Central Asian Journal of Plant Science Innovation **2** (2021) 76–90

REVIEW PAPER

Studies on polyploidy induction for improvement of quality traits in ornamental and medicinal plants

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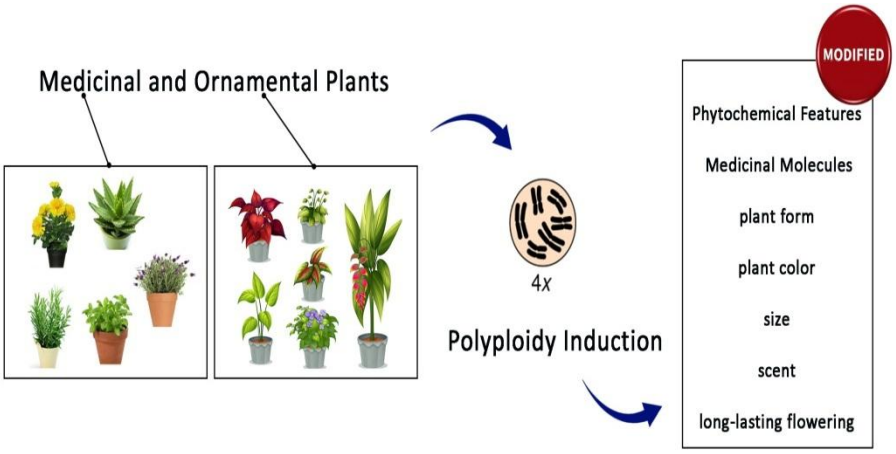
Highlights

- Using natural and induced mutations in improving gene resources is very effective and as a result, helps in the development of improved and new cultivars of ornamental and medicinal plants.
- One of the correction works to improve the valuable properties of plants is artificial polyploidy induction.
- Medicinal and ornamental plants with a complete set of duplicate chromosomes have more distinctive features such as plant form, color, size, aroma and long flowering.

Graphical Abstract



Article Info

Receive Date: 10 December 2020
Revise Date: 09 May 2021
Accept Date: 05 June 2021
Available online: 17 June 2021

Keywords:
Antimitotic agent
Chromosomes
Ornamental and medicinal plants
Whole-genome duplication

Abstract

Plants provide food, medicine, fuel and they have a positive impact on human life. Ornamental plants have a fundamental role in the human relationship with nature. Medicinal plants as genetic reserves can be considered as the precious national resource for each country and can be the most primary agricultural product. These two groups of plants are part of the natural wealth and have great economic values. Modern breeding methods are invented to resolve the need to diversify ornamental plants. These methods shorten the length of the breeding period to a good extent as well as affecting in the breeding of some plants whose improvement is not possible due to traditional methods. Creating a genetic mutation to improve quality is a necessity in any breeding program. Using natural and induced mutations in improving gene resources is very effective and as a result, helps in the development of improved and new cultivars of ornamental and medicinal plants. Haploid, double haploid and polyploidy plants are the new sources of germplasm that can be introduced as new cultivars or can be used in breeding programs. One of the breeding works to improve the valuable properties of plants is artificial polyploidy induction. Medicinal and ornamental plants with the complete set of duplicated chromosomes (not the usual ones) consist of more distinctive features such as modified phytochemical features, higher content of medicinal molecules, plant shape, plant color, size, scent and long-lasting flowering. To develop a successful protocol for duplicating the chromosomes, some important factors must be considered such as plant genotype and sample type. The type, amount and duration of mitotic inhibitors must also be considered as principal factors. In this article, significant advances in polyploidy are investigated using various mitotic inhibitors in ornamental and medicinal plants.

