



Review

Research Progress of Chromosome Doubling and $2n$ Gametes of Ornamental Plants

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Abstract: Polyploid plants, an important source for the selection of ornamental plants for their advantages of faster growth, higher yields, and stronger adaptability to an adverse environment, play an essential role in the development of economic plants for agricultural stress. The methodology has been improved to decrease blindness and increase the efficiency of ornamental plants' polyploid breeding in the long term. The progress of research on ornamental plants related to natural polyploidy, chromosome doubling, the $2n$ gametes pathway, and plant ploidy identification are reviewed in this paper. The main routes in polyploid breeding are chromosome doubling and sexual hybridization. Screening of suitable induction methods and plant material should be enhanced in chromosome doubling to improve induction efficiency. Regarding polyploid breeding, the utilization and research on $2n$ gametes produced by natural conditions or artificial induction should be strengthened to shorten the breeding years. Therefore, further research should strengthen the combination of chromosome doubling and sexual hybridization to improve breeding efficiency and strengthen the research and application of polyploid plants. This thesis review provides some reference value for polyploid breeding in ornamental plants.

Keywords: ornamental plant; polyploid; chromosome doubling; $2n$ gametes



Citation: Cui, L.; Liu, Z.; Yin, Y.; Zou, Y.; Faizan, M.; Alam, P.; Yu, F. Research Progress of Chromosome Doubling and $2n$ Gametes of Ornamental Plants. *Horticulturae* **2023**, *9*, 752. <https://doi.org/10.3390/horticulturae9070752>

Academic Editor: Chao Ma

Received: 8 May 2023

Revised: 4 June 2023

Accepted: 24 June 2023

Published: 28 June 2023



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1. Introduction

Polyploidy refers to organisms that consist of three or more complete sets of chromosomes. Polyploidy is widespread in nature, and it is estimated that approximately 70% of plants in nature have undergone polyploidy in evolution and approximately 35% of angiosperms have a polyploid origin [1–5]. Studies have shown a high proportion of polyploid species in many families and genera. Notably, there are many genera of polyploidy in nature, for example, *Chrysanthemum* [6], *Magnolia* [7], *Phalaenopsis* [8,9], *Alilium* [10], etc. Polyploids can produce more duplication genes, nonfunctional genes, and pseudogenes than diploids [11,12]. Usually, polyploids have fast growth, huge organs, high yield, higher content of active ingredients, and higher resistance to adversity. In addition, triploids usually have the advantage of being seedless or having fewer seeds, so from the last century to the present, breeders have used polyploid breeding to produce a large

number of varieties [13–17]. Moreover, polyploid breeding is now widely used in ornamentals, such as triploid and tetraploid lilies [18,19], triploid and tetraploid tulips [15], triploid narcissus [20], triploid and tetraploid *Paeonia* [21], tetraploid *Magnolia* × *soulangeana* [7], etc.

This paper describes the current status of research related to ploidy breeding in ornamental plants. The research on the origin and induction of polyploids and sexual polyploidy in ornamental plants is primarily explained, and research on polyploid breeding of ornamental plants has been carried out with the aim of providing a reference for ornamental plant breeding research.

2. Natural Occurrence of Polyploid Cells

Polyploidy is ubiquitous among plants, for example, the frequency of polyploidy is as high as 53% in bryophytes and 95% in ferns, while it is 4% in gymnosperms, which is relatively small [5,22,23]. Estimates of the frequency of polyploidy in angiosperms are approximately 35% [5]. In annual herb species, polyploidy occurs at a frequency of 28%; in perennial herb species, the appeared polyploid is 39%; in woody species, polyploidy is found in 22% of plants [3]. As previously reported, chromosome evolution is a reflection of species evolution and species adaptation to their habitat. [24,25]. Plants become polyploid as a result of adaptation to changes in their natural environment.

Furthermore, polyploids are pervasive in ornamental plants (Table 1). It has been found that there are a large number of polyploids in *Magnolia* spp. with high ploidy variation. The *Magnolia* is mostly tetraploid and hexaploid in polyploid, and *Magnolia acuminata*, *Magnolia biondii*, *Magnolia cylindrica*, and others are tetraploids, while *Magnolia campbellii*, *Magnolia dawsoniana*, *Magnolia sargentiana*, *Magnolia sprengeri*, and others are hexaploids [7,26]. Some studies have found tetraploids ($2n = 4x = 76$) and hexaploids ($2n = 6x = 114$) in *Magnolia denudata*, and similar phenomena have been found in *Magnolia liliflora* [26]. It can be inferred that the earliest *Magnolia liliflora* and *Magnolia denudata* were diploids, and that through a process of cultivation over time, they evolved into polyploids with tetraploid and hexaploid sequences.

Table 1. Natural polyploidy of some ornamental plants.

