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Review paper

Artificial polyploidy in the improvement of horticultural crops

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Abstract
Polyploids are organisms that have more than two paired homologous sets of chromosomes. Polyploidy has played a significant role in the evolution and diversification of higher plants. Artificial polyploidization has been induced using a few antimitotic chemicals such as colchicine, trifluralin and oryzalin. The type of mitotic inhibitors and their concentration and duration time are variable and species-dependent. This technique can be applied *ex vitro* or *in vitro* of which, tissue culture is simpler and more efficient. Changes in nuclear DNA content, gene expression and developmental processes due to ploidy manipulation can lead to morphological, anatomical and physiological changes in polyploid plants. In general, polyploid plants exhibit larger organs, greater biomass, higher yield, superior tolerance to biotic and abiotic stresses as well as higher primary and secondary metabolites. Also, polyploidy often reduces fertility and allows the production of seedless fruits. In ornamental plants, increasing the size of polyploid flowers is aesthetically and economically important. There are two direct and indirect methods for the ploidy determination of plants. Indirect methods are simpler but more inaccurate, involving the relationship between ploidy level and morphological (i.e. plant height, leaf size and pollen diameter) and anatomical (i.e. stomatal frequency and size as well as chloroplast number in guard cells) properties. In contrast, direct assay methods, such as chromosome counting in mitotic cells of root-tips and flow cytometry are accurate techniques for the determination of ploidy level in plants. Overall, polyploidy manipulation has long been used in improving the yield of many crop plants and can be considered as one of the most promising tools in plant breeding programs.

Keywords: Antimitotic agent; Flow cytometry; Genome doubling; Plant breeding; Polyploid.

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Introduction

Polyploidy refers to an organism that contains three or more complete sets of chromosomes per cell nucleus (Ranney 2006). It was first discovered in 1907 and is one of the more prominent mechanisms of speciation and diversification in higher plants (Lutz 1907; Wendel and Doyle 2005). The estimation of polyploidy frequency in angiosperms (flowering plants) is widely variable in the literature, ranging from 30-80% for with most estimates about 50-70% (Masterson 1994; Soltis *et al*. 2015). In gymnosperms, polyploidy is rare; however, Smarda *et al*. (2018) found a viable spontaneous tetraploid *Ginkgo* seedling in a Botanical Garden in the Czech Republic. The evolution of polyploidy is a continuous process, and not only a rare occurrence but also produce at a relatively high frequency, as it is estimated that the total rate of autotetraploid formation is the same as the rate of genetic mutation (10^{-5}) in flowering plants (Ramsey and Schmeske 1998;

Soltis *et al*. 2015). Since many important crops are polyploid, the attention of plant breeders has led to utilize it as a tool for crop improvement and induce desirable traits (Sattler *et al*. 2016; Salma *et al*. 2017; Miri and Roughani 2018a). Polyploids often possess novel biochemical, physiological, morphological and ecological traits, and presumably, it is the greater environmental adaptability of the polyploids that has allowed them to establish and sustain the severity of environmental change over the evolutionary time scale (Levin 1983). It has been demonstrated that polyploids show, although not always, increased gene activity and enzyme diversity, photosynthetic capacity, yield and biomass, tuber, rhizome, root, fruit and flower size, leaf length, leaf thickness, color intensity, flowering time, resistance to nutrient deficiency, diseases, pests, drought and cold stresses, increased secondary metabolite production as well as primary metabolism in medicinal plants, may break the self-incompatible system and restore fertility and cause dwarfism (Levin 1983; Parida and Misra 2015; Hannweg *et al*. 2016; He *et al*. 2016).

Traditionally, the polyploidy could be chiefly classified into two major groups: (i) allopolyploidy - more typically in nature, by hybridization of two or more different species and consequent duplication of the chromosomes of the resulting hybrids, and (ii) autopolyploidy - doubling homologous genomes of a species (Ramsey and Schmeske 1998; Hegarty *et al*. 2013). For instance, the AAA genomic group forms the major autopolyploids valued for its commercial uses in the *Musa acuminata* (A-genome) industry. Interspecific crosses occurred between *M. acuminata* diploids and *M. balbisiana* (B-genome), resulting in different allopolyploids including AB, AAB, ABB and ABBB (Subbaraya *et al*. 2011).

This paper provides an overview of the applications and advantages of polyploidy in plant improvement and the techniques for artificial polyploidization and detection, as well as examples of the effects of induced polyploidy on some horticultural crops.

Applications of polyploidy in plant breeding

Polyploidy induction has played a credible approach to plant improvement. Many vegetatively propagated flowers and fruits as well as crop plants are polyploids (Dhooghe *et al*. 2011; Corneillie *et al*. 2019). The effects of polyploidy in plants often seem to be associated with observable changes in phenotype such as increases in vigor and adaptation of the newly formed polyploid to novel conditions (Levin 1983; Sattler *et al*. 2016). These interesting features exhibited by polyploid individuals have led to an increased interest in developing artificial polyploids (Dhooghe *et al*. 2011; Sattler *et al*. 2016).

Reports indicate that natural and artificial polyploids have shown rapid and dynamic changes in genomic structure and gene expression after polyploidization (Song and Chen 2015). Genotypic changes in polyploid plants may occur due to heterozygosity, gene silencing and gene dosage effect or because of epigenetic and genetic interactions (Dewitte *et al*. 2011).

Genomic changes include structural chromosome rearrangements, aneuploidy, DNA sequence change, loss of duplicated genes and gene conversion. Epigenetic modifications including

DNA methylation, histone acetylation, chromatin remodeling and RNA interference also occur, promoting alterations in gene expression (Sattler *et al*. 2016; Ding and Chen 2018). The affected traits are diverse, including flowering time, biomass, leaf morphology, etc., which are subject to selection and can lead to the domestication of crop plants (Osborn *et al*. 2003; Ding and Chen 2018).

The most widespread consequence of polyploidy in plants is the increase in cell size, due to genome duplication, which is known as the *gigas* effect. Therefore, organs of polyploid individuals may exhibit an increase in size compared to their diploid progenitors, such as roots, leaves, tubercles, fruits, flowers and seeds. An increase in cell size is typical in polyploids, with tetraploid cells approximately have twice the volume of their diploid counterparts, but it does not necessarily lead to increased size of the whole plant or its organs, since the number of cell divisions in polyploids is often reduced (Hegarty *et al*. 2013; Sattler *et al*. 2016). Volume is ultimately a cubic function of the linear dimensions of the cell, while the area is a square function (Epstein 1986). Indeed, by doubling the cell genome, the nucleus volume increases up to 1.6 fold in the nuclear surface area, which can disrupt the balance between the chromosome and nuclear components. It is assumed that metabolism and overall growth would be inhibited in polyploids due to the altered ratios of nuclear/cytoplasmic volume. Therefore, high levels of polyploidy, for instance in octoploids, can cause stunted and malformed plants due to somatic instability and extreme gene redundancy (Manzoor *et al*. 2019). Also, it was revealed that autotetraploids of several plants possessed, on average, 30% less hormone content than diploids (Gustafsson 1944). Because of this, polyploid plants have typically lower growth rates, which tend to flower later or over a longer period than related diploids, which is a desirable feature for ornamental breeding (Sattler *et al*. 2016). However, this is different at specific stages of life, so the larger endosperm of polyploid seed can lead to faster growth in the early stages of seedling development, but not necessarily in adult stages of the plant development (Hegarty *et al*. 2013).