10.22034/CAJPSI.2021.02.03
E-ISSN: 2783-1310

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1. Introduction

Plant domestication had started since the primitives quit hunting and food collecting and started civilized life (Niazian, 2019). This process began with a simple selection of the basic products and continued until the discovery of the founders of genetics (Mendel) and modern statistics (Fisher). Connecting these research fields has led to some strong plant breeding methods. Various schemes were designed including average generation, diallel crossing and North Carolina confluence for genetic decomposition and analysis and the desired quantitative traits of various products' improvement. Moreover, other statistical methods such as simple correlation analysis, course decomposition, step-by-step and reverse regression are used by plant breeders (geneticists) to explain complicated relations and find a selection criterion to improve desired traits (Niazian and Molaahmad-Nalousi, 2020). Ornamental plants world trade is very valuable. The competition to produce high-quality cultivars and reduce the production costs in the world is on the rise. A large number of cultivars with new traits have been supplied to the global market so far, but to deal with limitations like gene-pool deficiency, incompatibility between species and the reduction of plant mutation time, genetic engineering can be used to increase the variety quality. Modern biotechnology scientists have tried to produce transgenic ornamental plants by using various methods. Genetic engineering objectives to mutate ornamental plants varies in different cultivars and species such as flower discoloration, production of cultivars resistant to fungal and viral diseases, production of cultivar resistant to ethylene and flower lifetime increase and flower deformation. Medicinal plants are one of the most significant medical sources that have been used for thousands of years and one of their most important problems is their improper harvest from nature (Iannicelli et al., 2018). Although the demand for these compounds has increased, the low density of them in the plant, natural source limitation, deforestation, pastures and green space, extinction of diverse plant species, problems related to domestication and cultivation of these plants made the researchers use biotechnology solutions to increase the production and efficiency of medicinal plants. Modern breeding methods provide rapid and massive propagation of plants and the production of secondary metabolites in vitro. By using in vitro planting, besides having access to the primary source of the medicine under the controlled condition, the increase of compounds compared to plants and the production of new compounds will be possible as well (Kumar and Gupta, 2008). The modern breeding of ornamental plants including the production of soma clonal variations, induction mutations, production of polyploidy and haploid plants and genetic engineering are all to create variety in color increase the durability and fragrance of the flower and also increase the plant resistance to diseases and pests.

Individuals and cells with a number of gamete chromosomes are called haploids and can be grouped into monoploid and polyhaploid. Haploidy has been observed in both animals and plants. Although haploid plants are sterile, their production is of great importance in plant breeding (Jauhar, 2003). From a cytogenetic point of view, haploid is a general term used to refer to individuals, tissues, and cells with a number of gametes (n) chromosomes. But since there are several sets of base (x) chromosomes, especially in polyploid plants, the term haploid can be defined in two main groups.

A) Monoaploids (x): Individuals are haploids that can be created from true diploid species.

B) Polyploids (4x, 3x, 2x, etc.): are Individuals who can be caused by any particular type of polyploidy; Such as creating 2x haploid from 4x species or creating 3x haploid from 6x species (D'Amato and Bayliss, 1985). Haploid individuals can occur spontaneously or inductively. The main origin of spontaneous haploids is unknown, but haploids may be caused by the asexual reproduction of real diploids. Among animals, the most common species in which haploidy has been observed is Drosophila. Other animals in which spontaneous and induced haploidism have been observed include salamanders, newts, frogs, mice, axolotl, chickens, and onion flies. Haploid animals usually have physiological abnormalities and die during embryonic development (Croser et al., 2006).

Spontaneous haploidy has been found in many plants such as tomatoes, wheat, cotton, coffee, sugar beet, barley, flax, coconut, millet, turnip and asparagus. In addition, researchers can create monoploid plants from diploid plants using methods such as pollen, anther, or female seed cultivation (Dunwell, 2010). As mentioned, monoploids have only one base (x) genome and most of them are sterile. This causes these plants to have very

irregular meiotic divisions because meiosis requires the presence of two homologous chromosomes. Because there is no homologous chromosome for mating during meiosis I, the chromosomes are randomly attached to spindle strands and randomly to cell poles. The result is gametes that are defective due to the absence of certain chromosomes. The higher the number of chromosomes in a cell, the less likely it is that healthy gametes will form. However, this probability is not zero, and in rare cases, all chromosomes are at the same pole (Appels et al, 1998). The production of monoploid plants is very important in plant breeding because it is possible to observe alleles directly and without the need for genetic crosses (Germana, 2011).

Polyploidy is created via two processes of endomitosis and endoreduplication (D'Amato, 1964), other processes like a fusion of nuclei, ineffective mitosis or the emergence of multinucleated cells are also involved in creating polyploidy cells but are limited to epidermis tissues (Joubès and Chevalier, 2000). Unlike normal mitosis, the endomitosis process occurs when the cell membrane is not destroyed and mitosis takes place within the cell membrane nucleus, chromosome numbers doubles and sister chromatids are likely to separate and return to interphase mode, except that chromosomal spindles are not created and as a result sister chromatids are not stretched to the side of the cell and polyploidy cells are not created. The endometriosis process mostly occurs in animal cells and is rare in angiosperms (flowering plants). In the endoreduplication process which normally occurs in plants, the cell nucleus DNA proliferates but the constriction of chromatids doesn't occur and as a result, 2n chromatids are formed without any change in the number of the cell chromosomes. The endoreduplication nucleus is capable of replicating the DNA without entering the mitosis process and create 4n, 8n, 16n and so forth (Jafarkhani Kermani and Emadpour, 2019).

