

## *Review Article*

# **Applications of Artificial Polyploid Induction for Genetic Improvement of Plants**

### **Abstract**

Polyploidy is the presence of more than two homologous sets of chromosomes. It has been one of the strong driving forces for higher plant evolution and also significant transforming tool for plant breeding programs. The review deals with the involvement of natural and artificial polyploids in enhancing traits and diversifying the species of plants. Artificial polyploidy induction (APPI) is mainly done using antimitotic agents like colchicine, trifluralin, and oryzalin. This has been very highly utilized for improving yield and tolerance to biotic and abiotic stresses. Besides, it has been utilized in aesthetic and economic traits in crops, medicinal, and ornamental plants. In many cases, polyploid organisms show better vigor, bigger organs, more biomass, and higher primary and secondary metabolite production. Ornamental plants tend to produce bigger flowers with better fragrance, color, and vase life. The chromosome doubling alters the phytochemical profile and increases the content of desired compounds in medicinal plants. Tissue culture methods for polyploid induction are efficient, and direct (chromosome counting, flow cytometry) and indirect (morphological and anatomical markers) techniques are available for ploidy determination. However, optimizing the type, dosage, and duration of antimitotic agents is critical for successful polyploid breeding. Reduced fertility, a frequent consequence of polyploidy, facilitates the production of seedless fruits, while genome doubling in hybrids restores fertility for further breeding applications. This paper reports recent advances in polyploidy research and its application in creating custom-designed plants with higher marketability, thus emphasizing the economic potential of APPI. Genetic diversity and advanced protocols in polyploidy manipulation provide great opportunities for improving plant yield, quality, and stress tolerance, which will ensure its place as a cornerstone of modern plant breeding.

**Keywords:** Polyploidy, antimitotic agents, colchicine, oryzaline, chromosome doubling, autopolyploid, allopolyploid, flow cytometry

## **Introduction**

Polyploidy, or possessing more than two complete sets of chromosomes per nucleus, is a common feature in the evolution and diversification processes in plants (Leitch & Leitch, 2008). Different studies have indicated varying degrees of polyploidy prevalence in angiosperms, ranging from 30–35% to as high as 70% (Otto & Whitton, 2000; Wood et al., 2009). Using transition models with odd and even numbers of chromosomes, researchers have determined that polyploidy occurs in approximately 2–4% of speciation events in angiosperms and up to 7% in ferns, emphasizing the importance of polyploidy in sympatric speciation (Mayrose et al., 2011). Ancient polyploidy events, such as whole-genome duplications identified through genomic studies, occurred approximately 319 and 192 million years ago, prior to the divergence of seed and flowering plants (Jiao et al., 2011). These findings confirm that the ancestor of all seed plants was a paleopolyploid (Soltis et al., 2015). Over the past century, researchers have analyzed several aspects of polyploidy, including its classification, frequency, origins, and ecological, genetic, and evolutionary consequences. A striking discovery is the ubiquity of polyploid genomes in plants, including primary crops, raising interest in artificial polyploidy as a pathway for crop improvement (Comai, 2005). This review highlights the applications of polyploidy in plant breeding, detailing methods for its induction and detection. Examples of commercially successful polyploid plant underscore the transformative potential of polyploidy in modern plant breeding, offering new avenues for enhancing plant yields and resilience (Chen, 2010).

## **Different classes of polyploids**

Generally, there are two classes of polyploids i.e. autopolyploidy and allopolyploidy. Autopolyploids involve individuals that can be formed inside a single species or between two populations of a specific species (Stebbins, 1947). Allopolyploids are from hybridization products of different species (Clausen et al., 1945). Usually, these two categories have been differentiated from each other in the context of chromosome pairing as well as through inheritance patterns. In autopolyploids, the presence of multiple homologous chromosomes may lead to the formation of multivalents during meiosis. Moreover, due to non-preferential pairing, polysomic inheritance may occur in the offspring which deviates from the typical Mendelian ratio (Ramsey & Schemske, 1998). True and segmental are two sub-classes of allopolyploids. True allopolyploids are produced by hybridization between distantly related

species. During meiosis, chromosomes of related species do not pair, which results in disomic inheritance (Grant, 1981). Segmental allopolyploids arise from hybridization between closely related species with partially differentiated genomes, which can allow different kinds of chromosome pairing, such as univalent, bivalent, and multivalent, thus lying between autopolyploids and true allopolyploids (Stebbins, 1947).

### **Origin of polyploids**

In nature, polyploidy arises through two primary processes: somatic doubling and the formation of unreduced gametes (Harlan & de Wet, 1975). The former process is more commonly utilized in artificial polyploid creation but plays a minor role in natural polyploid formation. The more common method involves the production of unreduced reproductive cells, which can fuse with either another unreduced gamete or a reduced gamete (Bretagnolle & Thompson, 1995). They arise from two types of mechanisms: either through first division restitution (FDR) or second division restitution (SDR), each associated with different consequences in terms of genetic heterozygosity that is critical in the success of polyploid establishment in breeding programs and wild populations (Bretagnolle & Thompson, 1995; Otto & Whitton, 2000).

### **Different methods of polyploidy induction**

Sexual polyploidization and somatic doubling can be employed in plants to produce polyploidy (Ramanna & Jacobsen, 2003). Until the discovery of colchicine in the 1930s, sexual polyploidization was the main method for the production of polyploids (Blakeslee & Avery, 1937). Sexual polyploidization involves the fusion of gametes without diminishing the number of their chromosomes. These methods have great economic value for breeding programs like those of potatoes, alfalfa, yams, roses, and lilies (Ramanna & Jacobsen, 2003). Sexual polyploidization is believed to be favored over somatic polyploidization due to the interaction of increased ploidy levels, such as genome buffering and gene expression, with meiotic recombination, which supports genetic variability and high heterozygosity (Bretagnolle & Thompson, 1995). It is either unilateral or bilateral, where one gamete is unreduced and the other reduced, or both gametes are unreduced (Ramanna & Jacobsen, 2003). The greatest drawback of sexual polyploidization is the relatively low rate of producing unreduced gametes, which averaged 0.56% in non-hybrid species but is significantly higher, at 27.52%, in hybrids (Bretagnolle & Thompson, 1995). The production of gametes can be improved through selective breeding. Research has also shown that treatments with chemicals and methods, such as temperature manipulation, nitrous oxide, and RNA interference, can improve the production

of unreduced gametes and enhance the efficiency of sexual polyploidization (Dewitte et al., 2012). Somatic polyploidization, on the other hand, involves doubling the chromosomes of somatic cells and has been used in a few crops (Ramanna & Jacobsen, 2003). Before the development of colchicine as a potent agent for polyploidy induction, treatments were conducted at extreme temperatures (Blakeslee & Avery, 1937). Colchicine, an alkaloid extracted from the meadow saffron plant, inhibits microtubule polymerization during cell division by binding to tubulins, thereby blocking chromosome movement during anaphase and preventing normal cytokinesis, resulting in polyploid cells (Eigsti & Dustin, 1955). Other antimitotic agents, such as trifluralin and oryzalin, have also been used for polyploidy induction (Morejohn et al., 1987). Initially, colchicine was applied by soaking seeds or twigs in the solution or spraying it on leaves (Blakeslee & Avery, 1937). Later, researchers explored in vitro techniques. Among them, Murashige and Nakano (1966) pioneered the use of tissue culture for polyploidization in tobacco calli. The in vitro method provides better control over the polyploidization process, thereby improving efficiency and consistency. Optimization for other species requires rigorous testing of parameters, including the concentration of antimitotic agents, duration of exposure, and the nature of tissue used (Murashige & Nakano, 1966). Following the induction of polyploidy, confirmation of success is necessary.

