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Review

Breeding Aspects of Selected Ornamental Bulbous Crops

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Abstract: This article provides an overview of the origin, genetic diversity and methods and trends in breeding of selected ornamental geophytes (*Lilium*, *Tulipa*, *Narcissus* and *Hippeastrum*). The role of interspecific hybridisation and polyploidisation in assortment development is reviewed. A great variety of cultivars with traits of interest have been generated over the last century by using classical breeding. Geophyte breeders have been interested in a diversity of traits, including resistance to diseases, flower colour and shape, long lasting flowering and a long vase life. Shortening the long breeding process of many geophytes by reducing the juvenile phase and using in vitro techniques are reviewed. Currently, the breeding process has been enhanced by using modern molecular cytogenetic techniques. Genomic in situ hybridisation is frequently used, among other techniques, for genome differentiation in interspecific hybrids, and for assessment of the extent of intergenomic recombination in backcross progenies. Furthermore, several molecular marker techniques are used for verification of hybrid status, identification of genetic diversity, confirmation of the genetic fidelity of in vitro propagated plants and construction of high-density linkage maps. Recently, a myriad of new plant breeding technologies, such as cisgenetics and genome editing technologies have been used to improve the traits of ornamental geophytes, an endeavour that is discussed here. Breeding trends, cultivar novelties as well a new cultivars registered by international authorities during the last five years are presented in detail.

Keywords: amaryllis; breeding trends; cultivar registration; cytogenetics; genome editing; *Hippeastrum*; *Lilium*; introgression breeding; molecular markers; *Narcissus*; ploidy manipulation; *Tulipa*

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1. Introduction

The huge group of geophyte plants was classified by Raunkiaer (1934) after [1] cryptophytes. They are plants with annually renewable buds located in special storage organs: bulbs, tubers and rhizomes, and include over 800 botanical genera [2], although not all are economically important. The dozen with high economic importance includes lilies (*Lilium* L.), narcissi (*Narcissus* L.), tulips (*Tulipa* L.) and hippeastrum (*Hippeastrum*), all of which are the focus of this review. The genera are popular worldwide as cut flowers, bedding and border plants as well as potted plants. They are grown in the field and in greenhouses. Many of these plants are forced. Globally, the value of production has been estimated at over $1 billion [3], with the total area of nearly 21,400 ha dedicated for bulb, corm and tuber production in the Netherlands, the leading producer of flowers [4–7]. Tulips and lilies have been among the top five cut flowers sold on the Dutch flower auctions for many years, with sales of 243 million euros (third highest position) and 144 million euros (fourth highest position) in 2020, respectively [8]. The United Kingdom is the world leader in the commercial production of narcissi (daffodils), with over 4000 ha grown. The narcissus industry is estimated to have an annual output value of around GBP 45 million [9]. *Hippeastrum*—commonly known as amaryllis on the global market—occupied the eleventh highest position in 2016 among the cut flowers sold on the Dutch flower auctions

[10]. However, the dominance of the Netherlands on the flower bulb market is decreasing, as new countries such as Chile and New Zealand (from the southern hemisphere) and China [11] have recently joined the group of significant producers of ornamental geophytes. Production is also expanding in other Latin American and Asian countries and in southern Africa [12].

Although geophytes include hundreds of botanical genera and species, and the cultivars of the economically most important ones number in thousands, their diversity continues to increase because of the purposeful breeding of new cultivars. This phenomenon is related both to changing growing conditions and new threats and to the constant market demand for original and surprising decorative features of flowers.

Geophyte breeding is not easy, especially because of the long juvenile phase of seedlings and the often low natural vegetative propagation rates of bulbs and tubers. This makes the process of developing a new cultivar and bringing it to market as long as 20–25 years in the case of tulips [13–15] and 15–25 years in the case of narcissi [16]. It is known that the juvenile phase to first flowering can take as long as 3–8 years for *Narcissus* [17–19], 4–7 years for tulips [18] and 2–3 years for lilies [20]. However, hybrids of *Lilium* × *formolongi* flower in the year of sowing was reported by Mynett [21] and by Anderson et al. [22]. *Hippeastrum* spp. propagated by seeds flower for the first time after 2–3 years [23], while the genus *Amaryllis* propagated by seeds needs 5–6 years to produce the first flowers [24]. In addition, in our own trials [25], seedling populations of *Hippeastrum* produced flowers for the first time in the second year after sowing (flowering at 6%) and flowered en masse in the third year (Figure 1a–c).