Species	Ploidy	Reference
<i>Lilium lancifolium</i>	$2n = 3x$	[27]
<i>Magnolia</i> spp.	$2n = 4x, 6x, 8x$	[7]
<i>Lycoris</i> spp.	$2n = 3x, 4x$	[28,29]
<i>Narcissus</i> spp.	$2n = 3x$	[20]
<i>Primula</i> spp.	$2n = 4x, 6x$	[30]
<i>Chrysanthemum</i> spp.	$2n = 4x, 5x, 6x, 7x, 8x, 10x$	[6]
<i>Habenaria aitchisonii</i>	$2n = 4x$	[31]
<i>Phalaenopsis amabilis</i>	$2n = 4x$	[8]
<i>Malus</i> spp.	$2n = 3x, 4x, 5x$	[32]
<i>Styrax</i> spp.	$2n = 4x, 5x$	[33,34]
<i>Fragaria</i> spp.	$2n = 4x, 6x, 8x, 10x$	[35]
<i>Oxalisterosa</i>	$2n = 8x$	[36]
<i>Zephyranthes grandiflora</i>	$2n = 8x$	[37]
<i>Sequoia sempervirens</i>	$2n = 6x$	[38]
<i>Paeonia mairei</i>	$2n = 4x$	[39]
<i>Camellia reticulata</i>	$2n = 4x, 6x$	[40]
<i>Rosa</i> spp.	$2n = 4x, 5x, 6x, 10x$	[41–43]
<i>Hibiscus paramutabilis</i>	$2n = 4x$	[44]
<i>Ilex theezans</i>	$2n = 4x$	[45]
<i>Oenothera</i> spp.	$2n = 4x, 6x, 8x$	[46]
<i>Deutzia</i> spp.	$2n = 4x$	[47]
<i>Alnus</i> spp.	$2n = 4x, 6x, 8x, 16x$	[48]
<i>Neolamarckia cadamba</i>	$2n = 4x$	[49]

3. Artificial Induction of Polyploidy in Ornamental Plant

The probability of polyploidy arising in nature is extremely low, so polyploids are induced by artificial means in breeding, and the induction of polyploids includes physical and chemical methods (Figure 1). Chromosome doubling can be induced through physical means such as thermal excitation, radiation, ultrasound, environmental stress, and mechanical damage. It can also be induced chemically using substances such as colchicine, oryzalin, trifluralin, 3-indoleacetic acid, and pronamide [50,51].