#### Different Antimitotic Agents for Polyploid induction in Plants

Induction of polyploid plants via in vitro manipulation is very reliant on antimitotic chemicals that interfere with cell division, with colchicine being the most universal chromosome doubling treatment used conventionally. Colchicine accomplishes this by destabilizing microtubules during metaphase by binding to  $\beta$ -tubulin, inhibiting chromosome migration, at the cost of low affinity for plant microtubules and possible human toxicity (Morejohn et al., 1984). As the science evolved, herbicides, specifically dinitroanilines such as oryzalin and trifluralin, were identified as possible alternatives with high specificity for plant tubulin (Morejohn et al., 1987; Hugdahl and Morejohn, 1993). These compounds bind to  $\alpha$ -tubulin, effectively inhibiting microtubule polymerization at much lower concentrations than colchicine. The efficacy of these chemicals is dependent on a variety of factors such as concentration, exposure time, tissue type, specific plant species, media composition, pH, and presence or absence of plant growth regulators (Ma et al., 2010). Supplementing dinitroanilines, other antimitotic chemicals such as phosphoric amides (amiprofos-methyl), benzamides (propyzamide), and cyanoacrylates have been explored, each exhibiting different mechanisms of cell cycle disruption (Murthy et al., 1994; Cai et al., 2016; Tresch et al., 2005). Nitrous oxide is an interesting alternative, possibly inhibiting microtubule polymerization by tyrosine interactions (Kitamura et al., 2009; Neill et al., 2003). While some such as carbamates and proteasome inhibitors were less effective (Vaughn and Lehnen, 1991;

Planchais et al., 2000), the range of available agents provides researchers with increasingly advanced tools for polyploid induction. The continued exploration and optimization of these antimitotic approaches continue to expand the potential for crop improvement via controlled chromosome manipulation, underlining the dynamic nature of plant biotechnology research (Touchell et al., 2020).

**Table1: Diverse Antimitotic Agents in Plant Polyploid Induction: Species, Tissues, and Methodological Variations**

Antimitotic Agent	Species	Tissue	Concentration	Exposure	Reference
Colchicine	<i>Actinidia chinensis</i>	Petioles	1.25–2.5 mM	4 h	Wu <i>et al.</i> , 2011
	<i>Clivia miniate</i>	Immature embryos	10–50 mM	10–30 days	Wang and Lei, 2012
	<i>Echinacea purpurea</i>	Petioles	300 mM	28 days	Nilanathi <i>et al.</i> , 2009
	<i>Malus x domestica</i>	Axillary buds	10 mM	2 days	Hias <i>et al.</i> , 2017
	<i>Triticum aestivum</i>	Microspore culture	3 mM	24–48 h	Hansen and Andersen, 1998
Oryzalin	<i>Spathiphyllum wallisii</i>	Somatic embryos	10 $\mu$ M	16 h	Eeckhaut <i>et al.</i> , 2004
	<i>Rudbeckia maxima</i>	Shoot apices	60 $\mu$ M	3 days	Palmer <i>et al.</i> , 2008
	<i>Cannabis sativa</i>	Shoot apices	20–60 $\mu$ M	24 h	Parsons <i>et al.</i> , 2019
	<i>Rosa rugosa</i>	Nodal segments	5 $\mu$ M	12 h	Allum <i>et al.</i> , 2007
Trifluralin	<i>Spathiphyllum wallisii</i>	Somatic embryos	10 $\mu$ M	16 h	Eeckhaut <i>et al.</i> , 2004
	<i>Rhododendron</i>	Seedlings	0.3 mM	3 days	Eeckhaut <i>et al.</i> , 2002
	<i>Ranunculus asiaticus</i>	Shoots	2.0 $\mu$ M	6–10 weeks	Dhooghe <i>et al.</i> , 2009a
Amiprophos-methyl (APM)	<i>Raphanus sativus x Brassica oleracea</i>	Shoots	10–30 $\mu$ M	24 h	Niimi <i>et al.</i> , 2015
	<i>Dianthus caryophyllus</i>	Nodal segments	32.9 $\mu$ M	24 h	Nimura <i>et al.</i> , 2006

Pendimethalin	<i>Plumbago auriculata</i>	Shoot apices	800 $\mu\text{M}$	7 days	Jiang <i>et al.</i> , 2020
Ethylfluralin	<i>Citrullus lanatus</i>	Shoot apices	25–100 $\mu\text{M}$	3–9 days	Nasr <i>et al.</i> , 2004
Propyzamide	<i>Vitis davidii</i>	Nodal segments	Not specified	Not specified	Cai <i>et al.</i> , 2016
Nitrous Oxide	Seeds	Not specified	Not specified	Taylor <i>et al.</i> , 1976	
Dinitramine	<i>Citrullus lanatus</i>	Shoot apices	25–100 $\mu\text{M}$	3–9 days	Nasr <i>et al.</i> , 2004
Butralin	<i>Citrullus lanatus</i>	Shoot apices	25–100 $\mu\text{M}$	3–9 days	Nasr <i>et al.</i> , 2004

### Methods for detection of polyploidy

Polyploid plants can be detected using both indirect and direct approaches. Indirect methods, such as inspecting morphological characteristics like stomata size and chloroplast number, are easy to execute but may not always be conclusive (Eigsti & Dustin, 1955). Direct methods include chromosome counting and flow cytometry. Chromosome counting is the most accurate method, although it is time-consuming. Flow cytometry, on the other hand, is quicker and reliable for measuring DNA content and determining the ploidy level of plants, making it effective for screening large numbers of plants in a short time (Doležel *et al.*, 2007).

### Applications of polyploidy in plant breeding

Polyploidy has greatly influenced plant evolution and speciation, often conferring advantages that enhance plant vigor and adaptability to new environments (Soltis & Soltis, 2000). Polyploid plants are frequently more vigorous than their diploid counterparts due to phenomena like transgressive segregation, which results in extreme phenotypes, and increased overall vitality (Rieseberg, Archer, & Wayne, 1999). Polyploid plants might have been better suited to survive dramatic environmental shifts, such as those during the Cretaceous-Tertiary (KT) extinction event (Fawcett, 2009). Some of these advantages include increased phenotypic variation, heterosis, mutational robustness, and changes in reproductive modes, which all contribute to their competitive edge over diploids (Chen, 2010). Because of this, synthetic polyploids develop into a promising area of interest for plant breeding programs (Madlung, 2013). A commonly observed effect of polyploidy is the enhancement of cell size, commonly known as the "gigas" effect (Masterson, 1994). Such a phenomenon can produce large organs in plants, such as roots, leaves, flowers, and fruits. Large cells are not indicative of large total

size for a plant because a cell division rate decrease often confines plant growth as well (Stebbins, 1971). Second, polyploids have slowed plant growth in many species as well as flower blooming often much later in maturity compared to a euploid crop; such tendencies might be preferable during ornamental plant development (Allard, 1960). Whereas in many cases of polyploidy, sterility is commonly manifested, especially in autopolyploids, it does offer advantages when used in crops in which vegetative reproduction is a dominant mode rather than seed reproduction (Ramsey & Schemske, 1998). For example, stress tolerance is among the most observed impacts of polyploidy, including tolerance to drought, nutrient deficiencies, temperature changes, and even pest attacks (Leitch & Leitch, 2008).