Polyploidy is a condition that there are more than two sets of chromosomes in each cell. Each organism has a specific number of chromosomes that fall into several categories. The number of chromosomal clusters is called the ploidy. Chromosomes in this situation may change in number. These changes generally fall into two groups: euploidy and aneuploidy (Ravichandran et al., 2018). The mechanism of polyploidy formation includes two pathways: somatic doubling and the production of unreduced gametes (2n) due to the failure of Dermius (Ramsey and Schemske, 1998). Paleopolyploidy as normally done in plants and is the most important feature in the evolutionary history of all land plants. It is seen that almost every plant somehow experienced polyploidy at a particular level. However, recently most plants experienced chromosome copying which is known as neoploploidy (Wendel et al., 2018; Zhang et al., 2014).

Polyploidy has attracted many researchers' attention to itself for various reasons: 1) Often with features that define new species, for instance, fertility separation and morphological differentiation 2) it can give rise to the new species 3) It can affect the evolution of the species because of the genome proliferation and the potential benefits of its compatibility (Wood et al., 2009). Polyploidy's can be classified as auto-polyploidies or allopolyploids according to their origin. Auto-polyploidies originate from the proliferation of chromosomes in one type of diploid resulting in two (or more) homologous chromosome pairs. Allopolyploids originate from the hybridization of two various species and subsequent chromosomal proliferation (i.e. it combines two different genomes) (Yang et al., 2011).

In general, the terms euploid and aneuploid are used to refer to organisms with different ploidy statuses, which will be described below (Tolmacheva et al., 2020). In euploidy, the total number of chromosomes is the exact multiple of the base chromosome series. Euploids can be divided into three groups: monoploids, diploids, and polyploids. But most euploids have two sets of chromosomes and are diploid (Dar et al., 2017). However, some euploid species have more than two chromosomal classes and are polyploid. It is worth noting that geneticists use the letter x to indicate the number of base chromosomes, which are sets of different single chromosomes that make up a complete single set. The letter n is also used to indicate the number of chromosomes in each gamete or ploidy surface (Elliott et al., 2018).

In past decades, new and modified cultivars of economically important species have been produced by inducing artificial polyploidies using mutagenic agents (Eng and Ho, 2019; Dhooghe et al., 2011), in addition, (Adams and Wendel, 2005) stated that polyploidy is not as easy as genome proliferation, in fact, it causes a wide

range of molecular and physiological changes. Most of the polyploidies (natural and artificial) show phenotypes that are different from their progenitor. Some of these traits like larger organ size (leaf, flower, etc.), higher range of biomass, dryness tolerance, disease tolerance, diverse flowering time and other changes can enable the polyploidies to make new environmental changes. Besides, these distinctive polyploidy traits improve their possibility of being selected for agriculture purposes (Osborn et al., 2003). In medicinal plants, polyploidy causes an increase in secondary metabolites, and more and better adaptation to the environment (Iannicelli et al., 2020) and in ornamental plants, it increases the thickness of the petals and the size of the flower, height, etc. (Manzoor et al., 2019; Niazian and Molaahmad-Nalosi, 2020). In fact, polyploidization is one of the most significant tools used in plant breeding, where the development of artificial polyploidy enables rapid genetic improvement of plants. The purpose of this article is to review the progress made using polyploidization techniques as a breeding tool in some ornamental and medicinal species.

2. Breeding of ornamental and medicinal plants

For breeding of ornamental and medicinal plants, particularly the species with the least breeding, various biotechnological interventions such as polyploidization, haploids, mutations and somaclonal changes in vitro may accelerate the production speed and selection of new mutations. Among these methods, polyploidy is used in asexually reproduced products and achieving various genetic changes. Indeed, polyploidies have been successfully used in cosmetic and pharmaceutical products over the past few decades (Sajjad et al., 2013). Polyploidy breeding compared to mutations leads to a change in the whole genome, which causes more phenotypic changes than a single gene mutation (Eng and Ho, 2019). Increasing cell size is the most important result of polyploidy which occurs due to the additional versions of the gene. This polyploidy effect is known as the "Gigas effect" (Sattler et al., 2016). "Gigas" phenomenon refers to the size of cells (larger organ size) due to the doubling of the chromosomes (Eng and Ho, 2019; Salma et al., 2017). Although, cell size in polyploidies is usually larger and increasing cell size is the most well-known polyploidy effect in plants. However, the final biomass size may not always increase in plants (Iannicell et al., 2020).

Polyploidy intensifies the color of the flower, increases the size of the flower and deforms the plant (Sajjad et al., 2013). Due to the increased sterility of induced tetraploids, vegetative propagation of ornamental plants is still the main system of proliferation and prevents contamination of flowers with external pollen (Nagahatenna and Pieris, 2008). Polyploidy in medicinal plants increases the secondary metabolite production and amount of biomass (Iannicelli et al., 2020). In other study reported that tetraploid increases the biomass and amount of triterpenes in *Centella asiatica* (L.) and suggested this to researchers (Kaensaksiri et al., 2011). Following the previous work, was compared a tetraploid plant with three diploid plants and in this research described that in comparison to the diploids the amount of biomass has increased in tetraploid and the amount of triterpenes has increased by 70% (Thong-on et al., 2014).