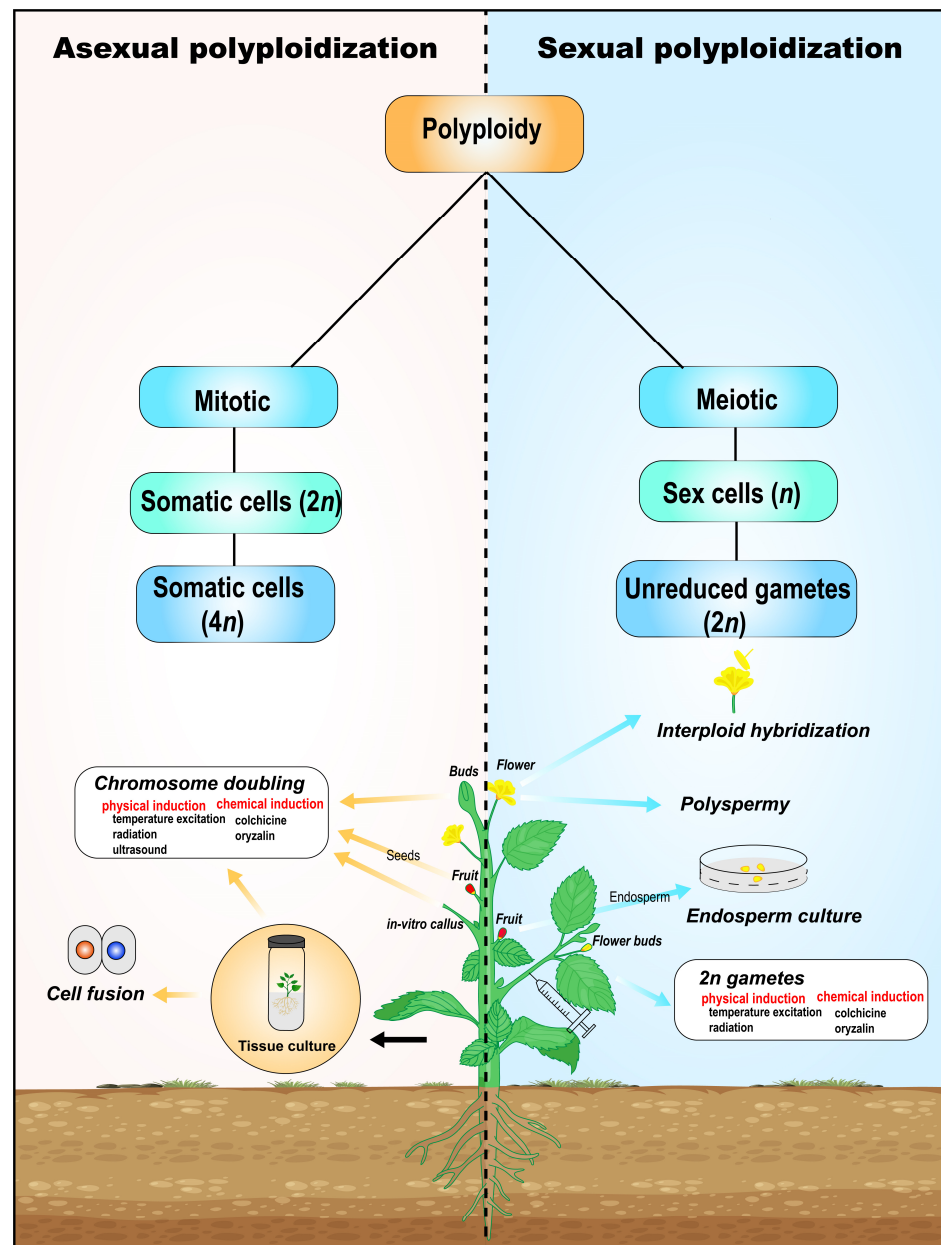


Figure 1. Systematic diagram of plant polyploid breeding [51].

3.1. Physical Induction Polyploidy/Mutation

Physical induction is a popular technique for obtaining plant polyploids, with radiation being the most frequently employed method. The sources of radiation mutagenesis currently utilized include γ -rays, X-rays, and ultraviolet rays.

The selection of the mutagenesis source is crucial for achieving successful results. Among the different options available, ^{60}Co - γ radiation induction is the most widely

used and effective method of physical induction. The low rate of X-rays and ultraviolet mutagenic polyploids has been previously reported. Initially, seed induction was the primary focus of research. However, with the development of technology, induction can now be carried out in various plant tissues such as roots, stems, and healing tissues. Almost all plant organs can be used as mutagenic material [52]. Radiation sensitivity varies among different organs of plants, with young tissues and organs generally being more sensitive to radiation. Seeds, on the other hand, are the least sensitive due to their low water content and dormancy. To minimize damage to the plant while achieving the highest mutagenic frequency, it is important to identify the most suitable radiation measurement. Research has shown that the induction of *Actinidia arguta* ($2n = 2x$) was used to obtain three mutants by using ^{60}Co - γ radiation, and all the mutants used in the study were chimeras [53]. Jiang et al. [54] used γ -rays to induce *Magnolia denudata* callus to produce two chimeric seedlings. The success rate of physically inducing chromosome doubling is low in plants and there is also a high occurrence of chimeras. Plants can show greater physiological damage after being treated with radiation.

3.2. Chemical Induction Polyploidy/Mutation

Chemical induction is currently the most popular method for inducing chromosome doubling, with colchicine being the most commonly used chemical for polyploidy induction (Table 2).

Colchicine functions by binding to microtubules and hindering the movement of chromosomes during mitosis, leading to the prevention of cell division and doubling of chromosomes. The plant material being tested may have varying tolerances to different chemical concentrations and treatment times, depending on the tissues, organs, and growth conditions involved. It has been reported that the most successful transformation of *Cymbidium hybridum* protocorms at 5 days of induction with 0.05% colchicine was 23.7%, and the success rate of transformation of young *Cymbidium hybridum* shoots at 0.05% colchicine for 24 h was 97% [55,56]. Feng et al. [57] induced diploid *Lilium davidii* var. *unicolor* bulbs with 0.05% colchicine for 48 h and finally succeeded in inducing 33.3% of tetraploids. Likewise, the diploid Asiatic hybrid lilies bulbs were soaked in 0.003% and 0.005% oryzalin for 16 h, and tetraploids were prevalent in 19% and 23% of Asiatic hybrid lilies, respectively [58].

When inducing plant polyploids, it is recommended to select actively dividing tissues as the mutagenic material. These can include seeds, seedlings, young shoots, bulbs, cluster buds, and corms, among others (Table 2). The mutagenic plant material also largely affects the efficiency and outcome of mutagenesis, so a rational choice of mutagenic material is needed in the experiment. It has been found that the use of seeds as a material to induce polyploids is prone to chimerism [51]. In a study of the chromosomes doubling in diploid *Dendrobium officinale*, it was found that 40%, 40%, and 30% of tetraploid *Dendrobium officinale* were obtained by the induction of seed embryos, primary bulbs, and stems, respectively, with 0.3% colchicine [59]. Therefore, according to the induction material, different treatments such as immersion, co-culture, absorbent cotton wrapping, smearing, and spraying can be used to induce chromosome doubling in plants, with the method of immersion being one of the most common methods at present. Colchicine is currently known for its high induction efficiency, but it also has drawbacks such as causing high stress to the plant and being expensive. This is the reason that amylin and trifluralin have also been used to induce polyploidy in plants (Table 2). Side effects of chemical mutagenesis methods for polyploid induction include plant chromosome instability issues, which could happen after polyploid induction by colchicine. For instance, there may be a trend of decreasing chromosome numbers after polyploid induction by colchicine in successive generations. Moreover, other side effects include slow or abnormal growth, which can be induced by different concentrations of colchicine in polyploid induction experiments.