Application in breeding relates more to polyploidy's effects on genome buffering and heterozygosity. Such redundancy in genes does protect polyploid plants against deleterious mutations and inbreeding depression, as an excess copy of the genes can hide deleterious alleles (Comai, 2005). Moreover, polyploidy can facilitate functional diversification by allowing duplicated genes to acquire new functions without making critical processes essential, making these genes redundant (Chen, 2007). Heterozygosity is also greatly increased in polyploids, especially in allopolyploids, where hybridization between divergent genomes enhances genetic diversity (Soltis & Soltis, 1999). There exists a correlation in this increase in genetic diversity with vigorosity in crops such as potato, maize, and alfalfa (Sattler, Carvalho, & Clarindo, 2016). Interspecific hybridization produces allopolyploids due to its widespread role in evolution and speciation of plants; in most cases, these hybrids exhibit superior vigor compared to the two diploid contributing parents (Otto & Whitton, 2000). Due to heterosis, hybrid vigor may bring about higher levels of growth and fertility, an increase in biomass, and elevated stress tolerance, playing significant roles in the improvement of crops (Chen, 2010). For instance, the hexaploid bread wheat *Triticum aestivum* is a natural allopolyploid that comes from hybridization events of several different species; it produces a highly productive plant for bread-making purposes (Peng et al., 2011). The two tetraploid cotton species, *Gossypium hirsutum* and *Gossypium barbadense*, have their A and D genomes combined, making them very productive for fiber yield and quality (Paterson et al., 2012). Sterility in hybrids may also be induced to overcome barriers and allow genetic transfer between species at different ploidy levels through induced polyploidy (Ramsey & Schemske, 1998). Successful application of induced polyploidy for restorability of fertility in sterile hybrid plants includes the *Catalpa bignonioides* by *Chilopsis linearis* hybrid, where the polyploidization results in fertile and stable hybrids (Olsen, 2006). Crossing bridges, in which polyploid hybrids are used to transfer

genes between species, has also been successful in crops such as Italian ryegrass and tall fescue (Buckner, 1961). This allows for the introduction of desirable traits into new plant varieties. Polyploidy offers several benefits for plant breeding, including enhanced vigor and stress tolerance, enabling genetic diversification, and facilitating the transfer of beneficial traits between species (Sattler, Carvalho, & Clarindo, 2016).

### **Implications of Induction of Polyploidy into Plant Improvement**

It is reported that approximately 40% of cultivated plant species appear to be polyploids (Ramsey & Schemske, 1998). In natural polyploids, such as wheat (*Triticum aestivum*) and cotton (*Gossypium hirsutum*), polyploidization has led to ecological and agronomic gains, enabling the establishment of polyploid cultivars (Chen, 2010; Soltis et al., 2015). The discovery in the 1930s of colchicine's ability to induce polyploidy sparked a wave of interest in artificial polyploidization of crop species during the 1930–1970 period. Despite initial enthusiasm, most artificially induced polyploids did not meet expectations, with only a few successes, such as sugar beet (*Beta vulgaris*) and red clover (*Trifolium pratense*) (Comai, 2005; Stebbins, 1971). Quality or yield is not always superior in polyploid crops compared to their diploid relatives. The effects of polyploidy vary based on factors such as the plant's initial ploidy, genome structure, reproductive mode, and the organ under consideration (Leitch & Leitch, 2008). For instance, polyploidization may enhance ornamental traits, such as larger and more vibrant flowers, but it can also lead to low fertility in crops that depend on reproductive organs like fruits and seeds. However, in ornamental plants, low fertility is less of a concern, as the primary focus is on aesthetics (Chen, 2010).

Genetic and epigenetic changes induced by polyploidy, including structural chromosome rearrangements, point mutations, and changes in DNA methylation patterns, can influence gene expression and generate new phenotypes (Comai, 2005; Ramsey & Schemske, 1998). These genetic modifications may account for the underperformance of some artificially induced polyploid plants in terms of traits and productivity relative to their diploid counterparts. Nevertheless, these changes also offer opportunities to exploit new genetic variations through breeding strategies (Soltis et al., 2015). A notable example of polyploidization's failure is Raphanobrassica, an early attempt to combine radish (*Raphanus sativus*) roots with cabbage (*Brassica oleracea*) leaves. Although a fertile allopolyploid was created, it exhibited undesirable traits, such as radish-like leaves and cabbage-like roots (Stebbins, 1971). On the other hand, triticale—a synthetic allopolyploid derived from wheat and rye—demonstrates the

success of polyploid crops. Triticale combines rye's hardiness with wheat's yield potential. However, its grains are primarily used for animal feed due to inferior quality for human consumption. Current breeding efforts aim to improve triticale's grain quality, making it more suitable for human diets (Chen, 2010; Soltis et al., 2015).

**Table 2: Few examples of artificially induced polyploidy ornamental and medicinal plants**

Plant Species	Antimitotic agent	Plant Receptor	Polyploidy Induction Efficiency (%)	Changes	Reference
<b>Ornamental Plants</b>					
<i>Gerbera jamesonii</i>	Colchicine	2 weeks old multiple shoots	53.3	Reduced stomata density, wider size, larger flower, longer stalks, broader leaves	Khalili <i>et al.</i> (2019)
<i>Zephyranthes rosea</i>	Ethanol extract of <i>Catharanthus roseus</i>	Tuber	Not specified	Increased root mass, number of broad leaves, stomata density	Slamet <i>et al.</i> (2019)
<i>Impatiens walleriana</i>	Oryzalin	Shoot tip	68.75	Increased leaf area, leaf thickness, ovary width, pollen cell length, stomatal size; decreased plant height, flower number	Ghanbari <i>et al.</i> (2019)
<i>Lilium distichum</i> and <i>Lilium cernuum</i>	Colchicine	Somatic embryos	46.15–57.14	Broader leaves, longer guard cells, larger stomata, higher chlorophyll content	Fu <i>et al.</i> (2019)
<i>Paphiopedilum villosum</i>	Colchicine	Nodal explants	19.88	Increased stomatal size and guard cell size	Huy <i>et al.</i> (2019)
<i>Lilium regale</i>	Colchicine	Bulb scales	27.3	Increased length of stomatal guard cell, lower stomatal number	Jeloudar <i>et al.</i> (2019)

<i>Catharanthus roseus</i>	Colchicine	Leaf	Not specified	Increased lateral branch diameter, leaf dimensions, petal size, flowering duration, root characteristics	Hosseini <i>et al.</i> (2018)
<i>Chrysanthemum carinatum</i>	Colchicine	Apical meristem	2.08	Slower growth, stronger stem, thicker leaves, larger flowers and seeds	Kushwah <i>et al.</i> (2018)
<i>Anemone sylvestris</i>	Oryzalin	Plants	Not specified	Stronger, more vigorous plants with early flowering; increased polyphenols	Zahumenická <i>et al.</i> (2018)
<i>Hedychium coronarium</i>	Colchicine	Thin cell layer (TCL)	Not specified	Increased leaf width/length, plant height, rhizome growth, stomata size, flower size, prolonged flowering	Tu <i>et al.</i> (2018)
<b>Medicinal Plants</b>					
Ajowan	Colchicine	Germinating seeds	11.53	Thymol (19.53% increase)	Noori <i>et al.</i> (2017)
<i>Andrographis paniculata</i>	Colchicine	Internode, seedlings, seeds	40	Andrographolide (28% increase)	Qi-Qing <i>et al.</i> (2018)
Anise ( <i>Agastache foeniculum</i> )	Colchicine, trifluralin, oryzalin	Apical meristem, seeds	16-20	-	Talebi <i>et al.</i> (2017)
Buckwheat ( <i>Fagopyrum tataricum</i> )	Colchicine	Apical meristem	54.55	-	Wang <i>et al.</i> (2017)
<i>Cannabis sativa</i>	Oryzalin	Axillary bud	100	Cannabidiol (9% increase)	Parsons <i>et al.</i> (2019)
<i>Citrus limon</i>	Colchicine	Seeds	36.36	-	Bhuvaneshwari <i>et al.</i> (2020)