3. Gene expression in polyploidy

The major genetic information of organisms is stored in DNA molecules, which form a compact structure called a chromosome during cell division. The structure of chromosomes can change in a variety of ways, and these changes are caused by genetic mutations. Similarly, the number of chromosomes can change, some of which are beneficial and some of which are harmful (Palozola et al., 2019). Gene amount manipulation and subsequent gene effect can occur in two main ways: a) introducing the targeted gene(s), b) genomic shock via the proliferation of the whole chromosome set (Niazian and Molaahmad- Nalousi, 2020). Gene transfer and gene silencing increase and decrease the gene expression in plants, respectively. Gene transfer by methods like agrobacterium increases the targeted gene in various species such as *Chrysanthemum morifolium* (Wang et al., 2017), *Gerbera aurantiaca* (Broholm et al., 2008), and *Pelargonium crispum* (Kanemaki et al., 2018). The converse of the above action, reduction of gene expression or suppression (gene silencing via artificial RNAi) can also be created through agrobacterium (Ban et al., 2019; Cheng et al., 2018). The mechanism of RNA interference

(RNAi) can destroy the expression of the targeted gene and affect the relevant phenotype in transgenic plants. Micro RNA (miRNA) - small interfering RNA (siRNA) and (miRNA) are responsible for silencing the messenger RNA (mRNA) through specific sequence inhibition or cleavage in the mechanism of RNAi gene silencing. RNAi-mediated gene silencing can be used to study gene function and plant modification by manipulating desirable or undesirable genes (Sabu and Nadiya 2020).

Silencing of Gigantea 1 gene in Atlas hybrid via RNAi mechanism led to the formation of larger plants with modified internode length, increased leaf size and reduced number of flower buds, but their flowering time didn't change (Brandoli et al., 2020). Increased ploidy in the plant is associated with increased production of specialized metabolites (terpenoids, flavonoids, alkaloids) in various medicinal plants (Lavania, 2005) which shows that these pathways respond to an increase in the net amount of the gene. Recently, transcriptome and metabolic studies have shown that autopolyploidy can alter transcript and metabolome (Fasano et al., 2016; Tan et al., 2017). Based on the research done on *Solanum commersonii* and *S. bulbocastanum* by Fasano et al., 2016, the production of antioxidants increases as the result of the polyploidization because of the genomic stress (for instance Chlorogenic acid (CGA)). About medicinal plants, over-expression of the different genes is an effective approach to increase the efficiency of the desired medicinal molecules using metabolic engineering. However, due to the involved genes, manipulation of the entire biosynthesis pathway is the most powerful approach. Therefore, the proliferation of the whole genome for the manipulation of the entire metabolic pathway can be more effective than one gene (Niazian, 2019). Polyploidy plants in terms of phenotypic expression which includes morphologic, physiologic, biochemical and cell change are significantly different or sometimes superior to diploid plants.

These polyploidy plants can also be used as a new species or genotype, which can be used in future reproduction programs to improve products. Due to the great importance of polyploidy, it has been inducing in many economically important products, but orchid, chrysanthemum (Lertsutthichawan et al., 2018), and bougainvillea ornamental plants were reported to be the most successful cases (Manzoor et al., 2019). However, this is a generalization, since multiple outputs can occur after polyploidy induction and the desired characteristics or phenotypes may not be expressed (Niazian and Molaahmad-Nalousi, 2020). Fast epigenetic changes, chromosome rearrangement, regulators network change and loss of duplicated genes can also alter the gene expression and lead to an unexpected change in polyploidy (Iannicelli et al., 2020). The CpYGFP gene have modified by CRISPR/Cas9 system in chrysanthemum that changed the flower color (Kishi-Kaboshi et al., 2016). In Petunia (Mirage Rose Cultivar) by modification of PhACO1 gene through CRISPR/Cas9, ethylene production has decreased and flower life (flower quality) has improved (Xu et al. 2020). CRISPR/SpCas9 approach was used to destroy the OMT2'4 gene in opium poppy (*Papaver somniferum* L). Results have shown that in transgenic plants by removing the OMT2'4 gene, BIAs biosynthesis (for instance morphine thebaine) reduces significantly (Alagoz et al., 2016).