Table 2. Polyploid induction of some ornamental plants.

Species	Explant	Induction Method	Method	Concentration, Duration	Most Successful Conversion rate	Reference
<i>Actinidia arguta</i>	hardwood	^{60}Co γ -ray		^{60}Co Gy, 1 Gy·min $^{-1}$	6.67% chimera	[53]
<i>Magnolia denudata</i>	callus	γ -ray		2.475×10^{-10} C/kg	chimera	[54]
<i>Cymbidium hybridum</i>	protocorm-like bodies	colchicine	co-culture	0.05% for 5 d	23.7%	[56]
<i>Cymbidium hybridum</i>	young shoots	colchicine	immersion	0.05% for 24 h	28.2%	[55]
<i>Lilium davidii</i> var. <i>unicolor</i>	bulb	colchicine	immersion	0.05% for 48 h	33.3%	[57]
Asiatic Lilies	bulb	oryzalin	immersion	0.005% for 5 h	23%	[58]
<i>Phalaenopsis amabilis</i>	cluster bud	colchicine	co-culture	0.05% for 10 d	3%	[60]
<i>Tagetes erecta</i>	seeds	colchicine	immersion	0.1% for 6 h	100%	[61]
<i>Impatiens walleriana</i>	seeds	colchicine	immersion	0.05% for 48 h	1.5%	[62]
<i>Gladiolus grandiflorus</i>	corms	colchicine	immersion	0.2% for 24 h	18%	[63]
<i>Chrysanthemum carinatum</i>	apical buds	colchicine	absorbent cotton wrapping	0.2% for 6 h/d, 3 d	2.08%	[64]
<i>Clematis heracleifolia</i>	apical buds	colchicine	spraying	0.2% for 2 d	80%	[65]
<i>Agastache foeniculum</i>	seeds, apical buds	colchicine	immersion	17.5 mM for 6 h	16%	[66]
<i>Agastache foeniculum</i>	seeds, apical buds	oryzalin	immersion	50 μM for 12 h	14%	[66]
<i>Agastache foeniculum</i>	seeds, apical buds	trifluralin	immersion	50 μM for 12 h	12%	[66]
<i>Hibiscus moscheutos</i>	seedlings	colchicine	immersion	0.025% for 12 h	22.5%	[67]
<i>Dendrobium officinale</i>	seeds	colchicine	co-culture	0.05% for 4 months	50%	[59]
<i>Dendrobium officinale</i>	embryo	colchicine	immersion	0.3% for 36 h	40%	[59]
<i>Dendrobium officinale</i>	protocorm-like bodies	colchicine	immersion	0.3% for 36 h	40%	[59]
<i>Dendrobium officinale</i>	stems	colchicine	immersion	0.3% for 36 h	30%	[59]
<i>Neolamarckia cadamba</i>	nodal segments	colchicine	immersion	0.3% for 48 h	20%	[50]
<i>Ziziphus jujuba</i>	in vitro callus	colchicine + dimethylsulphoxide	absorbent cotton wrapping	0.05% + 1% for 50 d		[53]
<i>Buddleja lindleyana</i>	seeds	colchicine	immersion	0.3% for 48 h	3%	[68]

3.3. Others

Cell fusion is also known as somatic hybridization, protoplast fusion, parasexual hybridization, and parasexual fusion. It refers to the fusion of protoplast to hybrid cells under certain conditions. At present, cell fusion is one of the important tools for ploidy breeding in plants, and there have been many studies on obtaining polyploids through cell fusion technology [69,70]. Zhang [70] successfully obtained allotetraploid by cell fusion using diploid citrus ‘Nova’ and ‘HBP’, and the allotetraploid hybrid citrus traits were more intermediate between their parents. Although cell fusion can transcend distant hybridization disorder, some distant hybridization plants may undergo random chromosome loss and organelle separation or merge after fusion, thus forming chimeras that affect their genetic stability [71].