Garden thyme ( <i>Thymus vulgaris</i> )	Oryzalin	Nodal segments	7.5	Thymol, carvacrol	Shmeit <i>et al.</i> (2020)
Iranian poppy ( <i>Papaver bracteatum</i> )	Colchicine	One-month-old seedlings	11.44	Thebaine, noscapine	Madani <i>et al.</i> (2019)
Ispaghul ( <i>Plantago ovata</i> )	Colchicine, trifluralin	Terminal bud	20 ± 3.3	-	Sabzehzari <i>et al.</i> (2019b)
<i>Lippia alba</i>	Colchicine	Nodal segments	-	-	Julião <i>et al.</i> (2020)
Roman chamomile ( <i>Chamaemelum nobile</i> )	Colchicine	In vitro induced calli	42.9	-	Tsuro <i>et al.</i> (2016)
<i>Eucalyptus polybractea</i>	Colchicine	Axillary bud	-	1,8-cineole/sabinene	Fernando <i>et al.</i> (2019)
Orchids ( <i>Dendrobium Phalaenopsis</i> )	Colchicine, amiprophos-methyl	Protocorm like bodies	80	Shihunidine-hircinol	Grosso <i>et al.</i> (2018)
<i>Plantago psyllium</i>	Colchicine, trifluralin	Terminal buds	23 & 19	Mucilage	Sabzehzari <i>et al.</i> (2019a)
<i>Tetradenia riparia</i>	Colchicine	Seeds	-	Fenchone	Hannweg <i>et al.</i> (2016)

**Table 3: Few examples of artificially induced polyploid agricultural crops**

Crop	Utility	Type	Ploidy Level	Chromosome Number	References
Tetraploid Rye	Grain	Autopolyploidy	4x	28	Müntzing (1951)
Triticale	Grain	Allopolyploidy	6x, 8x	42, 56	Mergoum and Gómez-Macpherson (2004)

Tetraploid Westerwolds Ryegrass	Forage	Autopolyploidy	4x	28	Armstrong (1981)
Tetraploid Italian Ryegrass	Forage	Autopolyploidy	4x	28	Armstrong (1981)
Tetraploid Red Clover	Forage	Autopolyploidy	4x	28	Levan (1948)
Triploid Sugar Beet	Industrial	Autopolyploidy	3x	27	Kinoshita and Takahashi (1969)
Triploid Cassava 'Sree Harsha'	Tubercle	Autopolyploidy	3x	54	Sreekumari et al. (1999)
Triploid Watermelon	Fruit	Autopolyploidy	3x	33	Crow (1994)

### Commercially viable Artificially induced polyploids

Many induced autopolyploids have become commercially significant in cultivation and trade, serving as prime examples of the practical value of polyploidy in crop improvement. Sugar beet (*Beta vulgaris*) is a crucial crop for sugar production in temperate regions, existing as diploid, triploid, and tetraploid varieties (Smulders et al. 2010). Since the 1970s, triploid (3x=27) varieties—developed by crossing diploid sterile plants with tetraploid pollinators—have gained prominence in Europe and the U.S. These triploids produce larger roots than diploids while retaining the same sugar content, thereby increasing sugar yield per unit area. Moreover, their sterility is an advantageous trait, as only the roots are harvested for commercial purposes (Dabholkar 2006). The first seedless triploid watermelon (*Citrullus vulgaris* Schrad.; 3x = 33) was created in 1939 by treating diploid plants with colchicine and crossing them with diploids to produce triploid offspring (Crow 1994). Though triploid watermelons are sterile and require hand-pollination, they are widely marketed in Israel, Japan, and the U.S., accounting for approximately 10% of the U.S. market (U.S. Department of Agriculture 2013). While their production costs are high due to specialized pollination techniques and expensive seeds, the consumer demand for seedless watermelons justifies these efforts (Boyhan et al., 2000). Colchicine-induced tetraploid red clover (*Trifolium pratense* L.; 4x = 28) varieties have been developed since the 1930s, mainly in Europe. Tetraploids surpass diploids in disease resistance, persistence, and forage yield. For example, the cultivar 'Sally' demonstrates a 72% higher seed yield than diploids, highlighting the advantages of tetraploids. However, challenges such as

low seed production and high seed prices limit their widespread adoption (Boller et al., 2010; Taylor & Quesenberry, 1996). Artificial tetraploid rye (*Secale cereale* L.;  $4x = 28$ ) has been bred since the 1930s, with varieties such as 'Dubbelstal' and 'Tetra Petkus' gaining commercial importance. These tetraploid cultivars are utilized for both grain and forage production in Europe and the U.S (Hancock and Overton 1960). Tetraploids exhibit improved baking properties and stiffer straw than diploids, although they generally yield less (Schlegel, 2006). Polyploid ryegrass, produced through colchicine treatment, is extensively cultivated as forage. Tetraploid ryegrass (*Lolium* spp.;  $4x = 28$ ) varieties offer enhanced drought tolerance, disease resistance, and palatability, making them highly productive in regions like New Zealand. However, the choice between diploid and tetraploid varieties depends on the specific environmental conditions of the region (Nair, 2004; White & Lemus, 2014).

### **Artificial polyploid induction in Vegetable crops**

Induced mainly through colchicine, polyploidy greatly improves the morphological and nutritional characteristics of many vegetable crops. *Trigonella foenum-graecum* plants displayed increased leaf area, seeds, and pods with more potassium, sodium, calcium, and phosphorus contents than in the diploids (Marzougui et al., 2009). Tetraploid *Cucumis melo* had an increased fruit weight, sugar, vitamin C, and total soluble solids (Zhang et al., 2010). Polyploid *Physalis ixocarpa* showed increased vitamin C content but changed the fruit shape in an unfavorable way (Ramírez-Godina et al., 2013). *Capsicum frutescens* was reported to have bigger fruits with a greater capsaicin content (Pliankong et al., 2017). Tetraploid *Petroselinum crispum* also presented leaves and stems of greater sizes (Nasirvand et al., 2018). Tetraploid *Lepidium sativum* and *Raphanus sativus* also presented enlarged leaves, increased stem thickness, elevated vitamin C, and antioxidant activities but presented lower seed germination and altered gene expression in relation to flowering (Aqafarini et al., 2019; Pei et al., 2019).