4. Polyploidy induction

Various vegetatively propagated products are polyploid. However, polyploidy doesn't naturally exist in all plant materials, thus, over the past few decades; it has been artificially induced in many important economic products (Dhooghe et al., 2011). Induced polyploidy formation is an effective method to create genetic diversity for genetics study and plant breeding. Polyploidy can be artificially created through interspecific combination, in vitro endosperm culture or doubling of somatic cells via mitotic inhibitors such as colchicine (Fatima et al., 2015). The laboratory system is the most common method of polyploidy that can lead to the rapid development of polyploidy in confined space (Eng and Ho, 2019). In the laboratory system, mitosis inhibitors can be added to the culture medium and simply apply to plant issues (Touchell et al., 2020). Low mortality rate, doubling and low mixoploidy percentage are other benefits of inducing chromosome doubling in vitro (Fu et al., 2019). There are two various ways to induce polyploidy artificially: 1) meiosis (sexual), 2) mitosis (somatic). In meiosis polyploidization, 2n (gamete) pollens are produced. Fig. 1 A wide range of micro samples, such as branch tip

meristems (Zhang and Gao. 2020), seeds (Carbajal et al., 2019), node parts (Shmeit et al., 2020), and parts of the leaf (Zhang et al., 2020) are exposed to mitotic inhibitors for in vitro polyploidy induction in various plant species. This type of chromosome proliferation is widely used to induce tetraploidy through mitotic inhibitory chemicals (Podwyszy et al., 2015).

Still, chromosome proliferation forms somatic cells with extra copies of existing chromosomes and genes, but several changes occur after chromosome doubling which leads to a change in plant phenotype. Yet, in many products, lots of polyploidy plants have been created which shows the higher operation in comparison to diploid parents that can be due to improvements happening in the plant's organs which are not economically viable. Each product has different responses to polyploidy, according to the genomic structure, reproductive patterns, ancestral attenuation levels, and the purpose for which the crop is grown. The field approach (in vivo) is completely different from in vitro reconstruction protocol. Therefore, less expertise is needed. Immersion, soaking, intercropping and drip cultivation are different methods for mitotic spindle inhibitors in the field system. Higher concentrations and volumes of mitotic inhibitors result from increased mortality of mixoploid plants is higher (Castillo et al., 2009).

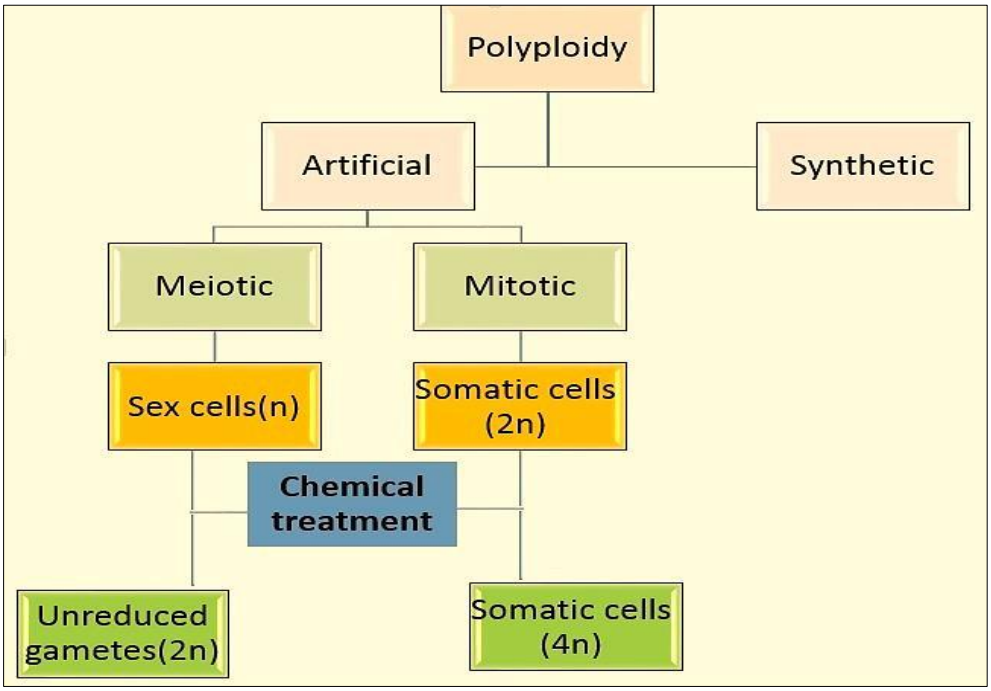

Figure 1. Polyploidy artificial induction systematic chart (Manzoor et al., 2019).