Endopolyploidy is the phenomenon of multiple ploidy cells in one organism, and polyploid cells are formed by intracellular replication in some organs in plants. Currently, some studies have found that endo polyploids are common in angiosperms, for example, *Arabidopsis thaliana* [72], tomato (*Lycopersicon esculentum*) [73], *Phalaenopsis aphrodite* subsp. *Formosana* [74], etc. The regeneration of polyploid plants from polyploid cells in endo polyploids by plant tissue culture also offers a new way of cultivating polyploids.

4. Sexual Polyploidization

Sexual polyploidy breeding is a reproductive technique used to produce polyploid offspring by crossing unmeiotic gametes ($2n$ gametes) or polyploid parents. This method can also be achieved to a lesser extent through endosperm culture (Figure 1).

4.1. $2n$ Gametes

Sexual polyploidy breeding is widely used to produce polyploids from unmeiotic gametes ($2n$ gametes) [75]. The process of the $2n$ gametophyte pathway is straightforward, but in natural conditions, most plants produce $2n$ gametes at a low frequency. This has led to extensive screening work to identify $2n$ gametes. However, few plant species produce a significant proportion of natural $2n$ gametes [76].

In general, undoubled interspecific distant hybrids with abnormal meiosis in the F1 generation are usually highly sterile but can produce a small number of $2n$ gametes. Polyploidy can be obtained by interbreeding. Previous research has demonstrated that polyploidy can arise from $2x \times 2x$, $2x \times 4x$, and polyploidy $\times$ polyploidy intersectional crosses. In lily ploid breeding, F1 distant hybrids have abnormal meiosis and are usually sterile, but the production of $2n$ gametes occurs in a small number of distant diploid F1 hybrids; for example, the allotriploid lily hybridization offspring of LA $\times$ AA and OT $\times$ OO [77,78]. Studies have also reported the use of LA $\times$ AAAA to obtain allotetraploid (LAAA) lilies. [19]. In the current study, a large number of triploids were produced using $4x \times 2x$ or $2x \times 4x$. A large number of polyploid varieties of *Magnolia* species have been successfully bred as polyploids by intersectional crosses, for example, *Magnolia* $\times$ *soulangeana*, *Magnolia* $\times$ *galaxy*, *Magnolia* $\times$ *atlas*, *Magnolia* $\times$ *sunsation*, etc. [7,79].

The $2n$ gametes are pollen or egg cells with specific cell chromosome numbers that result from certain factors during meiosis, also known as unreduced gametes. It has been shown that most plants in nature can produce $2n$ gametes, and polyploid species are also an important source of the $2n$ gamete pathway [80]. There are three main ways to produce $2n$ gametes: First-division restitution (FDR), second-division restitution (SDR), and indeterminate meiotic restitution (IMR) (Figure 2) [19,78,81]. During the normal meiotic process, homologous chromosomes pair up to form bivalents at metaphase I. Later, they form four n pollen. If separation at anaphase I is skipped and meiosis proceeds directly from bivalent to meiosis II, the resulting gametes are called FDR-type $2n$ gametes. If the first meiotic division results in normal segregation and there is no segregation during anaphase II, SDR-type $2n$ gametes are produced. On the other hand, if the bivalent formed during meiosis is able to separate normally while the monovalent also separates, IMR-type $2n$ gametes are produced. Sexual polyploidy through the $2n$ gametophytic pathway has a high rate of chromosomal rearrangements. Furthermore, parental trait genes can interpenetrate to achieve progressive breeding of target genes, leading to genetic improvement [19].

Artificial induction can increase the probability of $2n$ gamete production. The $2n$ gametes can be artificially produced by treating pollen or macrospores with high or low temperatures, colchicine, laughing gas (N_2O), etc. (Table 3). Tian [82] reported the induction of poplar $2n$ pollen production using high temperatures ($38\text{--}41^\circ\text{C}$), and 42 triploid hybrid offspring were obtained. Recent research has found that $2n$ gametes were induced from *Eucommia ulmoides* macrospores using high temperatures to obtain 23 triploid hybrid offspring [83]. Using the induction of $2n$ pollen production in Longan (*Dimocarpus longan*), the $2n$ pollen ratio reached 19.46% using 0.9% colchicine for 2 days; however, although the high temperature induced $2n$ pollen at 5.7%, triploid longan was eventually obtained for intersectional crosses [84,85]. It is worth noting that high-temperature (30°C) treatment of lily buds for 4h and 8h can significantly increase the ratio of $2n$ pollen [86]. In addition, $2n$ gametes have been utilized to achieve sexual polyploidy in various ornamental plants such as tulips, *Phalaenopsis amabilis*, and others (Table 3). Currently, the induction frequency of $2n$ gametes is low among most plants, so the application is limited.