Figure 1. A breeding programme of *Hippeastrum* × *chmielii* at Warsaw University of Life Sciences, Poland. (**a**) Seed germination on petri dishes; (**b**) hybrid seedlings in their juvenile phase under greenhouse conditions; (**c**) flowering of the seedlings' population en masse in the third year after sowing.

The initial life cycles for geophytes are essentially connected with annual warm–cold–warm sequences, leading to maximal growth rates until meristem competency for flower initiation occurs [26]. Because speed is crucial for breeding, marketing new cultivars or adapting new species, a great deal of research has been conducted to shorten the juvenile phase of *Lilium* [27,28], *Narcissus* [29] and *Tulipa* [30–32]. Most often, shortening this period is primarily associated with accelerating seed germination itself (especially for

Narcissus [29]). Moreover, in many but not all species, early seed germination (1–3 weeks after sowing) is highly correlated with leaf unfolding rates and early flowering, a phenomenon that was observed for *Lilium* × *formolongi* and was used as selection tool [33]. Conversely, the majority of the above-mentioned studies focused on physiological factors, and relatively limited molecular and genetic studies have been performed until recently. This deficit could be connected to the large genome size of *Lilium* and *Tulipa*: 25 and 36 GB, respectively [34]. Most of the current knowledge on the genetic and molecular mechanisms underlying flower initiation has been obtained from model species, mainly *Arabidopsis thaliana*, and now it can be translated to non-model species, such as geophytes [35]. Therefore, in recent years, studies related to the search for the molecular basis of change of vegetative phase and flowering initiation and regulation of certain geophytes have begun (*Lilium* [36], *Narcissus* [37], *Lilium* and *Tulipa* [38] and *Tulipa* [39]).

2. Origin, Natural Occurrence and Genetic Diversity of Selected Geophyte Genera

2.1. Hippeastrum

The present genus *Hippeastrum* (Amaryllidaceae) was included in the genus *Amaryllis* until the 14th International Botanical Congress in 1987, and they are now two separate botanical genera. *Hippeastrum* spp. often assume many names depending on the colour and shape of the perianth, the species or where it occurs. 'Butterfly Amaryllis', 'Green Amaryllis', 'Lily of the Palace' or 'Mexican Lily' are several names by which these plants are known [24]. The proper name—*Hippeastrum*–derives from two Greek words: *hippe* meaning horse and *aster* meaning star [40]. In horticultural production, there is *Hippeastrum hybridum* hort. [41–43], which was obtained by crossing several species (*H. vittatum, H. leopoldii, H. reginae, H. aulicum* and *H. pardinum*) [44]. The genus includes 50–60 [45] or 55–75 [42,46] taxa, which occur naturally in the Americas, mostly in tropical areas, Argentina and Chile. The natural habitat of *Hippeastrum* is steppe or wooded regions with a distinct dry season, a factor that has had a great impact on the physiology and morphology of this genus [40]. The first species of *Hippeastrum* that was imported to Europe in 1689 was *H. puniceum* (Lam) O. Kuntze, followed by *H. reginae* (L.) Herb. in 1725 and *H. vittatum* (L'Herti.) in 1769. In 1799, a cross between *H. vittatum* and *H. reginae* resulted in the first hybrid species, namely *H.* × *johnsonii* [24,44,47].

2.2. Lilium

The genus *Lilium* (*Liliaceae*) is considered to have originated in eastern Asia, similarly to the genera *Fritillaria, Nomocharis, Cardiocrinum* and *Notholirion* [48]. According to a study by Nishikawa et al. [49], lilies are closely related to the genera *Fritillaria* and *Nomocharis*. The name *Lilium* is from the Latin *li* (white) and *lium* (flower) and is connected with pure white flowers of *L. candidum* and *L. longiflorum*. Lily has also become a girl's given name in different languages, as Lilian in English, Susan (from the lily Shoshan) in Hebrew and Yuri and Sayuri in Japanese, in which 'yuri' means lily [50]. The genus comprises approximately 100 species native predominantly to Asia, but also to North America and Europe.