5. Effective elements on polyploidy induction

5.1. Plant tissue

Tissues with intense cell division are the most suitable plant receptors. Different explants have different potentials to induce artificial chromosome duplication (Fu et al., 2019) plant genotype is the most important factor influencing the induction of artificial polyploidy. The final polyploidy can be affected by the plant genotype in response to the parameters of mitotic inhibitors and in vitro regeneration (Niazian and Molaahmad-Nalousi, 2020). In order to increase the ploidy level of three rose species and create autopolyploids in them, Khosravi et al., (2008) used three substances: oryzalin, trifluralin and amiprophos methyl and showed that there was a significant difference between cultivars and ploidy surface enhancers. The different densities of colchicine on micro samples and somatic embryos of Lilium was examined and showed somatic embryos responded better to mitotic inhibitors than micro samples (Fu et al., 2019). The plant growth process (age of micro sample) is another important factor to create a successful artificial chromosome induction, especially in laboratory systems (Xu et al., 2018).

5.2. Ploidy surface enhancers

In artificial chromosome induction experiments, the mitotic inhibitor is the second most important factor (after plant factor). There are some of the effective parameters that should be considered such as the least effective density, the length of exposure time and the way of using. Various spindle inhibitors can be used in an artificial polyploidy induction study (Niazian and Molaahmad-Nalousi, 2020). A suitable ploidy enhancer in addition to being fast, effective and reliable for a wide range of plant species, should not cause physiological problems or genetic changes other than increasing the ploidy level in the plant. Almost a quarter of all herbicides on the market affect mitosis as their primary mechanism of action. These herbicides react directly or indirectly with microtubules to form a combination of herbicides and tubulin and stop the microtubules from lengthening. In a way that the microtubules open on one side and shorten progressively until they disappear (Jafarkhani Kermani and Emadpour, 2019).

The most common chemical is colchicine, an alkaloid compound that was first extracted from the wild species of the autumnal colchicum in 1883 by Zixel. The application of the chemical is in the meristematic region, that is, where cells are undergoing mitosis. Colchicine is highly toxic to animal cells and has anti-cancer potential, and is used in chemotherapy to prevent the growth of cancer cells in human medicine (Sivakumar et al., 2017). Another mitotic inhibitor is oryzalin which has recently been used as an alternative to colchicine. Oryzalin is more effective and less toxic to animals than colchicine because it is more dependent on plant tubulin-binding (Miguel and Leonhardt, 2011). Trifluralin, like oryzalin, is a herbicide of the dinitroaniline group, which pulls chromosomes to both sides of the cell by preventing the formation of protein strands during cell division and prevents the cell division (Zimmet and Ravid, 2000). Cerflen is a new combination of mitotic inhibitors used to induce polyploidization (Takamura et al., 2000). The ploidy surface enhancers have divided into two groups (Dhoogh et al., 2011).

The first group which includes the chemicals colchicine, colcemid, vinblastin, acenaphthene, oryzalin, trifluralin, benfluralin, ethafluralin, pendimethalin, butralin, dinitramin, amiprophos-methyl, chlorthaldimethyl, dithiopyr, thiazopyr and pronamide are used to prevent the polymerase of microtubules, and the second group contains chlorpropham, propham, and carbetamide that disrupts the cell cycle by disrupting and fragmenting the center of microtubule organization. While Amiprophos-methyl and oryzalin have higher properties for plant tubulin in mitotic inhibitors, they do not tend to bind to animal tubulin, therefore are safer than colchicine (Ebrahimzadeh et al., 2018; Grosso et al., 2018) and heir effective density is 50 to 250 times less than colchicine (Eng and Hu, 2019). Higher density than the effective level has a lethal effect on the treated plants, so finding the minimum effective density of mitotic inhibitors is critical (Touchell et al., 2020), because the mechanism of action of ploidy enhancers is not fully understood. Therefore, it is not possible to provide optimal instruction to increase ploidy levels as different factors affect polyploidy induction (Niazian and Molaahmad-Nalousi, 2020).

6. Polyploidy effects on morphology, physiology and cytology of medicinal and ornamental plants

The most important effect of polyploidy in plants is to increase cell size. The increase in cell size is due to the increase in nuclear content, which causes cell splitting reduction whilst their growth. The "Gigas effect" is most commonly seen in various commercially targeted plant organs like leaves, seeds, and flowers (Botelho et al., 2015). Tetraploid cells are about twice the size of diploid progenitors and increase the cell surface for about 1.5 times (Lavania, 2013). Fig. 2 on account of cell enlargement, polyploidies are faced with increasing plant body size in terms of morphological characteristics (Ramsey and Schemske, 2002; Dhooghe et al., 2011; øvrebø and Edgar, 2018). Fig. 3 Phenotypic differences are associated with a couple of inherently polyploidy topics which help the most compatible effectiveness of the "polyploidy phenotype": DNA content increase and consequently, gene rate rise (Salma et al., 2017). To maintain a constant relation between cytoplasmic and nucleus volume, cells develop with more chromosomes and consequently more DNA. As a result, these increases can be spotted in the size of the plant body and even the whole plant (Rauf et al., 2006).

