gene encoding key biosynthetic enzyme for metabolites that are expressed in the diploid genotype. This over-production of newly expressed key biosynthetic enzymes enhances the production of major metabolites or induces new metabolites in the phytochemical composition of plants that show strong binding or combined interactions with targeted proteins, and it enhances biological activities such as anti-inflammatory, antioxidant, and antibacterial activities.

In medicinal plants, the production of the per unit biomass of secondary metabolites is of immense economic importance [76]. Synthetic polyploidization involves alterations of cellular dynamics that are positively influenced by increased cell size, organelle size and numbers, transcriptome products, net photosynthetic rate, and upraised metabolic pathways [77]. The accumulation of favourable alleles in one organism, along with the induced doubling of chromosome number, further adds to the pharma-chemical productivity, which promotes partitioning of cell energy resources for secondary metabolism and cuts down lengthy pathways via improvised enzyme kinetics [66]. The polyploidization of *Catharanthus roseus* enhances the expression of genes related to alkaloid biosynthesis, boosting vindoline, catharanthine, and vinblastine content in leaves by 130.9%, 188.6%, and 122.6%, respectively, compared to the diploid genotype. These alkaloids, particularly vinblastine and vincristine, are prized for their potent anticancer properties, making *C. roseus* a crucial species for the pharmaceutical industry [78]. On the other hand, in hop tetraploids, the composition of the major chemical compounds used in beer production had an insignificant change with genome doubling compared to diploids where the essential oil content was lower, but the proportion of beneficial components such as humulene, limonene, caryophyllene, and farnesene was higher [79]. Some other studies also reported enhanced metabolite induction in plants with increased ploidy levels, such as a 56% increase in artemisinin content in *Artemisia annua* [44], high triterpenoid levels in *Centella asiatica* [80], and a 50% increase in morphine in *Papaver somniferum* [81].

Metabolites interact with proteins via binding, allosteric regulation, or post-translational modifications, influencing protein function, stability, localization, and cellular processes like signal transduction, gene expression, and metabolism. The specificity and affinity of these interactions determine their impact on protein function and cellular responses. As the concentration of bioactive secondary metabolites increases, their affinity for binding with targeted proteins also rises. This enhanced interaction can significantly boost the biological activity of plant extracts. The overall effect largely depends on the synergistic interplay among these bioactive metabolites [82,83] (Figure 1). Many studies reported that an enhanced phytochemical profile in the polyploid genotype resulted in enhanced biological activities such as antibacterial, antioxidant, or anti-inflammatory activities [3,40,41].

In conclusion, synthetic polyploidization boosts gene copy numbers for key enzymes in biosynthetic pathways, elevating protein expression. This regulation enhances secondary metabolite production and augments biological activities in polyploid plants (Figure 1).

4. Summary and Outlook: Current Challenges and a Way Forward

In this mini-review, we attempted to highlight the potential of synthetic polyploidization as one of the powerful breeding approaches for improving MAP species. In this study, we have already proven its success in different MAP species for enhancing secondary metabolites as well as the biological activity of plants (Table 1). However, induced polyploids may show a reduced level of metabolite production due to unpredictable gene duplication and increased concentrations of certain secondary metabolites do not guarantee enhanced biological activity, as component synergy is crucial [53,84]. Polyploidization in MAPs enhances both agronomic performance and biochemical activities. For example, nearly one-quarter of global chamomile varieties are now colchicine-induced tetraploids, highlighting the effectiveness of this method [85]. In addition, synthetic polyploidization offers substantial benefits for MAP species by enhancing agronomic traits, improving stress tolerance, and increasing genetic diversity, which facilitates the development of novel traits. Simultaneously, it boosts secondary metabolite production, thereby augmenting medicinal and aromatic properties, and promotes hybrid vigour. Furthermore, it bridges reproductive barriers, enabling the creation of new hybrids, fosters novel phenotypes, and extends growth cycles, resulting in crop varieties with specialized and optimized characteristics tailored to specific agricultural or industrial requirements. In summary, the industrial implementation of polyploidization supports sustainable production practices and meets the growing demand for natural bioactive compounds in diverse industries.

Despite so many advantages, this uncertainty and selection of the desired genotype is one of the major challenges for plant breeding. However, the genomic selection process for polyploid screening and integration of multi-omics strategies can overcome this issue. Utilizing multi-omics data, including genomics, transcriptomics, proteomics, and metabolomics, can offer not only valuable insights but also novel approaches to estimating the genomic correlation in polyploids and helping in precision screening for desired traits. However, multi-omics applications for genomic selection can themselves be a challenge because of their complex data output, which makes it difficult to manage and interpret, and also due to the limited data availability of MAP species, specifically transcriptome, genome, reference genome, as well as their integrated approach.

Numerous studies have highlighted the influence of geographical, environmental, agroclimatic, and genetic factors in shaping both the quantity and quality of secondary metabolite production in plants [86]. Synthetic polyploids demonstrate superior adaptability across diverse environments compared to diploids, which can influence the screening of superior polyploid genotypes [87]. So, analyzing the interaction between genotypes and various environmental factors is crucial. Additionally, synthetic polyploidization can affect the selection of desired genotypes through epigenetic instability, disrupted genomic imprinting, and unwanted epistasis. Epigenetic instability can cause variable gene expression, complicating trait selection. Disrupted genomic imprinting may lead to inconsistent trait outcomes, while unwanted epistasis can obscure genotype-phenotype relationships, making it challenging to stabilize and select beneficial traits. These complexities need to be managed to effectively achieve the desired polyploid genotypes. Another potential challenge is that substantial non-additive effects in MAP species during vegetative propagation can influence biochemical traits and stress responses. Hence, understanding additive effects is essential for effective genomic selection. For that, disruptive technologies like CoPhMoRe (corona phase molecular recognition) nanosensors can measure targeting molecules in real time, offering high sensitivity, and non-destructive analysis of plant signalling pathways. This is achieved by creating various corona phases based on the structure of amphiphilic polymers. When combined with machine learning models like random forest, support vector machine, and deep neural network, these tools can accurately capture complex marker–trait relationships and improve marker selection, which can be highly useful for agricultural precision [88]. Despite having several challenges, synthetic polyploidization with genomic selection offers significant potential for enhancing the medicinal properties of plants. However, further investigation is required to optimize ideal polyploidization protocols, elucidate genomic and epigenomic alterations, and perform the comprehensive phenotypic and metabolite profiling of synthetic polyploids. Additionally, research should focus on assessing fertility, reproductive viability, and the environmental and economic implications of polyploidization. Also, integrating the polyploid approach with advanced biotechnological tools, such as CRISPR/Cas9 and marker-assisted selection, is essential. A deeper understanding of these mechanisms could facilitate the development of superior MAP cultivars with enhanced medicinal properties.

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