Polyploids frequently demonstrate disrupting of self-incompatibility systems, allowing self-fertilization (Comai 2005; Hegarty *et al*. 2013). The reduction in seed sterility is another common consequence of autopolyploidy and may result from meiotic irregularities (Sattler *et al*. 2016). Therefore, autopolyploidy induction in breeding programs is usually favorable in species grown for their vegetative organs and those with vegetative propagation, e.g. triploid watermelon and banana (Acquaah 2015; Sattler *et al*. 2016). For ornamental breeding, reduced fertility is not a problem, since larger and more beautiful flowers may offset the lower number of flowers and seed production (Sattler *et al*. 2016). Triploidy promote vegetative growth by conserving a large amount of photosynthetic energy consumed by seed and fruit production, hence they are very important in trees and shrubs that are used for biomass and soil conservation (Hoshino *et al*. 2011).

Interspecific hybrids are usually sterile due to the failure of chromosome pairing during meiosis. Chromosome doubling provides a pathway to resolving major chromosomal differences in interspecific hybrids by preparing each

chromosome gets its exact copy and chromosomal homology, which result to overcome hybrid sterility and produce viable offspring after interspecific or interploidy crosses (Aversano *et al*. 2012; Hegarty *et al*. 2013; Manzoor *et al*. 2019). Artificial polyploidization has been utilized as a means to resolve the difficulties in crossing between two *Vitis* subgenus: *Euvitis* (2*n* = 2x = 38) and *Muscadinia* (2*n* = 2x = 40) (Owens 2008).

In addition to the alterations in the morphology, polyploid plants exhibit larger stomata with lower density, enlarged vessel diameter, bigger vacuole, thicker leaves, denser pubescence, lower transpiration rate, higher rates of photosynthetic activity and lower specific hydraulic conductivity that may result in higher capacity to drought stress (Levin 1983; Maherali *et al*. 2009; Dhooghe *et al*. 2011; Manzoor *et al*. 2019). Autopolyploids may also be more compatible with the environmental conditions such as nutrient deficiency, temperature, pests and pathogens stresses (Levin 2002).

Furthermore, by increasing the nuclear content in the polyploid plants, gene expression is increased, which eventually leads to increased production of secondary metabolites. These metabolites not only enhance the plant resistance and tolerance mechanisms but are also valuable in pharmacology (Manzoor *et al*. 2019).

Although polyploidy has been obtained in many crops, they do not always present higher quality and/or quantity than their diploid relatives or this improvement occurs in organs that are not of commercial interest. Each plant species responds differently to polyploidy induction, depending on their ploidy level, genome structure, reproduction state, perennially and the plant organ for which the crop is cultivated (Sattler *et al*. 2016). Polyploidy also has disadvantages, including changes in cellular architecture and regulatory implications, problems in mitosis and meiosis, regulatory changes in gene expression and epigenetic instability (Comai 2005).

M Methods of polyploidy induction

It was generally assumed that polyploids in plants are induced through two mechanisms: sexual (meiotic) polyploidization or somatic (mitotic) doubling in meristem tissue of sporophytes (Ramanna and Jacobsen 2003).

1. Sexual polyploidization

Before the discovery of colchicine in the 1930s, meiotic polyploidization was commonly used for obtaining polyploids (Ramsey and Schmeske 1998; Ramanna and Jacobsen 2003). This process involves the generation of unreduced (2*n*) gametes during gametogenesis, which contain the full somatic chromosome number. Meiotic aberrations related to spindle formation, spindle function and cytokinesis have been implicated as the cause of unreduced gamete production (Ramsey and Schmeske 1998).

The mechanisms of 2*n* gamete formation can be divided into three developmental-specific classes: pre- and post-meiotic genome doubling and meiotic restitution. Pre- and post-meiotic genome duplication is only rarely recorded in plants, whereas meiotic restitution is the predominant mechanism of unreduced gamete formation. In this process, meiotic cell division is converted into a mitosis-like nonreductional

process, generating dyads (and triads) instead of the normal tetrads at the end of meiosis II (De Storme and Geelen 2013). The fusion of reduced (*n*) and unreduced gametes, or of two unreduced gametes, can produce triploid and tetraploid embryos, respectively. It is believed that unreduced gametes (2*n* pollen or 2*n* eggs) to be a major mechanism of polyploid formation (Ramsey and Schmeske 1998). In cacti, most polyploids originated by fusion of 2*n* gametes (Karle *et al*. 2002). Triploids are also produced by crossing diploids and tetraploids (Ramsey and Schmeske 1998). Sedov *et al*. (2017) reported that in diploid × tetraploid apples crosses, 31.8% diploid, 68.0% triploid and 0.1% tetraploid plants were formed, whereas, in tetraploid-diploid crosses, 22.4% diploid, 54.0% triploid and 23.4% tetraploid were obtained.

Polyspermy, the fertilization of an egg by multiple sperm, is a widespread phenomenon in angiosperms (Vigf´usson 1970). The polyspermy-derived triploids are taller and produce bigger organs than monospermic plants, however, it is lethal in many eukaryotes and generally considered as an uncommon mechanism of polyploid formation (Grant 1981; Nakel *et al*. 2015). The major advantage of sexual polyploids against somatic polyploids is that they enhance genetic variation of the progeny, allowing the maintenance of high levels of heterozygosity and, therefore, a potentially higher degree of expression of traits (Ramsey and Schmeske 1998; Sattler *et al*. 2016).

For some crops, such as triploid *Musa acuminata* and *M. balbisiana*, sexual polyploidization may be the most efficient way to produce polyploids. However, the application of sexual polyploidization is restricted by the low frequency of unreduced gametes production (Sattler *et al*. 2016). It has been found that unreduced pollen production is mainly controlled genetically, however, several pieces of evidence have shown that the genes involved in the control of the 2*n* pollen production are highly influenced by environmental conditions such as temperature, light, herbivory, wounding and water and nutrient stress, of which light and temperature, especially changes in temperature during gametogenesis, have particularly large effects on meiotic abnormalities (Ramsey and Schmeske 1998; Guerra *et al*. 2016; Martin *et al*. 2019).

Besides, several attempts to increase the production of 2*n* gametes have been made, by applying nitrous oxide (N_2O), anti-tubulin agents and ethyl methane sulphonate (EMS), as well as gene silencing by RNA interference and virus-induced gene silencing (Dewitte *et al*. 2011). Induction of 2*n* pollen in tulips was obtained by the treatment of bulbs with N_2O for 24-48 h at 6 atmosphere when meiosis in the anthers reached metaphase I (Okazaki *et al*. 2005).

2. Somatic polyploidization

Somatic polyploidization implies the induction of chromosome doubling in somatic tissues, and has been performed in several crop species (Sattler *et al*. 2016). There is a wide range of natural and synthetic compounds that are reported to interrupt the cell cycle mainly in the late metaphase stage and known as the antimitotic agents (Ascough *et al*. 2008; Salma *et al*. 2017). Initial efforts to induce somatic polyploidy were made through other methods, such as exposure to high or low

temperature (Blakeslee and Avery 1937). An increased frequency of tetraploid cells was observed in root-tips of *Pisum sativum* and *Zea mays* plants which had been exposed to hot water at 40 °C (Randolph 1932). Nevertheless, the advancement in artificial polyploidy induction was accomplished only after the introduction of colchicine by Blakeslee and Avery (1937) (Sattler *et al*. 2016; Salma *et al*. 2017).