Doubling through ploidy surface enhancers has increased leaf and branches number, plant height and stem length in salvia (*Salvia coccinea cv Coral Nymph*) (Kobayashi et al., 2008), tobacco (*Nicotiana alata*), prunella (*Prunella vulgaris*) (Kwon et al., 2014), *Zingiber officinale* (Prabhukumar et al., 2015), orchid (*Dendrobium nobile*) (Vichiato et al., 2007) and Lilium. The resulting polyploidy also increases the color and leaf area of *Impatiens balsamina*, prunella (*Prunella vulgaris*) (Kwon et al., 2014), pot marigold (*Tagates erecta*) (Sadhukhan et al., 2014), and chrysanthemums (*Dendranthema grandiflora*) (Lertsutthichawan et al., 2017). Duplication of mitotic chromosomes through colchicine treatment leads to large-scale inflorescence production and growth in flower parts in Salvia (*Salvia coccinea cv. Coral Nymph*), but blooming is put back for 10 to 30 days (Kobayashi et al., 2008). The flower weight and diameter is enlarged by feverfew tetraploid plants (*Tanacetum parthenium*) but in comparison with diploid plants just 50% of flowering is produced (Majidi et al., 2010). Also in *zingiber officinale* species (*Larsenianthus careyanus*) leaf number and shape, and flower size together with inflorescence and spike length have been grown with the chromosome duplication (Prabhukumar et al., 2015). *Chrysanthemum carinatum* has had larger flowers with thicker petals that increased their pot life (Kushwah et al., 2018).

In gladiolus, larger flower size and utmost postharvest lifetime could be seen in colchicine putative derived from polyploidies, as well. In addition, new changes in flower morphology such as serrated margins with prominent growth in gladiolus petals have been observed (Manzoor et al., 2018). Due to the higher density of colchicine in African marigolds (*Tagetes erecta*) flowering in treated plants happened earlier (59 days) than the control plants (80 days). Furthermore, more flowers with increased diameter and weight have been produced in polyploidy (Rathod et al., 2018). Phenotypic changes in plant body size have been observed in many species. Even though the plant body size increases, an increase in biomass doesn't always lead to the net result. An increase in dry and fresh weight often causes higher biomass (Gao et al., 2002; Kim et al., 2004; Mishra et al., 2010). Still in some cases, organ numbers reduce and the biomass doesn't increase (Xu et al., 2014; Kaensaksiri et al., 2011). After polyploidy, the thing we are looking forward to is the plant body size change. Therefore, a connection between the production of secondary metabolites and the change in plant body size caused by polyploidy has been established by recent studies (Lavania, 2013; Lavania et al., 2012).

An intense connection between the quality combination of essential oil of lemongrass (Cymbopogon spp) and the unpleasant changes in plant body size and biomass have been found by other researchers. They have shown that in those species with alcohol-rich essential oil, plant body size had a greater rise (There is an intense and positive relationship between the source diploid alcohol density and plant biomass rise in the derived Autotetraploidy), whereas, in those species aldehyde-rich oil was greatly reduced (There is a strong negative relation between aldehyde density in source diploid and changes in plant biomass in Autotetraploidy derivative) (Iannicelli et al., 2020). About the change in cell size, it is assumed that autopolyploid makes a general increase in cell size (the size of the cells secreting the essential oil) but their number reduces (Lavania et al., 2012).

In addition to obvious changes in the morphology of ornamental plants, polyploidy can also have a significant effect on several plants physiological processes such as water relations. Larger pores with lower frequency per unit area have been observed in polyploidy plants of feverfew (*Tanacetum parthenium*), phlox (*Phlox drummondi*) (Majidi et al., 2010; Tiwari and Mishra, 2012), common sage (*Salvia hains*) (Grouh et al., 2011), petunia (*Petunia hybrida*) (Ning et al., 2009), Mexican marigold (*Tagates erecta*) (Sadhukhan et al., 2014), chrysanthemum (*Aromaticum Dendranthema indicum var*) (He et al., 2016), and amaranth (*Celosia argentea*) (Mostafa and Alahmad, 2016). The results show that these changes reduce the rate of transpiration (overall gas exchange rate) in polyploidy plants. In addition, increasing the size of the vacuole and thicker leaves preserve more water content, which can be used in drought conditions. Therefore, these polyploidy plants can be grown in areas with limited water and can also be modified by producing other species to produce drought-tolerant genotypes. This method can be useful for the domestication of some species in hot climates (Manzoor et al., 2019).

Induced autotetraploid stomata are also larger and are used as one of the main indicators of ploidy level change in species because their size is simple to determine and does not require advanced equipment (Salma et al., 2017). Corneillie et al., (2019) by working on Arabidopsis thaliana found that polyploidy also alters cell wall and sugar composition. These changes can affect the structures involved in the production, secretion, storage of essential oils, regardless of their size, it should also be considered when analyzing various patterns of secondary metabolites. The stress response is another trait that can affect polyploidy levels. In other study reported a higher capacity of autoploids to adapt to oxidative stress by increasing the activity of enzymes with antioxidant activity (Zhang et al., 2014).