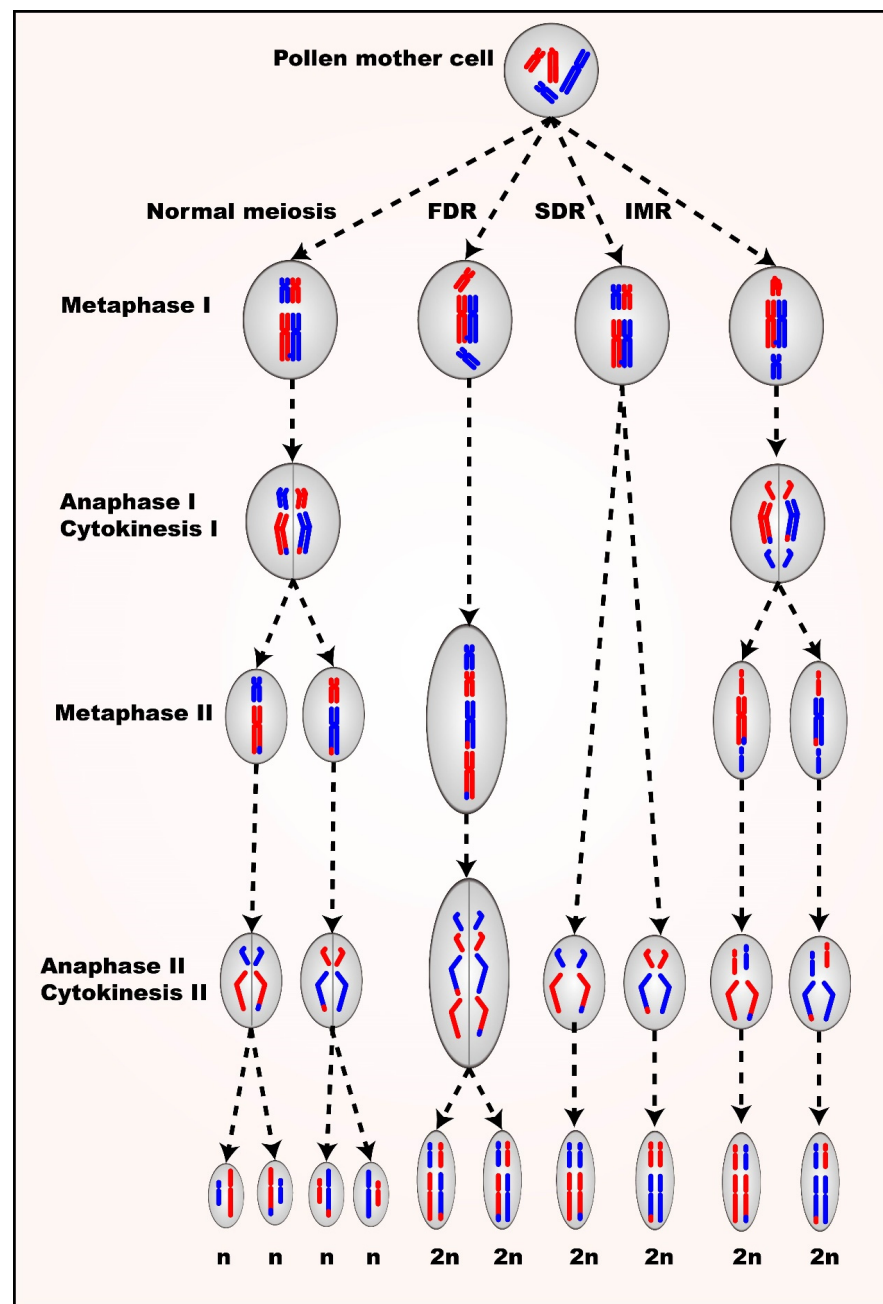


Figure 2. The diagram illustrates normal meiosis resulting in n gametes and abnormal meiosis to FDR, SDR, and IMR $2n$ gametes, modified from the reference [78].

Table 3. $2n$ gamete induction of some ornamental plants.

Species	Explant	Induction Method	Method	Concentration, Duration	Result	Reference
<i>Populus canescens</i>	pollen	high temperature	detached heat treatment	38–41 °C for 3 and 6 h	42 triploids seedlings	[82]
<i>Populus canescens</i>	pollen	colchicine	injection	0.5% for injection 11 times (2 h/time)	30.27% $2n$ pollen	[87]
<i>Populus tomentosa</i>	pollen	colchicine	injection	0.5% for injection 3, 5, 7 times (2 h/time)	68 triploids seedlings	[88]
<i>Dimocarpus longan</i>	pollen	colchicine	absorbent cotton wrapping	0.9% for 2 d	19.46% $2n$ pollen	[85]

Table 3. Cont.