Colchicine is a toxic alkaloid extracted from seeds and bulbs of *Colchicum autumnale* L. and the most widely employed antimitotic agent for polyploidy induction (Sattler *et al*. 2016). During metaphase, a microtubules network comprising of a dimer of α- and β-tubulin, forms a spindle fiber that emerges out from the microtubule-organizing center. The spindle fiber is essential during anaphase for proper polar migration of chromosomes (Salma *et al*. 2017). Colchicine is a metaphase inhibitor and its mechanism of action involves binding to α- and β-tubulin dimers, disruption of microtubule assembly during the cell cycle and prevention of polar chromosome migration during anaphase that results to raise the ploidy level (Sattler *et al*. 2016). It is very toxic to humans because it binds tightly to the microtubules of an animal cells. However, it has a poor affinity for plant tubulins and must be used in relatively high concentrations. These shortcomings necessitate searching for a substitute cell cycle inhibitor that has a higher affinity for plant tubulins (Salma *et al*. 2017). About 25% of all herbicides are likely to affect the mitosis of the plants that may be used as antimitotic agents at lower concentrations. They belong to different chemical classes, including vinblastin, acenaphthene, dinitroanilines (trifluralin, oryzalin, benfluralin, ethalfluralin, pendimethalin, butralin, dinitramin), pyridines (dithiopyr, thiazopyr), benzamides (pronamide, propyzamide), phosphoroamidates (amiprophos-methyl, butamiphos), benzoic acid (chlorthaldimethyl), carbamates (chlorpropham, isopropyl N-(3-chlorophenyl) carbamate) and others (Dhooghe *et al*. 2011; Salma *et al*. 2017; Roughani and Miri 2018b). Except for carbamates, these antimitotic agents, like colchicine, are metaphase inhibitors. Carbamates disrupt and fragment the microtubule organizing center, but do not depolymerize microtubuli. Instead, they alter the organization of the spindle microtubules so that multiple micronuclei are formed (Vaughn and Lehnen Jr 1991; Dhooghe et al. 2011). Pliankong *et al*. (2017) demonstrated that the induction of polyploidy in *Capsicum frutescens* by colchicine was more effective than oryzalin. Roughani *et al*. (2017) treated the seeds of *Spinacia oleracea* with colchicine, trifluralin and oryzalin and found that all three antimitotic agents could be effective in the increase of polyploidy induction, but oryzalin was preferred for its low toxicity, low cost and ability to increase ploidy levels at lower doses. On the other hand, plant materials responded differently to the chemicals, showing diversity in antimitotic sensitivity and effect indicating (Carvalho *et al*. 2016). For instance, Talebi *et al*. (2017) reported that the highest tetraploid plants (20%) were observed from seeds treated with 100 μM oryzalin for 24 h, whereas the highest tetraploid induction of apical meristems and seedlings (16%) was achieved with 17500 μM colchicine and 50 μM trifluralin, respectively.

Endopolyploidy is commonly observed in

many species of orchids (Chen and Tang 2018). Chen and Tang (2018) described a simple and effective technique for the induction of polyploids in *Phalaenopsis* orchids by horizontal sectioning of protocorms without using antimitotic agents.

2.1. *Ex vitro* polyploidy induction

Antimitotic agents are applied by two methods of *ex vitro* and *in vitro* (Roughani and Miri 2018b). Initially, colchicine was applied by dipping of seeds, shoot apices, or the whole plant in solution or repeated application of single drops of solution to a bud (Blakeslee and Avery 1937). The application of antimitotic agents is generally done by foliar spray or cotton plug method, but the most effective method for tetraploidy induction is through pre-germinated seeds having emerging roots (Manzoor *et al.* 2019; Salma *et al*. 2017). However, Noh *et al*. (2012) obtained a suitable method with high efficiency for inducing tetraploids in *Citrullus lanatus* by treating seed, shoot apex and inverted hypocotyl with 0.1% and 0.2% colchicine and concluded that the highest rate tetraploids (29.5%) were identified when hypocotyl portion of seedlings was placed at the inverted position in the glass tubes containing 0.2% colchicine for 15 h. He *et al*. (2016) examined plant materials, colchicine concentrations and duration time for improving the induction of polyploidy in *Dendranthema indicum* and found that soaking the germinated seeds in 0.1% colchicine for 24 h (14.5%) and dropping of 200 µl 0.1% colchicine by using a micropipette onto a cotton plug placed on shoot tips for 7 d (40%) were suitable method to induce chromosome doubling. It is believed that this technique is generally uneconomical due to the wastage of chemicals through evaporation (Salma *et al*. 2017). Even though the *ex vitro* system of regrowth is cheaper, it has a lower rate of polyploidy induction and high numbers of mixoploids and is more time-consuming than the *in vitro* techniques (Salma *et al*. 2017; Roughani and Miri 2018b). Shi *et al*. (2015) stated that the popular synthetic polyploidy induction methods for fruit trees cause difficulties and usually take at least 2-3 years to thoroughly separate mixoploids and obtain pure polyploidy, and another 3-4 years to get fruits from an *in vitro* plantlet. To overcome these challenges, Shi *et al*. (2015 & 2016) developed a mixoploid-free *in vivo* autopolyploid induction technique in the *Ziziphus sp.* by integrating *in vivo* bud regeneration via calli with polyploid induction. Their novel protocol included field callus induction from strong branches treated with 4 mg/L thidiazuron plus 2 mg/L $AgNO_3$, callus cell polyploidy induction with 0.05% colchicine, and shoot regeneration from a chromosome duplicated callus cell.

2.2. *In vitro* polyploidy induction

In vitro polyploidy induction is recommended since plant growth regulators in the supplemented media raise the regeneration of explants and also shortens the time and space (Salma *et al*. 2017). Murashige and Nakano (1966) first reported an *in vitro* protocol that resulted in the spontaneous polyploidy induction of tobacco calli and suggested that *in vitro* culture is a potential tool for artificial polyploidy. The *in vitro* technique is simpler to apply and more efficient in inducing polyploidy due to the controlled conditions than the greenhouse (Salma *et al*. 2017). The effectiveness

of an *in vitro* chromosome doubling depends on many factors, such as a well-established protocol for the multiplication of the target species, type and concentration of the antimitotic agent, exposure time, the method of antimitotic solution application, explant type and regrowth medium (Sattler *et al*. 2016; Salma *et al*. 2017). So, the development of an *in vitro* polyploidy induction protocol requires several tests to obtain the most appropriate combination of antimitotic agent concentration and exposure time for each species (Sattler *et al*. 2016). Dhooghe *et al*. (2011) summarized the most currently used methods for *in vitro* polyploidy induction of fruits, vegetables, ornamental and medicinal plants.

2.2.1. Genotype

Chromosome doubling is genotype-dependent and plants with lower ploidy levels have a higher propensity for polyploidy induction (Khosravi *et al*. 2008; Roughani and Miri 2018b). Some research has indicated that oryzalin was less efficient for chromosome duplication in cassava (Mondin *et al*. 2018) and chili pepper (Pliankong *et al*. 2017), but more efficient in *Alocasia* (Thao *et al*. 2003), ornamental ginger (Sakhanokho *et al*. 2009) and blueberry (Tsuda *et al*. 2018). Mondin *et al*. (2018) studied *in vitro* polyploidy induction of two cassava cultivars and found that Vassourinha genotype was more responsive to colchicine than Colombia 22. However, Chauvin *et al*. (2005) demonstrated that the percentage of *in vitro* tetraploid tulip clones was not influenced by the genotype.