### **Artificial Polyploid induction in Ornamental plants**

Polyploidy is extensively employed in ornamental plants, leading to larger flowers, extended flower longevity, and richer colors. For example, the tetraploid *Rhododendron minus* 'Epoch' produces larger and more durable flowers than its diploid counterpart (Kehr 1971). Other

ornamental species, such as *Antirrhinum* and *Magnolia*, have also been enhanced through polyploidy, making them commercially more attractive (Tolety and Sane 2011; Parris et al. 2010). Artificial polyploidy induction (APPI) has been widely applied in ornamental plants, thereby improving their genetic diversity and producing novel traits like unusual flower colors, improved fragrance, and enhanced morphological features (Azadi et al., 2016; Zahumenická et al., 2018). New varieties are bred using APPI, which is also derived from wild and medicinal plants to cater to consumer demand (Van Kleunen et al., 2018). For instance, leaves of oryzalin-induced tetraploid lines of *Rhododendron fortunei* were thicker and darker green than those of diploids (Lan et al., 2020). Colchicine treatment of triploid *Pennisetum × advena* resulted in hexaploids that were shorter and flowered later than the original triploids (Yue et al., 2020a). The most significant advantage of polyploid induction is seen in triploids and tetraploids, as these enhance traits such as fragrance, flower structure, and longer flowering periods (Podwyszyńska, 2011; Khalili et al., 2019). Colchicine-induced triploid *Lavandula × intermedia* (lavandin) has potential for oil production (Urwin, 2014), and tetraploid *Chrysanthemum boreale* showed better morphology with larger leaves (Hoang et al., 2020). Ploidy manipulation usually alters traits through gene silencing, gene dosage effects, and epigenetic interactions (Manzoor et al., 2019). Colchicine is the most commonly used antimitotic agent (AMA), effective across various species like *Gerbera jamesonii* and *Lavandula × intermedia* (Tong & Chen, 2012; Urwin, 2014; Khalili et al., 2019). Other AMAs, including oryzalin, have proven successful in inducing polyploidy with improved results in specific species (Esmaeili et al., 2020). Key characteristics such as flower size, organ growth, and flowering time tend to be enhanced as a result of polyploidization in *Hedychium coronarium* and *Chrysanthemum carinatum* as shown by Tu et al., 2018 and Kushwah et al., 2018. Nevertheless, APPI outcomes vary since, for example, *Hyoscyamus muticus* polyploids had smaller organs (Shahriari-Ahmadi et al., 2008). Consequently, APPI remains an essential breeding method in ornamental plant species.

### **Artificial Polyploid induction in Medicinal Plants**

Medicinal plants are of immense importance for their bioactive compounds, which are found to have excellent therapeutic potential (Pradhan et al., 2018). Due to safety, lower side effects, and affordable costs, plant-based medicines are being favored over synthetic drugs. For

instance, synthetic drugs in diabetes and obesity treatment are outperform the herbal remedies in terms of efficacy and affordability (Kooti et al., 2016; Mopuri & Islam, 2017). The increasing demand for medicinal plants requires sustainable production systems to avoid their extinction (Niazian et al., 2017b, 2018). Polyploidy has been utilized in medicinal plants to increase the production of secondary metabolites. For instance, tetraploid chamomile varieties like 'Lutea' and 'Goral' produce over 20% more chamazulene, a valuable compound in essential oils, compared to diploids. This has resulted in the widespread adoption of tetraploid chamomile in medicinal applications ((Gosztola et al. 2006; Das et al., 2011). Artificial polyploidy induction (APPI) is one of the promising breeding techniques for medicinal plants. Colchicine is the most common agent used in this process, but other antimetabolic agents (AMAs) such as oryzalin also produce successful results. For example, oryzalin-induced tetraploid garden thyme (*Thymus vulgaris* L.) contained higher amounts of essential oil, thymol, and carvacrol (Shmeit et al., 2020). Besides, colchicine-induced tetraploids of *Citrus limon* possessed new phytochemical profiles, by the appearance of  $\beta$ -bisabolene and some original components deletion (Bhuvaneshwari et al., 2020). The same happened in other crops, such as ajowan (Noori et al., 2017), and *Lippia alba* (Julião et al., 2020). Gene silencing by induction of polyploidy often changes the chemical profile in the way that creates new components, or removes present ones. For instance,  $\alpha$ -humulene and viridiflorol were documented in tetraploid *Tetradenia riparia*, which are absent in diploids (Hannweg et al., 2016). Moreover, polyploidy increases the tolerance to biotic and abiotic stresses, which can increase secondary metabolite production under stress conditions (Liu et al., 2011; Jaleel et al., 2008; Niazian et al., 2019). These benefits make APPI a useful tool for enhancing the pharmaceutical potential of medicinal plants.

### **Artificial polyploid induction in Fruits and flowers**

Polyploidy has been pivotal in plant improvement through interspecific hybridizations involving different ploidy levels. In apples (*Malus domestica*), triploids outperform diploids with consistent fruit production, larger fruits, higher commercial value, and scab resistance. Tetraploids, though not commercially preferred due to poor fruit quality and low cold resistance, play a crucial role in developing triploids (Sedov et al., 2014). About 10% of apple varieties, like 'Baldwin' and 'Gravenstein,' are spontaneous triploids, and Russia developed eight triploid cultivars through diploid-tetraploid hybridizations (Janick et al., 1996; Sedov et al., 2014). In grapes (*Vitis* spp.), polyploid breeding has produced large-berry and seedless varieties. Autotetraploid grapes, induced via colchicine, exhibit reduced fruitfulness and vigor but help bridge gaps between species such as *V. vinifera* and *V. labrusca*. The allotetraploid

'Kyoho' and seedless triploids like 'Osuzu' exemplify such advancements (Park et al., 2002; Morinaga, 2001). Tulips (*Tulipa* spp.) benefit from triploid and tetraploid forms with enhanced flower size and vigor. Using N<sub>2</sub>O instead of colchicine facilitates polyploidy in bulbous plants, as seen in cultivars 'Rambo' and 'Hunter' (Marasek-Ciolakowska et al., 2012; Eikelboom et al., 2001). Polyploidy also enhances kiwifruit (*Actinidia chinensis*), grapes, and watermelons, offering genetic resources for future breeding programs (Wu et al., 2012; Jaskani et al., 2005).

### **Conclusion and future prospects**

Polyploidy has been extensively studied and is considered a key mechanism for adaptation and speciation in plants. Its significance extends to agriculture, as many important crops are polyploid. This highlights the value of polyploidy for humans, especially in crop improvement. One notable consequence of polyploidy is the "gigas" effect, which leads to increased organ size, benefiting crops of commercial interest. Other phenomena, such as genome buffering, heterozygosity, and hybrid vigour - also known as heterosis, all contribute to the enhanced vigour of polyploid plants in comparison to their diploid counterparts. There are many induced polyploidization techniques which include colchicine-mediated *in vitro* polyploidization as well as sexual polyploidization, facilitated by the fusion of unreduced reproductive cells. Though polyploidy held a very bright promise for scientists, not so many plants resulting from such manipulations are able to make commercial impact. Although this, polyploidy plays a critical role in plant breeding, not only for the "gigas" effect in enhancing traits but also the ability to transfer genes between species or restoration of fertility in sterile hybrids. In the last few decades, a lot of research has been done on polyploidy, but still, many questions are left to be answered, and thus more research is needed. Further understanding of how genomic changes lead to new phenotypes may allow breeders to better exploit polyploidy for crop improvement.

### **DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

### **References**

1. Leitch, I. J., & Leitch, A. R. (2008). Genomic plasticity and the diversity of polyploid plants. *Science*, 320(5875), 481–483.