Species	Explant	Induction Method	Method	Concentration, Duration	Result	Reference
<i>Dimocarpus longan</i>	pollen	high temperature		38 °C for 10 d	5.7% 2n pollen	[84]
<i>Eucommia ulmoides</i>	megasporogenesis	high temperature	bagging	42~48 °C for 2~6 h	23 triploids seedlings	[83]
Lily	pollen	high temperature	greenhouse	30 °C for 4 h and 8 h	1~5% 2n pollen	[86]
<i>Eucalyptus urophylla</i>	pollen	colchicine	injection	0.5% for 3 and 6 h	28.71% 2n pollen	[89]
<i>Phalaenopsis</i>	pollen	colchicine	absorbent cotton wrapping	0.05% for 3 d	0.9~1.78% 2n pollen	[90]
Tulipa	pollen	N ₂ O	high-pressure container	6 atm for 24 h	16~26.7% 2n pollen	[91]
<i>Phalaenopsis amabilis</i>	pollen	N ₂ O	high-pressure container	48 h	35.6% triploids seedlings	[92]
<i>Populus</i>	pollen	gibberellins	injection	10 µM for 7 times	21.37% 2n pollen	[93]
<i>Paeonia lactiflora</i>	pollen	colchicine	injection	0.4% for 2 times	7.79~47.39% 2n pollen	[94]

4.2. Others

Generally, in double fertilization, angiosperms normally allow only one pollen tube to fertilize the ovule, and most plants suffer from multiple sperm fertilization disorders [95]. Recently, we have revealed the presence of multiple fertilizations in some plants, and triploid rice has been obtained through multiple fertilizations [96]. Therefore, multiple sperm fertilization can still be used as a reference when breeding polyploids.

Endosperm is a product of double fertilization. In Polygonum-type plants, the embryo is diploid and the endosperm is triploid. Therefore, endosperm culture can be used to obtain triploids quickly. So far, endosperm culture to obtain triploids has been reported in a variety of plants, for example, *Actinidia deliciosa* [97], *Lonicera caerulea* var. *emphyllcalyx* [98], *Citrus* plants [99], and *Acacia nilotica* [100], etc. In summary, diploid Polygonum-type plants culture can obtain triploids, and similarly, the use of tetraploids can breed hexaploids, thus achieving rapid plant polyploidy (Figure 3a). In Fritillaria-type plants, the central cell of the embryo sac is equal to the sum of the two somatic cells [18]. Therefore, it stands to reason that the use of endosperm cultures in Fritillaria-type endosperm plants allows rapid access to pentaploids and decaploids (Figure 3b). Endosperm culture is more challenging, therefore practical applications in combination with other breeding tools are needed in future research [101].

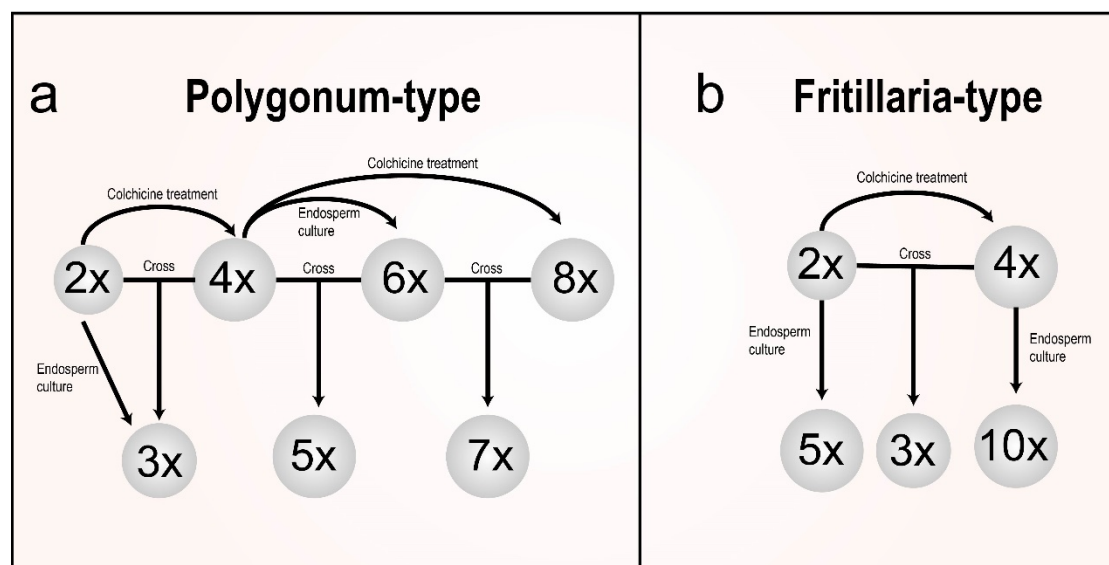


Figure 3. Diagram of polyploid cultivation using endosperm culture. (a) Polygonum type, (b) Fritillaria type.