2.2.2. Explant source

True choice of explant is the critical step to induce polyploidy. Several explants have been employed as the starting material for successful polyploidization, for instance, seedling, shoot tip, callus, somatic embryo, seed, single node, tuber segments, cotyledon, hypocotyl and root tip (Ascough *et al*. 2008; Dhooghe *et al*. 2011; Salma *et al*. 2017). As endosperm is a triploid tissue, it would be reasonable to assume that endosperm culture is a useful procedure for the production of natural triploids from diploid plants, however, the time needed for triploid plant production by this technique is lower than conventional methods, but regeneration from cultured endosperm is often technically challenging and factors such as explant stage, media composition and additives, especially plant growth regulators are important (Hoshino *et al*. 2011; Wang *et al*. 2016).

The success of polyploidization depends upon the cells remaining in the active division stage and permeability potential of the antimitotic agents through the cell membrane and their transport capability to the meristem. Even though several explants have been used to effectively induce polyploidy and further multiplication, but apical bud or shoot tips and then seeds have been the most accepted explant choice, and other explants are rarely employed (Dhooghe *et al*. 2011; Salma *et al*. 2017). In an experiment conducted on *Petroselinum crispum*, the induction of polyploidy was higher in the node explants compared with the seeds (Nasirvand *et al*. 2018). The reviewed reports about the comparison of the explant sources on their influential role in polyploidy induction are insufficient, so it is recommended to consider the

different types and stages of explant for optimization (Salma *et al*. 2017).

2.2.3. Antimitotic agent

Colchicine is the most common antimitotic agent to induce polyploidy in plants. Even after autoclaving it does not lose its polyploidizing ability. But, colchicine has some adverse effects on various plant species, including abnormal growth, reduced viability due to irregular shaped nuclei and micronuclei, sterility, chromosome aberrations and gene mutation. In many instances, trifluralin and oryzalin are a standard preference against colchicine, have increased survival of explants and are used at lower concentrations (Ascough *et al*. 2008; Salma *et al*. 2017). Ascough *et al*. (2008) reported that in *Watsonia lepida*, oryzalin is more effective in comparison to colchicine in inducing tetraploidy. Similarly, in *Ranunculus asiaticus*, oryzalin and trifluralin were more efficient for chromosome doubling than colchicine (Dhooghe *et al*. 2009).

2.2.4. Concentration and exposure time of the antimitotic agent

Polyploidization is often induced through the treatment of explants with low concentrations of antimitotic agents in the liquid or solid culture medium during one subculture, or a short application in liquid medium containing high concentrations of antimitotic agents followed by culture of explants on a fresh medium (Dhooghe *et al*. 2009; Dhooghe *et al*. 2011). There are reports that the survival rate of explants decreased with increasing concentration of the antimitotic agent and its treatment period, while too low concentrations were unsuccessful (Nasirvand *et al*. 2018). He *et al*. (2016) indicated that high concentrations of colchicine at short durations of treatment and long durations at low concentrations could achieve the same desirable effect on ploidy induction. Zhang *et al*. (2018) also confirmed it and found 10 and 20% tetraploids by treating the germinated *Stevia rebaudiana* seeds for 24 h in 0.1% colchicine or 48 h in 0.05% colchicine, respectively. Treatment of 24 h of 0.05% colchicine was inadequate to induce polyploidy, whereas 48 h of 0.1% colchicine had some toxic effects, as germination rate and survivability of seedlings were very low. The tetraploid *Petroselinum crispum* plants in 0.05% colchicine increased from 12.5 to 75% by enhancing the treatment duration from 8 to 48 h, respectively (Nasirvand *et al*. 2018).

The solvent, which is used to dissolve the antimitotic agent is also important. It contributes to the efficacy of the treatment or sometimes toxicity to the explant (Salma *et al*. 2017). In many reports, antimitotic agents are dissolved in dimethyl sulfoxide (DMSO) (Shi *et al*. 2015; Tavan *et al*. 2015; Roughani *et al*. 2017; Manzoor *et al*. 2018). The main function of DMSO is to increase the permeability of cell membrane and allow easy absorption of chemicals into the cell, but sometimes it may induce cell mortality at higher concentrations. Colchicine dissolved in 3-4% DMSO decreased the explant survival compared to water or liquid medium, however, the frequency of tetraploids increased. As an alternative to prevent over-toxicity, other solvents can be selected, such as oryzalin, which can be dissolved in ethanol (70%) or NaOH (1 M), trifluralin in acetone

(100%) and colchicine in water, liquid MS medium (for *in vitro* polyploidy induction) or ethanol (96%) (Dhooghe *et al*. 2011; Salma *et al*. 2017).

2.2.5. Regrowth medium

Growth recovery after appropriate treatment with antimitotic agents is an essential step in this technique. A range of protocols was followed for the regrowth of polyploids and maintenance (Salma *et al*. 2017). In addition to genotype and explant type, components of basal media including both organic and mineral compositions as well as growth regulators strongly influence the growth and development of tissue culture plantlets (Hasanloo *et al*. 2014; Fallahpour *et al*. 2015; Erfani *et al*. 2017; Abbaszadeh *et al*. 2018; Miri and Roughani 2018c). Most researchers have suggested Murashige and Skoog (1962) (MS) or modified MS media for shoot multiplication of the polyploids because it contains all of the essential nutrients for *in vitro* growth (Ascough *et al*. 2008; Dhooghe *et al*. 2009; Hannweg *et al*. 2016; Hias *et al*. 2017; Miri and Roughani 2018b; Parsons *et al*. 2019; Podwyszyńska and Pluta 2019). Silva *et al*. (2000) cultured young shoots of *Cattleya intermedia* in a modified liquid induction medium (Vacin & Ment 1949) supplemented with coconut water, myo-Inositol, vitamins (nicotinic acid, pyridoxine HCl, thiamine HCl), amino acids (glycine, tryptone) and sucrose. After four weeks, the explants were transferred to the above-mentioned medium containing BAP and NAA to grow. Then, the grown meristems were sub-cultured to the solid ½ MS salts proliferation medium containing BAP, NAA and the same vitamins and amino acids of the induction medium. After the colchicine treatment, the protocorm-like bodies were transferred to the colchicine free induction liquid medium for two days. Subsequently, the protocorm-like bodies were transferred onto the proliferation solid medium to multiply and plantlets to develop. In the immature endosperm, it is essential to add a nitrogen source such as yeast extract, casein hydrolysate, coconut milk, or tomato juice for endosperm multiplication (Hoshino *et al*. 2011).

Assessment system

After induction of chromosome doubling, it is important to confirm polyploidy status in plants as they might have several polyploid series or chimeras in their tissues (Sattler *et al*. 2016; Manzoor *et al*. 2019). The methods for detection of polyploidy are categorized as direct and indirect. Indirect methods involve physiological, morphological and anatomical traits that are rather rapid and simple but often inaccurate. Conversely, confirmation through direct methods is accurate and sometimes necessary, for instance, chromosome counting and measuring nuclear genome by flow cytometry (FCM) (Sattler *et al*. 2016; Salma *et al*. 2017).