2. Otto, S. P., & Whitton, J. (2000). Polyploid incidence and evolution. *Annual Review of Genetics*, 34(1), 401–437.
3. Wood, T. E., Takebayashi, N., Barker, M. S., Mayrose, I., Greenspoon, P. B., & Rieseberg, L. H. (2009). The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences*, 106(33), 13875–13879.
4. Mayrose, I., Zhan, S. H., Rothfels, C. J., Magnuson-Ford, K., Barker, M. S., Rieseberg, L. H., & Otto, S. P. (2011). Recently formed polyploid plants diversify at lower rates. *Science*, 333(6047), 1257.
5. Jiao, Y., Wickett, N. J., Ayyampalayam, S., Chanderbali, A. S., Landherr, L., Ralph, P. E., ... & Leebens-Mack, J. (2011). Ancestral polyploidy in seed plants and angiosperms. *Nature*, 473(7345), 97–100.
6. Soltis, P. S., Marchant, D. B., Van de Peer, Y., & Soltis, D. E. (2015). Polyploidy and genome evolution in plants. *Current Opinion in Genetics & Development*, 35, 119–125.
7. Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nature Reviews Genetics*, 6(11), 836–846.
8. Chen, Z. J. (2010). Molecular mechanisms of polyploidy and hybrid vigor. *Trends in Plant Science*, 15(2), 57–71.
9. Stebbins, G. L. (1947). *Types of polyploids: Their classification and significance in the evolution of higher plants*. The Botanical Review, 13(8), 422–456.
10. Clausen, J., Keck, D. D., & Hiesey, W. M. (1945). *Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autopolloidy, with examples from the genera Crepis and Nicotiana*. Carnegie Institution of Washington.
11. Ramsey, J., & Schemske, D. W. (1998). *Pathways, mechanisms, and rates of polyploid formation in flowering plants*. Annual Review of Ecology and Systematics, 29, 467–501.
12. Grant, V. (1981). *Plant speciation*. Columbia University Press.
13. Harlan, J. R., & de Wet, J. M. J. (1975). *On Ö. Winge and a prayer: The origins of polyploidy*. The Botanical Review, 41(4), 361–390.
14. Bretagnolle, F., & Thompson, J. D. (1995). *Gametes with the somatic chromosome number: Mechanisms of their formation and role in the evolution of autopolyploid plants*. New Phytologist, 129(1), 1–22.
15. Blakeslee, A. F., & Avery, A. G. (1937). Methods of inducing doubling of chromosomes in plants by treatment with colchicine. *Journal of Heredity*, 28(12), 393–411.

16. Ramanna, M. S., & Jacobsen, E. (2003). *Relevance of sexual polyploidization for crop improvement – A review*. *Euphytica*, 133(1), 3–18.
17. Bretagnolle, F., & Thompson, J. D. (1995). *Gametes with the somatic chromosome number: Mechanisms of their formation and role in the evolution of autopolyploid plants*. *New Phytologist*, 129(1), 1–22.
18. Eigsti, O. J., & Dustin, P. (1955). *Colchicine in agriculture, medicine, biology, and chemistry*. Iowa State College Press.
19. Dewitte A, Van Laere K, Van Huylbroeck J (2012) Use of 2n gametes in plant breeding. In: Abdurakhmonov IY (ed) *Plant breeding*. InTech Open Access Publisher, Croatia, pp 59–86.
20. Morejohn, L. C., Bureau, T. E., Mole-Bajer, J., Bajer, A. S., & Fosket, D. E. (1987). Oryzalin, a dinitroaniline herbicide, binds to plant tubulin and inhibits microtubule polymerization in vitro. *Planta*, 172, 252-264.
21. Murashige, T., & Nakano, R. (1966). Tissue culture as a potential tool in obtaining polyploid plants. *Journal of Heredity*, 57(4), 115-118.
22. Doležel, J., Bartoš, J., Voglmayr, H., & Greilhuber, J. (2007). *Nuclear DNA content and genome size of trout and human*. *Cytometry Part A*, 71(2), 85–91.
23. Soltis, P. S., & Soltis, D. E. (2000). The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences*, 97(13), 7051–7057.
24. Rieseberg, L. H., Archer, M. A., & Wayne, R. K. (1999). Transgressive segregation, adaptation and speciation. *Heredity*, 83(4), 363–372.
25. Fawcett, J. A., Maere, S., & Van De Peer, Y. (2009). Plants with double genomes might have had a better chance to survive the Cretaceous–Tertiary extinction event. *Proceedings of the National Academy of Sciences*, 106(14), 5737-5742.
26. Madlung, A. (2013). Polyploidy and its effect on evolutionary success: Old questions revisited with new tools. *Heredity*, 110(2), 99–104.
27. Masterson, J. (1994). Stomatal size in fossil plants: Evidence for polyploidy in the majority of angiosperms. *Science*, 264(5157), 421–424.
28. Stebbins, G. L. (1971). *Chromosomal evolution in higher plants*. London: Edward Arnold.
29. Allard, R. W. (1960). *Principles of plant breeding*. New York: John Wiley & Sons.

30. Ramsey, J., & Schemske, D. W. (1998). Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics*, 29(1), 467–501.
31. Chen, Z. J. (2007). Genetic and epigenetic mechanisms for gene expression and phenotypic variation in polyploids. *Annual Review of Plant Biology*, 58(1), 377–406.
32. Soltis, P. S., & Soltis, D. E. (1999). Polyploidy: Recurrent formation and genome evolution. *Trends in Ecology & Evolution*, 14(9), 348–352.
33. Sattler, M. C., Carvalho, C. R., & Clarindo, W. R. (2016). The polyploidy and its key role in plant breeding. *Planta*, 243(2), 281–296.
34. Peng, J. H., Sun, D., & Nevo, E. (2011). Domestication evolution, genetics and genomics in wheat. *Molecular Breeding*, 28(3), 281–301.
35. Paterson, A. H., Wendel, J. F., Gundlach, H., Guo, H., Jenkins, J., Jin, D., ... & Wang, B. (2012). Repeated polyploidization of *Gossypium* genomes and the evolution of spinnable cotton fibres. *Nature*, 492(7429), 423–427.
36. Olsen, R. T., Ranney, T. G., & Vilorio, Z. (2006). Reproductive behavior of induced allotetraploid× *Chitalpa* and in vitro embryo culture of polyploid progeny. *Journal of the American Society for Horticultural Science*, 131(6), 716–724.
37. Buckner, R. C., Hill, H. D., & Burrus Jr, P. B. (1961). Some characteristics of perennial and annual ryegrass× tall fescue hybrids and of the amphidiploid progenies of annual ryegrass× tall fescue 1. *Crop science*, 1(1), 75–80.
38. Azadi, P., Bagheri, H., Nalouisi, A. M., Nazari, F., & Chandler, S. F. (2016). Current status and biotechnological advances in genetic engineering of ornamental plants. *Biotechnology Advances*, 34(6), 1073–1090.
39. Zahumenická, P., Fernández, E., Šedivá, J., Žiarovská, J., Ros-Santaella, J. L., Martínez-Fernández, D., ... & Milella, L. (2018). Morphological, physiological and genomic comparisons between diploids and induced tetraploids in *Anemone sylvestris* L. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 132, 317–327.
40. Van Kleunen, M., Essl, F., Pergl, J., Brundu, G., Carboni, M., Dullinger, S., Early, R., González-Moreno, P., Groom, Q. J., Hulme, P. E., & Kueffer, C. (2018). The changing role of ornamental horticulture in alien plant invasions. *Biological Reviews*, 93(3), 1421–1437.
41. Lan, M. O., Chen, J. H., Fei, C., Tong, Z. K., Huang, H. H., Dong, R. H., Lou, X. Z., & Lin, E. P. (2020). Induction and characterization of polyploids from seeds of *Rhododendron fortunei* Lindl. *Journal of Integrative Agriculture*, 19(8), 2016–2026.