2.3. Narcissus

Narcissus (Amaryllidaceae) is used both as the name of a botanical genus written in italics and as a common English name (non-italics) [50]. 'Daffodil' is also generally used interchangeably with 'narcissus', but Rees [51] has suggested that it should be strictly reserved for the yellow trumpet narcissus (*N. pseudonarcissus*). 'Jonquil' and 'Paperwhites' are used for *N. jonquilla* and *N. tazetta*, respectively. In popular British usage, the term 'daffodil' is used for trumpet or large-cup *Narcissus*, and 'narcissus' is used for smaller flowered types [16]. The name of the genus comes from Narcissus, a son of the river god Cepheus and a forest nymph in Greek mythology [52]. The centre of density and diversity of the genus *Narcissus* is concentrated in the Iberian Peninsula, the Southern Alps and the Mediterranean. *N. pseudonarcissus* is only narcissus native to the United Kingdom, where

it is known as the 'Lent Lily' or the 'Old English Daffodil' [53]. *N. tazetta* var. *tazetta* (*chinensis*), known as 'Chinese Sacred Lily', originated also from the Mediterranean, but was transported to China by trade in ancient times [11]. The genus *Narcissus* presents great taxonomic problems, and there have been numerous attempts at its classification. The number of species varies from 26 [54] to 60 [55].

2.4. Tulipa

The number of species in the genus *Tulipa* (Liliaceae) varies depending on the author: 50–60 [56,57], 76 [58], 87 [59], 100 [60] or 105 [61]. The name for the tulip is derived from the Persian *dulband*, or Turkish *türbent*, meaning a turban, and one explanation is that the flower was compared with turbans commonly worn by Ottoman men in the 16th century. In truth, the word *lale* was used for tulip in the Turkish and also Arabic languages [58]. Tulips occur in nature from Anatolia and Iran in the west to north-eastern China. The centre of biodiversity of the genus is in the Pamir and Hindu Kush mountains and on the steppes of Kazakhstan. In natural locations, tulips also grow in Europe (especially in the south) and northern Africa. However, these are probably species imported by man and naturalised. *Tulipa sylvestris* is one such European species [58]. Tulips were cultivated as early as the 11th–12th centuries in the gardens of today's Turkey and modern Iran. In Turkey, species from natural sites were cultivated, also in addition to intensive breeding of new cultivars [60,62]. The tulip with slender, curving petals—of the current lily-flowered tulips—has become a symbol of the Ottoman Empire since the mid-16th century. Probably the first European who saw tulips in Istanbul (Constantinople at that time) was Marquis Ogier Ghiselin de Busbecq, ambassador of emperor Ferdinand I at the court of Suleiman I the Magnificent in 1554. Several years later, he brought the first tulip bulbs to the imperial garden in Vienna and put them under the care of the French botanist Charles de L'Écluse (Latin: Carolus Clusius). The latter took up a chair of botany at the University of Leiden (the Netherlands) in 1593, where he took the gift with him [60,63]. Tulips were rare in the early 1600s and then gained popularity, which led to Dutch 'Tulipomania'—to this day the most spectacular example of an economic speculative bubble that burst in 1637. In the 18th century, tulips regained popularity in the Netherlands [50] and later in other countries worldwide.

2.5. Basic Chromosome Number and Ploidy Level of Species

Ornamental bulbous crops differ in the basic chromosome number. In the genus *Hippeastrum* Herb., the species possess x = 11 chromosomes [64,65], whereas *Lilium* L. and *Tulipa* L. share the same basic chromosome number (x = 12) [66,67]. In *Narcissus*, the two subgenera, *Narcissus* L. and *Hermione* (Haw) Spach, differ in basic chromosome number. The species of the subgenus *Narcissus* have a basic chromosome number of x = 7. Conversely, the subgenus *Hermione* comprises species with different basic chromosome numbers of x = 5, 10 and 11 [66,68].

The majority of native species of bulbous flowers are diploid. In *Lilium*, the exception is a triploid form of *L. tigrinum* ($2n = 3x = 36$). In the subgenus *Narcissus*, the native species include diploids ($2n = 2x = 14$) and triploids ($2n = 3x = 21$) [69], but chromosome numbers rise to the hexaploid level ($2n = 6x = 42,43$) [70] or to the octoploid level ($2n = 8x = 56$) [66] in certain species. Subgenus *Hermione* species include tetraploids ($2n = 4x = 20$) and hexaploids ($2n = 6x = 30$), as in *N. serotinus* [71]. Recently, more polyploids, such as the tetraploid *N. papyraceus* ($2n = 4x = 22$) and the hexaploid *N. dubius* ($2n = 6x = 50$) [72], have been reported. Marques et al. [73] studied hybridisation and polyploidy frequency in the Mediterranean region, where *Narcissus* is native, and concluded that both phenomena seem be important for the genus. A remarkable karyological variability with basic chromosome numbers are the result of chromosomal rearrangements, natural hybridisation and polyploidisation, including between species not closely related. High variation in the number of chromosomes occurs in the genus *Tulipa*, where in addition to the predominant numbers of diploid species, there are also triploid genotypes ($2n = 3x = 36$) in *T. kaufmanniana*