1. Indirect assay

The indirect measures for polyploidy identifications include morphological and anatomical features. The morphological evaluation takes account of plant height, shoot number and length, root number and length, leaf size and pollen grain diameter (Salma *et al*. 2017). Morphological traits commonly associated with polyploidy are bigger flowers, higher yield, larger fruit size,

altered leaf length-to-width ratio, darker-colored green and ticker leaves, stouter stems and shorter internodes (Hias *et al*. 2017; Zhang *et al.* 2018). Hannweg *et al*. (2016) stated that the induced tetraploid *Tetradenia riparia* plants were characterized by thicker, stickier and more rounded leaves compared with the diploid plants. Anatomical assessment engrosses stomatal frequency and size as well as chloroplast density in guard cells (Salma *et al*. 2017). The measurement of stomatal dimensions is rapid, inexpensive, non-destructive, does not require expensive equipment and has a fairly high accuracy rate (Ascough *et al*. 2008). Polyploids typically exhibit larger stomata in lower density than the diploids and chloroplast number per guard cell is higher (Sattler *et al*. 2016; Salma *et al*. 2017). If chimeras (mixoploid) plants exist, then stomata size and density can be an unreliable method to select putative tetraploids and should be combined with another technique (Chen *et al*. 2006). Pliankong *et al*. (2017) observed an increase in the stomata size of guard cells and a decreased density of stomata per unit leaf area. Tetraploid *Stevia rebaudiana* plants had larger stomata, higher chlorophyll content index and approximately 2-4 times more glands of the diploid controls; however, they only had about half the number of stomata (Zhang *et al*. 2018). Similarly, Nasirvand *et al*. (2018) found that the size of the stomata and leaves in tetraploid *Petroselinum crispum* plants was larger as compared to the diploids, but the density of stomata was decreased. However, tetraploid *Dendranthema indicum* had greater values for stomatal density and size, and the chloroplast number of guard cells than the controls (He *et al*. 2016). Hias *et al*. (2017) noticed that the pigment content (especially chlorophyll *a* and *b*) considerably was higher in tetraploid apples. According to Salma *et al*. (2017), tetraploid-induced plantlets showed decreasing plant height, internode length and root length than the normal diploids, but a higher width/length ratio of the leaf and stem diameter was observed. Hias *et al*. (2017) suggested that the frequently observed lower growth rate and smaller organ size of tetraploids may be due to the decrease in cell density, which can be partially compensated by an increased cell volume. Polyploidy was also reported to affect pollen size, and pollen diameters in the polyploid plants were higher than diploids, but their viability and *in vitro* and *in vivo* germination were lower (Kuliev 2011; Martin *et al*. 2019). Thus, if diploid plants were pollinated with pollens of tetraploid plants, the fruit set rate, seed number and germination ability significantly reduced compared to reciprocal crosses and crosses between the controls (Zhang *et al*. 2016).

2. Direct assay

Chromosome counting has been considered the most accurate and efficient technique to detect ploidy levels (Sattler *et al*. 2016). Compared to diploids, polyploids exhibit multiple sets of chromosomes (Salma *et al*. 2017). However, cytogenetic techniques are often time-consuming, requiring highly specific protocols for each species (Sattler *et al*. 2016). Fixation is the most critical step in the cytological method on which the chromosome visibility depends. Counting the chromosome number in cells is sometimes hectic due to poor magnification of light microscope (Nagahatenna and Peiris 2008). Also, the

chromosomes appear so small, like dots, under the optical microscope that it is difficult to count (Tavan *et al*. 2015). Also, successful staining depends on temperature, pH, osmotic balance and fixation time. If there is a discrepancy in any of the factors, insufficient staining may result, and therefore, a limited number of cells could only be tested. For this reason, inaccuracy often occurs during the counting of small chromosomes (Bohanec 2003). Alternatively, FCM is a rapid, accurate and simple method to estimate ploidy level and genome size, which has been *used* extensively since the 1980s. Also, it is an efficient means of analyzing the nuclear DNA content of large populations within seconds and can be used in the early stages of plant growth (Xing *et al*. 2011; Hannweg *et al*. 2016; Zhang *et al*. 2018). The FCM technique follows an extraction of the cell nuclei using a razor blade chopping or bead beating method and subsequently, DNA is stained by a DNA fluorochrome such as DAPI (4′,6-diamidino-2-phenylindole) or PI (propidium iodide) that binds to the DNA (Salma *et al*. 2017; Roughani and Miri 2018a). The stained nucleus emits a fluorescence that can be measured with the aid of a flow cytometer (Figure 1) and the fluorescence intensity is directly related to the ploidy level (Roughani *et al*. 2017; Salma *et al*. 2017).

In the FCM analysis, the ploidy level is correlated with the accurate nuclear DNA content (Salma *et al*. 2017). Therefore, it is assumed that an increment in the DNA content is due to an increase in the chromosome number (Sattler *et al*. 2016). For flow cytometric genome size measurements, an internal reference standard (a plant with known nuclear DNA content, processed together with the sample) is needed. In many works, species such as *Petunia hybrida*, *Glycine max*, *Allium cepa*, *Pisum sativum* and *Petroselinum crispum* (Figure 2) which possess a stable genome size, serve as internal standards (Sattler *et al*. 2016; Roughani *et al*. 2017; Salma *et al*. 2017; Sliwinska 2018). FCM can efficiently discriminate the polyploids from diploids and mixoploids (Salma *et al*. 2017). Besides, flow cytometry requires expensive equipment and chromosome behavior cannot be observed with this method (Ascough *et al*. 2008).

Advances in molecular technologies have created opportunities for determining polyploidization. Guo *et al*. (2016) established an analytical toolkit for polyploid *Salix* identification by combining molecular markers and FCM. A total of 10 single-copy fully informative SSRs were chosen for marker-aided selection based on a segregation test with a full-sib willow pedigree and a mutability test with a collection of natural willow stands. They found that results from marker-aided selection were consistent with those from FCM measurements, and with this analytical toolkit, polyploids can be rapidly screened from a large number of natural stands. Zhang *et al.* (2019) developed a reliable real-time quantitative PCR (qPCR) technique by quantifying the highly conserved 5S rDNA sequence and its copy numbers for watermelon ploidy detection. qPCR is a mature method for gene expression detection and copy number analysis. They stated that this technique requires less sample collection and has comparable accuracy to FCM, it accelerates the analysis process and provides a new method for the identification of polyploidy of watermelon.