42. Yue, Y., Fan, X., Hu, Y., Han, C., Li, H., Teng, W., Zhang, H., Teng, K., Wen, H., Yang, X., & Wu, J. (2020a). In vitro induction and characterization of hexaploid *Pennisetum × advena*, an ornamental grass. *Plant Cell, Tissue and Organ Culture*.
43. Podwyszyńska, M. (2011, September). In vitro tetraploid induction in tulip (*Tulipa gesneriana* L.). In *VII International Symposium on In Vitro Culture and Horticultural Breeding 961* (pp. 391-396).
44. Khalili, S., Niazian, M., Arab, M., & Norouzi, M. (2020). In vitro chromosome doubling of African daisy, *Gerbera jamesonii* Bolus cv. Mini Red. *The Nucleus*, *63*, 59-65.
45. Urwin, N. A. (2014). Generation and characterization of colchicine-induced polyploid *Lavandula × intermedia*. *Euphytica*, *197*(3), 331–339.
46. Hoang, T. K., Hwang, Y. J., & Lim, J. H. (2020). Chemical polyploidization of *Chrysanthemum boreale*. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *140*, 677-683.
47. Manzoor, A., Ahmad, T., Bashir, M. A., Hafiz, I. A., & Silvestri, C. (2019). Studies on colchicine induced chromosome doubling for enhancement of quality traits in ornamental plants. *Plants*, *8*(7), 194.
48. Tong, J., & Chen, F. Z. (2012). Study on colchicines induced polyploid breeding of *Callicarpa bodinieri*. *Hubei Agricul Sci*, 2049-2053.
49. Esmaili, G., Van Laere, K., Muylle, H., & Leus, L. (2020). Artificial chromosome doubling in allotetraploid *Calendula officinalis*. *Frontiers in Plant Science*, *11*, 622.
50. Tu, H. Y., Zhang, A. L., Xiao, W., Lin, Y. R., Shi, J. H., Wu, Y. W., Wu, S. T., Zhong, C. H., & Mo, S. X. (2018). Induction and identification of tetraploid *Hedychium coronarium* through thin cell layer culture. *Plant Cell, Tissue and Organ Culture*, *135*(3), 395–406.
51. Kushwah, K. S., Verma, R. C., Patel, S., & Jain, N. K. (2018). Colchicine induced polyploidy in *Chrysanthemum carinatum* L. *Journal of Phylogenetics & Evolutionary Biology*, *6*(1), 2.
52. Shahriari-Ahmadi, F., Dehghan, E., Farsi, M., & Azizi, M. (2008). Tetraploid induction of *Hyoscyamus muticus* using colchicine treatment. *Pakistan Journal of Biological Sciences*, *11*(24), 2653–2658.
53. Pradhan, S. K., Gupta, R. C., & Goel, R. K. (2018). Differential content of secondary metabolites in diploid and tetraploid cytotypes of *Siegesbeckia orientalis* L. *Natural product research*, *32*(20), 2476-2482.

54. Kooti, W., Farokhipour, M., Asadzadeh, Z., Ashtary-Larky, D., & Asadi-Samani, M. (2016). The role of medicinal plants in the treatment of diabetes: a systematic review. *Electronic physician*, 8(1), 1832.
55. Mopuri, R., & Islam, M. S. (2017). Medicinal plants and phytochemicals with anti-obesogenic potentials: A review. *Biomedicine & Pharmacotherapy*, 89, 1442-1452.
56. Niaziyan, M., Sadat-Noori, S. A., Tohidfar, M., Galuszka, P., & Mortazavian, S. M. M. (2019). Agrobacterium-mediated genetic transformation of ajowan (*Trachyspermum ammi* (L.) Sprague): an important industrial medicinal plant. *Industrial Crops and Products*, 132, 29-40.
57. Shmeit, Y. H., Fernandez, E., Novy, P., Kloucek, P., Orosz, M., & Kokoska, L. (2020). Autopolyploidy effect on morphological variation and essential oil content in *Thymus vulgaris* L. *Scientia Horticulturae*, 263, 109095.
58. Sadat Noori, S. A., Norouzi, M., Karimzadeh, G., Shirkoool, K., & Niaziyan, M. (2017). Effect of colchicine-induced polyploidy on morphological characteristics and essential oil composition of ajowan (*Trachyspermum ammi* L.). *Plant cell, tissue and organ culture (pctoc)*, 130, 543-551.
59. Julião, S. A., Ribeiro, C. D. V., Lopes, J. M. L., Matos, E. M. D., Reis, A. C., Peixoto, P. H. P., ... & Viccini, L. F. (2020). Induction of synthetic polyploids and assessment of genomic stability in *Lippia alba*. *Frontiers in plant science*, 11, 292.
60. Bhuvaneswari, G., Thirugnanasampandan, R., & Gogulramnath, M. (2020). Effect of colchicine induced tetraploidy on morphology, cytology, essential oil composition, gene expression and antioxidant activity of *Citrus limon* (L.) Osbeck. *Physiology and Molecular Biology of Plants*, 26, 271-279.
61. Hannweg, K., Visser, G., De Jager, K., & Bertling, I. (2016). In vitro-induced polyploidy and its effect on horticultural characteristics, essential oil composition and bioactivity of *Tetradenia riparia*. *South African Journal of Botany*, 106, 186-191.
62. Liu, S., Chen, S., Chen, Y., Guan, Z., Yin, D., & Chen, F. (2011). In vitro induced tetraploid of *Dendranthema nankingense* (Nakai) Tzvel. shows an improved level of abiotic stress tolerance. *Scientia horticulturae*, 127(3), 411-419.
63. Jaleel, C. A., Sankar, B., Murali, P. V., Gomathinayagam, M., Lakshmanan, G. M. A., & Panneerselvam, R. (2008). Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus*; impacts on ajmalicine accumulation. *Colloids and Surfaces B: Biointerfaces*, 62(1), 105-111.