5. Identification of Ploidy

The most direct and basic method of polyploid identification is morphological identification, and preliminary plant ploidy identification can be performed according to plant size; leaf size, thickness, color, etc.; size and density of stomata and guard cells in the upper and lower epidermis of leaves; pollen grain size; and other characteristics [57,102,103]. Generally, polyploids have larger plants, leaves, flowers, and fruits than diploids. Research has reported that tetraploid gerberas have larger stomata, wider and thicker leaves, larger corollas, and longer petals than diploids [104]. Feng et al. [57] observed the tetraploid *Lilium davidii* var. *unicolor* and found that the tetraploid had larger bulbs, leaves, and stomata, but lower stomatal density relative to the diploid. In a study of $2n$ pollen induction in woody plants, for example, *Populus tomentosa*, *Populus alba*, *Populus canescens*, *Populus alba* $\times$ *P. glandulosa*, *Populus adenopoda*, *Populus pseudo-simonii*, *Eucommia ulmoides*, etc., induced $2n$ pollen is longer in diameter than n pollen [105,106]. Phenotypic observations on doubled *Phalaenopsis* have shown that leaf stomata length and width become larger, and guard cell length and width increase; however, the average stomatal number decreases, and stomata are round in tetraploids and oblong in diploids [107,108].

Chromosome count and estimation (Chromosome preparation) is the most accurate and useful method for ploidy identification, and there have been many studies that have reported chromosome count and estimation [20,27,109–111]. However, some plants have minute chromosomes, making chromosome preparations difficult and experiments time-consuming. Research on the use of fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) for ploidy identification has been well reported. In lily breeding, there is the use of FISH to identify lily ploidy and the veracity of hybrid progeny. [112]. In addition to identifying plant ploidy, GISH can also identify whether a phase is autopolyploid or allopolyploid [18,19,77,113]. Flow cytometry is the predominant method used for determining nuclear DNA content due to its ease and speed of use. Flow cytometry (FCM) can also be used to identify the ploidy of plants. However, it should be noted that some aneuploids may have C-values that are less distinguishable from those of the whole ploidy. [114]. Generally, C-values are proportional to ploidy, but some modern hybrids differ in species [8]. Therefore, although FCM can be used for the rapid detection of plant ploidy, there are limitations. The use of quantitative fluorescent polymerase chain reaction (QF-PCR) analysis for quantitative genotyping also enables karyotyping. By examining the genotype of two markers per chromosome, we were able to deduce the complete karyotype [20,44]. Henry et al. [115] used QF-PCR to identify euploids and aneuploids in *Arabidopsis thaliana*. Currently, simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) molecular markers are also widely used to identify ploidy [116]. The number of SSR alleles and the frequency of alleles in SNP markers can be analyzed to identify the plant's ploidy.

6. Conclusions and Further Prospects

Polyploid plants have excellent traits such as robust plants, broad leaves, large and colorful flowers, and high-stress resistance; however, polyploid plants also have disadvantages including delayed development and low fruit set. In future research, the study of molecular biology should be strengthened with the aim to produce more excellent polyploids and enriching ornamental plant germplasm. Colchicine is the main way to induce polyploids in plants. Two important factors for successful polyploid induction in plants are the concentration of colchicine solution and the time of treatment. The higher the concentration of colchicine solution, the lower the survival rate, and there is first an increase and then a decrease in the mutation rate, for the same duration of polyploid induction treatments. When colchicine concentrations are consistent, survival decreases with time, and mutagenesis increases at low concentrations and decreases at high concentrations. Currently, somatic cell induction is still primarily used in polyploid induction, but chimerism may also exist in somatic cell induction. However, the isolation of chimeras is still primarily performed using plant tissue culture, and the period in plant tissue culture is a longer

cycle duration. Some plants are difficult to propagate in plant tissue culture, so research on isolation and chimera isolation needs to be intensified. Different induction methods have an impact on the efficiency of polyploid breeding, as the three $2n$ gametes induced by FDR, SDR, and IMR can produce gametes with different heterozygosity. FDR-type $2n$ gametes are a hot research topic for breeding because they have relatively high heterozygosity and therefore allow for the more efficient transfer of parental heterozygosity and epistasis to progeny. Sexual polyploidy primarily consists of the $2n$ gamete pathway, and interploidy hybrids and sexual polyploidy can produce more variation and provide more conditions for breeding new varieties of ornamental plants. The combination of sexual polyploidy and chromosome-doubling techniques should be enhanced in future experiments. We are sure that we will be able to achieve the desired results in the future, which can be used in the breeding of new varieties of ornamental plants and innovations in cultivation.

Author Contributions: Conceptualization, L.C. and F.Y.; investigation, L.C., Z.L., Y.Z., M.F., P.A., Y.Y. and F.Y.; funding acquisition, F.Y.; software, L.C.; supervision, F.Y.; writing—original draft, L.C.; writing—review and editing, L.C., F.Y. and Y.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (3197140894), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), and the Postgraduate Research and Practice Innovation Program of Jiangsu Province (SJKY19_0882).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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