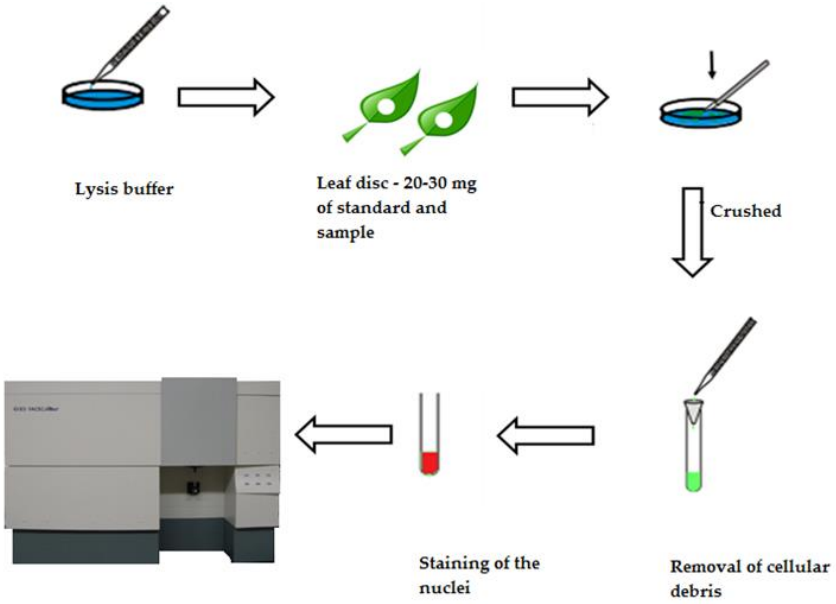

Figure 1. Diagram of the methodology used to analyze the nuclear DNA content from plant tissue (Pasqual *et al*. 2012).

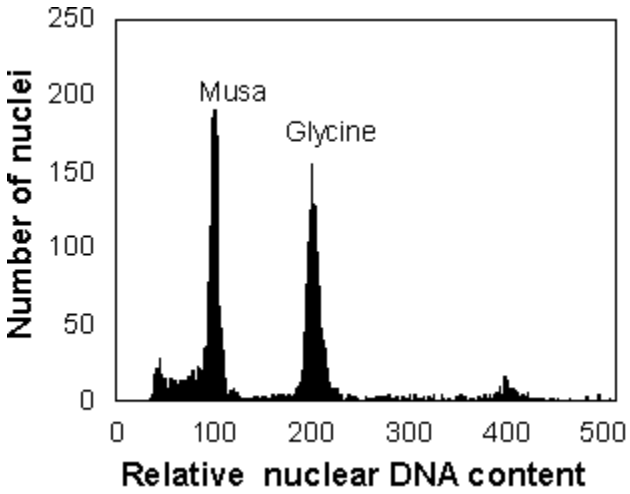

Figure 2. Histograms of nuclear DNA content obtained during the analysis of diploid *Musa* clones. Nuclei isolated from *Glycine max* were used as an internal reference standard (Doleel *et al*. 2004).

Impacts of polyploidy induction on crop improvement

Fruit Trees

In the breeding of fruit trees, many polyploid cultivars have been commercially successful due to their favorable horticultural characteristics, such as large fruit size, sturdiness, high productivity, better disease resistance and lack of or a small number of seeds (Shi *et al*. 2015). The ways of producing polyploid fruit crops mainly include the selection of natural variation, artificial mutation, sexual hybridization, endosperm culture and protoplast fusion (Tongkun *et al*. 2004). Polyploid breeding has been achieved widely in many species of fruit trees including *Citrus sp.* (Guerra *et al*. 2016), *Eriobotrya japonica* (Blasco *et al*. 2015; Liu *et al*. 2019), *Malus domestica* (Hias *et al*. 2017), *Ribes nigrum* (Podwyszyńska and Pluta 2019), *Vitis vinifera* (Kuliev 2011) and *Ziziphus jujuba* (Shi *et al*. 2015). Fruit of colchicine-induced tetraploids of *Actinidia chinensis* was 50-60% larger than those of their diploid progenitors (Wu *et al*. 2003). However, Notsuka *et al*. (2002) revealed no difference of induced tetraploid grapes and the original diploids in time of full maturity, cluster and berry shape, skin color, Brix and acidity, whereas, depending on the variety, a 1.1 to 1.5 fold increase in berry size was observed.

The study of a new polyploid variety of leafy and fruit-producing mulberry demonstrated that the fruit and leaf yield of the new variety were 45.8 and 40% higher than the control cultivar, respectively. Compared to the clonal diploid parents, synthetic tetraploids were superior and their net photosynthetic rate and chlorophyll content increased (Wang *et al*. 2011). Researchers in Sweden were the first to show promising prospects of apple breeding through polyploidy. During 1970–2015, a substantial study was conducted at All-Russian Research Institute of Fruit Crop Breeding on apple breeding using polyploidy: 455 crossing combinations were carried out; 660000 flowers were artificially pollinated; 124700 hybrid seeds were obtained; and 47900 one-year-old seedlings were grown, from which, after multiple rejections, 13200 seedlings were planted into selection gardens; 10 triploid varieties from crosses of di- tri- or tetraploid varieties and five triploid varieties from crosses of two diploid varieties were regionalized, that was promising in terms of triploid varieties, scab immunity and columnar habit (Sedov *et al*. 2017). Xue *et al*. (2015) showed that colchicine-induced autotetraploid of apple cultivars ‘Hanfu’ and ‘Gala’ had a better response to salt stress and higher relative water content (RWC) than that of the diploids. They suggested that it may be related to the higher levels of expression of aquaporin genes (*MdPIP1;1*, *MdTIP1;1*) in response to salt stress. According to Tan *et al*. (2015), tetraploid citrus rootstocks are expected to have stronger stress tolerance than diploid. They observed that doubled diploid of *Citrus junos* Sieb. ex Tanaka rootstock had typical morphological and anatomical features such as shorter plant height, larger and thicker leaves, bigger stomata and lower stomatal density compared to its diploid parent. They also concluded that the higher expression level of stress-related genes and higher content of stress-related metabolites (such as sucrose, proline and γ-aminobutyric acid (GABA)) in the doubled diploid could be beneficial for its stress tolerance.

Although polyploidization is a useful tool for crop improvement with the potential to generate new diversity, it should be noted that polyploid induction does not always increase and improve crop traits. For instance, Amah *et al*. (2019) evaluated induced tetraploids of banana genotypes for their agronomic traits and found that tetraploids generally displayed 20% lower bunch weights and a 50% decrease in fruit provitamin A carotenoids than their original diploids. Nevertheless, they indicated that induced tetraploids were pollen fertile, hence could provide an opportunity for utilizing useful variability in diploid bananas, which may otherwise remain inaccessible for breeding.