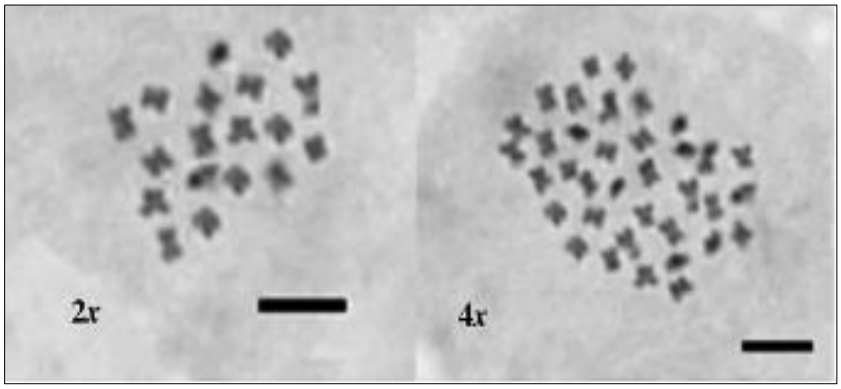

Figure 2. The chromosomes of diploid (2n = 2x = 18) and tetraploid (2n=4x=36) plants (scale bar = 10 μm) (Talebi et al., 2017).

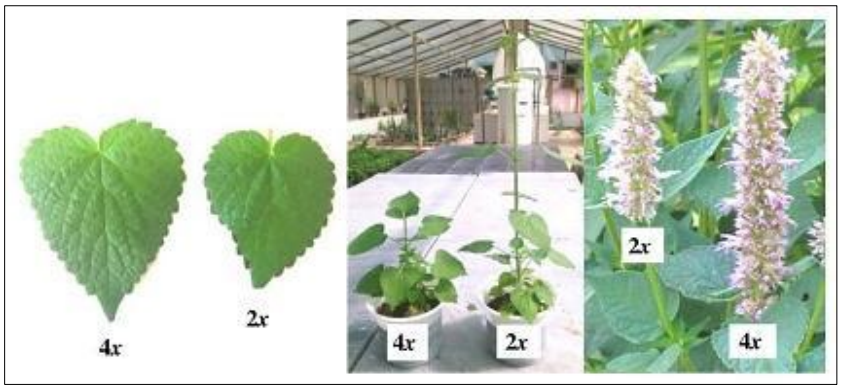

Figure 3. Morphological changes between the diploid and tetraploid anise hyssop plants (Talebi et al, 2017).

7. Disadvantages of polyploidy

Apart from the major advantages, there are still several disadvantages that can occur due to the increase in the number of chromosomes. Increasing the nuclear content of the cell increases the volume of the cell. Doubling the cell genome is expected to double the size of the nucleus, but at the nuclear volume level, it increases only 6.1 times, which can upset the balance between chromosomes and nuclear components. This imbalance can cause various abnormalities during mitosis and meiosis (Comai, 2005; Bharadwaj, 2015; Madlung, 2013). High levels of polyploidy, for instance in octoploids, cause poor appearance phenotypes due to physical instability and excessive gene overgrowth. Like the five-fingered tree (*Vitex agnus castus*) colchicine produced destructive effects by producing tetraploid dwarf plants with short internodes and smaller leaves without flower bud formation (Ari et al., 2015). Similarly, scaling and thickening of leaf tissue, deformation, reduced growth and loss of growth points were also observed in two cultivars of common zinnia (*Zinnia violacea*) due to colchicine use.

Decreased or no fertility associated with meiotic problems is an expected outcome of this process (Fake pairing between multiple chromosomes, unpaired chromosomes, and gametes with unbalanced chromosome counts) (Madlung, 2013), It is traditionally thought that autopolyploids are not more fertile than allopolyploids

and therefore are less likely to create and maintain a good population (Ramsey and Schemske, 2002; Soltis et al., 2007). In a study conducted on hollyhocks (Hibiscus moscheutos), flowers of colchicine-induced tetraploid plants did not produce any pollen, while triploid plants produced inanimate pollen grains but the fruits were aborted after pollination, resulting in triploid infertility (Li and Ruter, 2017).

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How to cite this paper:
Ghasemi, M., Aelaei, M., Akbari-Soltankohi, F., Minaei Chenar, H., Bahrami-Rad, E., 2021. Studies on polyploidy induction for improvement of quality traits in ornamental and medicinal plants. *Cent. Asian J. Plant Sci. Innov.*, **2**, 76-90.