64. Niazian, M., Sadat-Noori, S. A., Tohidfar, M., Galuszka, P., & Mortazavian, S. M. M. (2019). Agrobacterium-mediated genetic transformation of ajowan (*Trachyspermum ammi* (L.) Sprague): an important industrial medicinal plant. *Industrial Crops and Products*, *132*, 29-40.
65. Pradhan, S. K., Gupta, R. C., & Goel, R. K. (2018). Differential content of secondary metabolites in diploid and tetraploid cytotypes of *Siegesbeckia orientalis* L. *Natural product research*, *32*(20), 2476-2482.
66. Kooti, W., Farokhipour, M., Asadzadeh, Z., Ashtary-Larky, D., & Asadi-Samani, M. (2016). The role of medicinal plants in the treatment of diabetes: a systematic review. *Electronic physician*, *8*(1), 1832.
67. Mopuri, R., & Islam, M. S. (2017). Medicinal plants and phytochemicals with anti-obesogenic potentials: A review. *Biomedicine & Pharmacotherapy*, *89*, 1442-1452.
68. Niazian, M., Sadat-Noori, S. A., Tohidfar, M., Galuszka, P., & Mortazavian, S. M. M. (2019). Agrobacterium-mediated genetic transformation of ajowan (*Trachyspermum ammi* (L.) Sprague): an important industrial medicinal plant. *Industrial Crops and Products*, *132*, 29-40.
69. Shmeit, Y. H., Fernandez, E., Novy, P., Kloucek, P., Orosz, M., & Kokoska, L. (2020). Autopolyploidy effect on morphological variation and essential oil content in *Thymus vulgaris* L. *Scientia Horticulturae*, *263*, 109095.
70. Sadat Noori, S. A., Norouzi, M., Karimzadeh, G., Shirkoool, K., & Niazian, M. (2017). Effect of colchicine-induced polyploidy on morphological characteristics and essential oil composition of ajowan (*Trachyspermum ammi* L.). *Plant cell, tissue and organ culture (pctoc)*, *130*, 543-551.
71. Julião, S. A., Ribeiro, C. D. V., Lopes, J. M. L., Matos, E. M. D., Reis, A. C., Peixoto, P. H. P., ... & Viccini, L. F. (2020). Induction of synthetic polyploids and assessment of genomic stability in *Lippia alba*. *Frontiers in plant science*, *11*, 292.
72. Bhuvaneshwari, G., Thirugnanasampandan, R., & Gogulramnath, M. (2020). Effect of colchicine induced tetraploidy on morphology, cytology, essential oil composition, gene expression and antioxidant activity of *Citrus limon* (L.) Osbeck. *Physiology and Molecular Biology of Plants*, *26*, 271-279.
73. Hannweg, K., Visser, G., De Jager, K., & Bertling, I. (2016). In vitro-induced polyploidy and its effect on horticultural characteristics, essential oil composition and bioactivity of *Tetradenia riparia*. *South African Journal of Botany*, *106*, 186-191.

74. Liu, S., Chen, S., Chen, Y., Guan, Z., Yin, D., & Chen, F. (2011). In vitro induced tetraploid of *Dendranthema nankingense* (Nakai) Tzvel. shows an improved level of abiotic stress tolerance. *Scientia horticulturae*, 127(3), 411-419.
75. Jaleel, C. A., Sankar, B., Murali, P. V., Gomathinayagam, M., Lakshmanan, G. M. A., & Panneerselvam, R. (2008). Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus*; impacts on ajmalicine accumulation. *Colloids and Surfaces B: Biointerfaces*, 62(1), 105-111.
76. Niazian, M., Sadat-Noori, S. A., Tohidfar, M., Galuszka, P., & Mortazavian, S. M. M. (2019). Agrobacterium-mediated genetic transformation of ajowan (*Trachyspermum ammi* (L.) Sprague): an important industrial medicinal plant. *Industrial Crops and Products*, 132, 29-40.
77. Kehr, A. E. (1971). A tetraploid *Rhododendron carolinianum*. *Am Rhod Soc Bull*, 25, 4-7.
78. Tolety, J., & Sane, A. (2011). Antirrhinum. In *Wild Crop Relatives: Genomic and Breeding Resources: Plantation and Ornamental Crops* (pp. 1-14). Berlin, Heidelberg: Springer Berlin Heidelberg.
79. Parris, J. K., Ranney, T. G., Knap, H. T., & Baird, W. V. (2010). Ploidy levels, relative genome sizes, and base pair composition in *Magnolia*. *Journal of the American Society for Horticultural Science*, 135(6), 533-547.
80. Gosztola, B., Nemeth, E., Sarosi, S. Z., Szabo, K., & Kozak, A. (2006). Comparative evaluation of chamomile (*Matricaria recutita* L.) populations from different origin. *International Journal of Horticultural Science*, 12(1), 91-95.
81. Das, M. (2014). *Chamomile: medicinal, biochemical, and agricultural aspects*. CRC Press.
82. Sedov, E. N., Sedysheva, G. A., Serova, Z. M., Gorbacheva, N. G., & Melnik, S. A. (2014). Breeding assessment of heteroploid crosses in the development of triploid apple varieties. *Russian Journal of Genetics: Applied Research*, 4, 52-59.
83. Janick J, Cimmins JM, Brown SK, Hemmat M (1996) Apple. In: Janick J, Moore JN (eds) Fruit breed, Volume I: tree and tropical fruits. John Wiley & Sons, New York.
84. Park, S. M., Wakana, A., Kim, J. H., & Jeong, C. S. (2002). Male and female fertility in triploid grapes (*Vitis* complex) with special reference to the production of aneuploid plants. *VITIS-GEILWEILERHOF-*, 41(1), 11-20.
85. Morinaga K (2001) Grape production in Japan. In: Papademetriou MK, Dent FJ (eds) Grape production in the Asia-Pacific region. FAO, Thailand, pp 38-52

86. Marasek-Ciolakowska, A., Ramanna, M. S., Arens, P., & Van Tuyl, J. M. (2012). Breeding and cytogenetics in the genus *Tulipa*. *Floricult Ornamental Biotech*, 6, 90-97.
87. Eikelboom, W., Straathof, T. P., & Van Tuyl, J. M. (2001). Tetraploide "Christmas marvel" methoden om tetraploide tulpen te verkrijgen. *Bloembollencultuur*, 112, 22-23.
88. Wu, J. H., Ferguson, A. R., Murray, B. G., Jia, Y., Datson, P. M., & Zhang, J. (2012). Induced polyploidy dramatically increases the size and alters the shape of fruit in *Actinidia chinensis*. *Annals of botany*, 109(1), 169-179.
89. Jaskani, M. J., Kwon, S. W., & Kim, D. H. (2005). Comparative study on vegetative, reproductive and qualitative traits of seven diploid and tetraploid watermelon lines. *Euphytica*, 145(3), 259-268.
90. Marzougui, N., Boubaya, A., Elfalleh, W., Ferchichi, A., & Beji, M. (2009). Polyploidy induction in *Trigonella foenum-graecum* L.: morphological and chemical comparison between diploids and induced autotetraploids cultivars.
91. Zhang W, Hao H, Ma L, Zhao C and Yu X, 2010. Tetraploid muskmelon alters morphological characteristics and improves fruit quality. *Scientia Horticulturae* 125(3): 96-400.
92. Ramírez-Godina, F., Robledo-Torres, V., Pournabav, R. F., Benavides-Mendoza, A., Hernández-Piñero, J. L., Reyes-Valdes, M. H., & Alvarado-Vázquez, M. A. (2013). Yield and fruit quality evaluation in husk tomato autotetraploids (*Physalis ixocarpa*) and diploids.
93. Pliankong, P., Suksa-Ard, P., & Wannakrairoj, S. (2017). Effects of colchicine and oryzalin on polyploidy induction and production of capsaicin in *Capsicum frutescens* L.
94. Nasirvand, S., Zakaria, R. A., Zare, N., & Esmailpoor, B. (2018). Polyploidy induction in parsley (*Petroselinum crispum* L.) by colchicine treatment. *Cytologia*, 83(4), 393-396.
95. Aqafarini, A., Lotfi, M., Norouzi, M., & Karimzadeh, G. (2019). Induction of tetraploidy in garden cress: morphological and cytological changes. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 137, 627-635.
96. Pei, Y., Yao, N., He, L., Deng, D., Li, W., & Zhang, W. (2019). Comparative study of the morphological, physiological and molecular characteristics between diploid and tetraploid radish (*Raphanus sativus* L.). *Scientia Horticulturae*, 257, 108739.

UNDER PEER REVIEW