Flowers and ornamental plants

Ornamental plant breeders primarily focus on esthetically relevant parameters including plant shape, flower color, flower type, or fragrance (Roughani and Miri 2018b). Chromosome doubling has been frequently considered during the last few decades as a valuable strategy to improve ornamental characteristics, since it often results in the extension of flower longevity, increase in flower and leaf size, deep flower colors, delay in flowering, malformation of flowers and alter the plant shape, which is more attractive to consumers (Ascough *et al*. 2008; Sattler *et al*. 2016; Miri and Roughani 2018a; Manzoor *et al*. 2019). In ornamentals, mostly *in vitro* protocols are used for polyploidy induction, as good micropropagation protocols have already been established for many plant species (Leus *et al*. 2012). In *Zinnia violacea*, autotetraploid plants were produced in the 1930s and the first autotetraploid cultivar (‘State Fair’) was released in the 1950s. Several other autotetraploid cultivars have been introduced since that time. Compared to diploids, tetraploid zinnias have larger flowers, thicker and stronger stems but have poorer seed germination, less branching, delayed flowering and fewer capitula. Polyploidy was also used to restore fertility in allodiploid *Z. marylandica* (Stimart and Boyle 2007). Relative to diploids, polyploid cacti (*Schlumbergera* and *Hatiora*) typically have larger and thicker phylloclades and larger flowers but are slower to root, produce fewer phylloclades and show less branching (Boyle 2007). In cyclamen, both mitotic and meiotic polyploidy induction is possible, as *in vivo* and *in vitro* chromosome doubling by using colchicine treatments and tetraploid production via unreduced pollen in 4x × 2x cyclamen crosses has been reported. The colchicine induced tetraploid yellow-flowered plants had larger and deeper yellow petals than the diploid relatives, suggesting that polyploidy could be one of the techniques to improve flower characteristics (Takamura 2007). Most of the tulip cultivars are diploid ($2n = 2x = 24$), however, several attempts have been made to obtain polyploid tulips. Furthermore, most interspecific hybrids of tulip are highly sterile, but their fertility can be restored by artificial polyploidization. In tulip, mitotic chromosome doubling with colchicine is difficult, because the meristems are hidden in noses inside bulbs and colchicine is harmful to bulbous plants. Oryzalin was successfully used with *in vitro* chromosome-doubling in tulip *T. gesneriana* during a stem-disc regeneration process (Marasek-Ciolakowska *et al*. 2012). The tetraploid *Dendranthema indicum* exhibits larger and thicker leaves, greater flower

diameter and shorter plant than the diploid plants (He *et al*. 2016). Kushwah *et al*. (2018) showed that the application of 0.2% colchicine solution in cotton swab to the shoots of *Chrysanthemum carinatum* seedling is an effective method for inducing polyploidy. The tetraploid plants showed slightly slower growth, stronger stem, thicker and larger leaves, larger flowers and seeds. Unlike tulip, *Lilium* tends to replace diploids with polyploid cultivars. Therefore, a lot of research has been done to optimize the concentration of different chemicals and time of treatment used to induce polyploids or restore F1 fertility in lily hybrids. Oryzalin is more effective in comparison to colchicine to produce tetraploids in the lily species. An alternative to mitotic polyploidization is the use of unreduced gametes by utilizing flower buds of lilies with N_2O gas to inhibit the chromosome segregation in metaphase I during meiosis of pollen mother cells. There are several reports that tetraploid lilies have larger but fewer flowers, thicker leaves, higher tolerant to leaf scorch and bloom later than their diploid counterpart (Marasek-Ciolakowska *et al*. 2018). To improve the ornamental traits of tetraploid gladiolus 'White Prosperity', Manzoor *et al*. (2018) induced polyploidy by soaking gladiolus corms in 0.1-0.3% colchicine solution for 24 h and found that all colchicine concentrations were equally effective in the induction of octoploids (17-18%). They observed that 0.1% colchicine increased vase life whereas 0.3% colchicine was effective in increasing floret diameter. Zeng *et al*. (2020) evaluated nine cultivars of *Cymbidium* orchid and discovered that unreduced-gamete formation frequencies varied from 0.15 to 4.03% and interspecific hybrids generally produced more 2*n* gametes than the traditional cultivars. To generate sexual polyploid plants, they made seven pairs of crosses and produced five triploid and two tetraploid hybrids. Characterization of triploid plants showed that they exhibited improved ornamental value including a more compact growth style, produced fragrant flowers and rounder flowers with wider sepal, petals and lips.

Vegetables

The tetraploidization method for seedless watermelon production was invented by Kihara (1951). Seedless cultivars (2n = 3x = 33) are produced by crossing a tetraploid (female parent) with a diploid (male parent) inbred line. Since the triploid hybrid is female sterile, the fruit induced by pollination tends to be seedless. On the other hand, the triploid does not have viable pollen, so it needs to plant at least 20% diploid cultivar in the production field to provide the pollen that stimulates fruit set (Wehner 2008). Triploid seedless watermelon is popular on a commercial scale and has a high price in the world market, due to its distinctive characteristics such as small size, vigorous growth, higher fruit number per plant, high sugar content, flesh firmness, thin rind and possible longer shelf life (Noh *et al*. 2012). Marzougui *et al*. (2009) evaluated morphological and chemical comparison of diploids and induced autotetraploids of *Trigonella foenum-graecum* and found that the autotetraploids had higher leaf area and productivity concerning the number of seeds, pods and branches compared to the diploids. Its leaves also are richer in potassium, sodium, calcium and phosphorus. In

a study, tetraploid muskmelon (*Cucumis melo*) were induced from diploid plants by colchicine. The results showed that the fruit weight, total soluble solids (TSS), soluble sugar and vitamin C contents in the tetraploids were higher than those in the diploids (Zhang *et al*. 2010). Ramírez-Godina *et al*. (2013) indicated that colchicine-induced autopolyploidy can increase vitamin C and fruit quality in *Physalis ixocarpa*, even though, it was associated with undesired changes in the shape of fruits which became flattened at the poles with gaps between the endocarp and mesocarp. Pliankong *et al*. (2017) induced polyploidy in *Capsicum frutescens* and reported that the size and amount of capsaicin in the polyploid fruit were higher than the diploid fruit. Colchicine-induced polyploid has also been reported to be high in vitamin C content (Basu and Krishna De 2003). The *Petroselinum crispum* tetraploid plants obtained from the colchicine treatment had higher leaf size and stem diameter than diploid plants (Nasirvand *et al*. 2018). Induced tetraploids of *Lepidium sativum* plants as compared with diploid ones, were specified by the increase in leaf size and thickness, stem diameter, seed weight and on the contrary, decrease in plant height and percentage of seed germination (Aqafarini *et al*. 2019). The induced tetraploid plants of *Raphanus sativus* displayed larger leaves and taproots as well as higher soluble sugar and protein, vitamin C and antioxidant enzyme activity (peroxidase). Moreover, the qRT-PCR analysis showed that *FLC1.1*, the gene that has been known to play roles in suppressing flowering, was highly expressed in tetraploid plants at the flowering stage, whereas the expression levels of *VRN2* and *AGL24*, the genes that positively regulated flowering and bolting, were lower in the tetraploid compared to the diploid radish, at flowering and bolting stages (Pei *et al.* 2019).

Medicinal plants

Several factors including genotype, geography, climate and harvest period are responsible for the secondary metabolite content found in medicinal plants (Salma *et al.* 2017). Genetic improvement by chromosome doubling has been reported to affect not only oil quantity but also its components such as phenolics, terpenoids, phenylpropanoids, anthocyanins, flavonoids, alkaloids and polyketides (Parida and Misra 2015; Hannweg *et al*. 2016). Salma *et al*. (2017) presented a summary of the *ex vitro* and *in vitro* methods of producing synthetic polyploidy in medicinal herbs. In general, polyploids are larger than the normal plants, which may be due to increased biomass or yield. In those medicinal plants that accumulate the secondary metabolites in the vegetative tissues, polyploidy may be very beneficial and priceless due to the increased composition or biomass yield (Salma *et al*. 2017; Corneillie *et al*. 2019). Furthermore, due to the increased number of gene copies in polyploids, synthetically induced polyploidy may lead to increases in enzymatic activity, isozyme diversity and alteration in flavonoid profiles that contribute to enhanced production and qualitative changes in secondary metabolites (Dhawan and Lavania 1996). For instance, tetraploids induced in *Tetradenia riparia* produced 3.5 times higher oil than diploid plants. Also, tetraploids contained higher amounts of fenchone than diploids and had α-humulene, α-terpineol and viridiflorol which

were not present in the diploids (Hannweg *et al*. 2016). The increase of ploidy levels in *Dracocephalum moldavica* caused a decrease in plant height and an increase in leaf area, fresh and dry mass of plants, size of seeds as well as essential oil content (Omidbaigi *et al*. 2010). Xing *et al*. (2011) induced tetraploidy in *Catharanthus roseus* and reported that accumulation of terpenoid indole alkaloids (vindoline, catharanthine, vinblastine) increased in the tetraploid plants. They analyzed the expression of terpenoid indole alkaloids biosynthesis-related genes and transcript factors by QRT-PCR to explain the molecular mechanism of these metabolites enhancement, and found that the expression of *tdc*, *g10h*, *sls*, *str*, *dat* and *prx1* genes increased in the autotetraploids. Similarly, tetraploid *Stevia rebaudiana* plants showed higher contents of stevioside and rebaudioside A, in comparison to the diploid controls (Zhang *et al*. 2018). Zhou *et al*. (2020) studied the polyploid induction system of *Zingiber officinale*. Tetraploid gingers were larger than their diploid plants for leaf length, leaf width, leaf thickness, stem diameter and guard cell. The soluble sugar, soluble protein, proline and other substances were also higher; especially carotenoids concentration was near 1.4 times than the diploid plants, which showed that tetraploid gingers had potential to increase yield and adaptability.

Conclusions and future perspectives

Polyploidy is one of the most common phenomena of diversity, adaptation and evolution in flowering plants. The several advantages of polyploidy observed in natural species indicate that polyploids have a selective advantage over diploids. Polyploidy is associated with extensive structural, developmental, physiological and biochemical changes in plants that result in wide variation in these traits. Therefore, it can be said that polyploidization provides new options for plant breeders to induce *ex vitro* and *in vitro* synthetic polyploids and select suitable plants depending on the purpose such as medicinal, ornamental and resistance applications, and so on. The *gigas* effect is one of those direct consequences and, when occurring in organs of commercial interest, is a valuable feature to improve the crop. The phenomena of genome buffering, heterozygosity and heterosis deserve attention in plant breeding programs, as they may lead to the higher vigor observed by polyploid organisms than their diploid relatives. Polyploids are also important as bridges for germplasm transfer between species where direct crossing is not possible, as well as to restore the fertility of sterile hybrids. Polyploidy effectively affects the enzymatic activity of the pathways of production of secondary metabolites and consequently the quantitative and qualitative pattern of production of plant secondary compounds. Increasing biomass production coupled with the change in the production of secondary pharmaceutical compounds has made researchers an efficient option for selecting high-yielding plants.

Several protocols have been developed for possibility of artificial polyploidization by using different chemicals and methods in a wide range of crop species. Since the discovery of colchicine, *in vitro* polyploidy induction using this antimitotic agent has been one of the most important means of artificial polyploidy induction. The type of

antimitotic agent and its concentration acting for a time period for polyploidy induction is variable and species dependent. Although, colchicine at different concentrations and time durations has been widely employed as antimitotic agent both *in vitro* and *ex vitro*, other antimitotic agents like oryzalin, trifluralin and AMP (amiprophos-methyl) can also be investigated. It is likely that other antimitotic agents will be identified in future that will lead to superior polyploidy induction and also minimize harmful chemical effects. In the *ex vitro* polyploidy induction technique, antimitotic agents are applied by various methods including dipping, soaking, whole plant immersion or by cotton wool method. Sexual polyploidy induction is also achieved through the fusion of unreduced gametes, especially when high levels of heterozygosity are required. For successful polyploidization, various direct and indirect techniques have been used to identify ploidy level in plants

In recent decades, significant progress has been made in the field of polyploidy and several mechanisms have been explored in relation to its causes and consequences. Further study of the relationship between genomic changes with gene expression and regulation after polyploidy induction may allow plant breeders to obtain more accurate and desired results.

Conflict of Interest

The author declare that they have no conflict of interest with any organization in relation to the subject of the manuscript.

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پلی‌پلوئیدی مصنوعی در بهبود محصولات باغی

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چکیده

پلی‌پلوئیدها موجوداتی هستند که بیش از دو مجموعه کروموزوم زوج (همولوگ) دارند. پلی‌پلوئیدی نقش مهمی در تکامل و تنوع گیاهان عالی داشته است. با استفاده از چند ماده شیمیایی ضدمیتوزی مانند کلشیسین، تری فلورالین و اوریزالین، پلی‌پلوئیدی مصنوعی القا می‌شود. نوع مهار کننده‌های میتوزی و غلظت و مدت زمان آن متغیر و وابسته به گونه‌ها است. این روش می‌تواند به صورت برون شیشه‌ای یا درون شیشه‌ای باشد که روش کشت بافت ساده‌تر و کارآمدتر است. تغییر در محتوای DNA هسته‌ای، بیان ژن و فرایندهای رشد به دلیل دستکاری پلوئیدی می‌تواند منجر به تغییرات موفولوژیکی، آناتومیکی و فیزیولوژیکی در گیاهان پلی‌پلوئید شود. به طور کلی، گیاهان پلی‌پلوئید اندام‌های بزرگتر، زیست توده بیشتر، عملکرد بالاتر، تحمل برتر در برابر تنش‌های زنده و غیر زنده و نیز متابولیت‌های اولیه و ثانویه بیشتری دارند. همچنین، پلی‌پلوئیدی اغلب باروری گیاهان را کاهش می‌دهد و امکان تولید میوه‌های بدون بذر را فراهم می‌کند. در گیاهان زینتی، افزایش اندازه گل‌های پلی‌پلوئید از نظر زیبایی شناختی و اقتصادی از اهمیت بالایی برخوردار است. دو روش مستقیم و غیرمستقیم برای تعیین پلوئیدی گیاهان وجود دارد. روش‌های غیرمستقیم ساده‌تر اما غیر دقیق‌تر هستند که شامل ارتباط سطح پلوئیدی با ویژگی‌های مورفولوژیکی (مانند ارتفاع گیاه، اندازه برگ و قطر دانه گرده) و آناتومیک (تراکم و اندازه روزنه و همچنین تراکم کلروپلاست در سلول‌های محافظ) می‌باشد. در مقابل، روش‌های سنجش مستقیم مانند شمارش کروموزوم در سلول‌های مریستمی ریشه و فلوسایتومتری، تکنیک‌های دقیقی برای تعیین سطح پلوئیدی در گیاهان هستند. اخیرا، نشانگرهای مولکولی ابزارهای کارآمد، سریع و مقرون به صرفه‌ای برای تجزیه و تحلیل تعداد زیادی از نمونه‌ها ارائه می‌دهند. در این بررسی، برخی از اثرات پلی‌پلوئیدی در چنین محصول باغی نیز به طور خلاصه بررسی شده است. به طور کلی، دستکاری‌های پلی‌پلوئیدی مدت‌هاست که در بهبود عملکرد بسیاری از گیاهان باغی مورد استفاده قرار گرفته و می‌تواند یکی از امیدوار کننده‌ترین ابزارها در برنامه‌های اصلاح نباتات محسوب شود.

واژه های کلیدی: اصلاح نباتات؛ پلی‌پلوئید؛ دو برابر شدن ژنوم؛ فلوسایتومتری؛ ماده ضد میتوزی