and *T. clusiana* [59]; tetraploids (2*n* = 4x = 48) in *T. bifloriformis*, *T. sylvestris*, *T. kolpakowskiana* and *T. tetraphylla*; and pentaploids (2*n* = 5x = 60) in *T. clusiana* and hexaploids (2*n* = 6x = 72) in *T. polychrome* [74]. Similarly, in the genus *Hippeastrum*, apart from diploids (2*n* = 2x = 22), there are triploids (2*n* = 3x = 33) in *H. puniceum* (from Guyana); tetraploids (2*n* = 4x = 44) in *H. reginae*, *H. starkii* and *H. blossfeldiae*; pentaploids (2*n* = 5x = 55) in *H. scopulorum* and *H. cybister*; and hexaploids (2*n* = 6x = 66) in *H. puniceum* (from Brazil) [65,75,76]. A number of *Hippeastrum* species are euploids, including *H. forgeti* (2*n* = 23) and *H. iguazuanum* (2*n* = 24), and aneuploids—*H. blumenavia* (2*n* = 20) [75,77,78].

3. Classical Breeding: Cross-Pollination

Most of the new cultivars of ornamental bulbous crops introduced to the floriculture industry were developed via classical breeding strategies such as intra- and interspecific hybridisation, spontaneous mutation, haploid induction and polyploidisation, among other techniques. The breeding strategy varies according to the genus. Although interspecific hybridisation is the primary and most important source of variation in *Lilium*, *Narcissus*, *Hippeastrum* and *Tulipa*, these crops differ in the origin of polyploidy. Spontaneous polyploidisation has played an essential role in the origins of polyploid cultivars in narcissus [70] and Darwin hybrid (DH) tulips [79]. In the last few decades, polyploidisation in *Lilium* and *Tulipa* has been more manipulated by breeders (mitotic chromosome doubling, *2n* gamete induction, interpolyploidy crosses, etc.). In tulips, an important source of variation is also spontaneous mutations and mutation breeding [80]. For example, well known sports of the DH tulip 'Pink Impression' are: 'Apricot Impression', 'Design Impression', 'Red Impression' and 'Salmon Impression' [80].

In species of the Amaryllidaceae family, including *Hippeastrum* and *Narcissus*, propagation by artificial pollination is the only method for obtaining viable seeds. Therefore, many breeding programmes focus on cross-pollination of these plants. The use of diploid forms, which have the advantage of being easy to cross, provides the opportunity to introduce traits belonging to the species. The plants thus obtained are characterised by high vigour and a shorter juvenile period with respect to polyploids [81,82]. In classical breeding of *Hippeastrum* spp., it can be a big problem to obtain receptive maternal forms and pollinating paternal forms at the same time. This is due to flowering of cultivars at different times and the short-lived ability to pollinate and fertilise flowers. Additionally, the flowers of *Hippeastrum* are proterandrous, and flowering generally occurs once per year [83,84].

The storage capacity of *Hippeastrum* pollen grains increases with decreasing temperature. Studies by Ye and Shi [85] and Almeida et al. [84] have confirmed this rule. As the temperature decreases to −20 °C, the germination capacity is higher with respect to high temperatures (20 or 25 °C) and the storage time is about 3 months. For other species in the family Amaryllidaceae, such as *Narcissus poeticus*, pollen can be stored for 72 days, and pollen from *Galanthus nivalis* can be stored for 42 days [85]. Low temperatures do not harm the individual pollen grains. Instead, they stimulate pollen tube formation, without affecting their ability to fertilise. Conversely, high temperatures reduce the viability and germination of pollen grains. This phenomenon has been confirmed by studies on the degradation of pollen of narcissi grown in greenhouses during hot summers [86]. According to Marciniak et al. [87], the low viability of pollen grains of *Hippeastrum* may be affected by the high temperature in the greenhouse during the flowering period of the plants. The pollen viability could also be a cultivar feature. In *Narcissus*, Chwil [88] determined that the pollen grain viability was up to 92% in the cultivar 'Hardy' and only 22% in the cultivar 'The Sun'. Sanders [89] reported that the number of germinated pollen grains from the narcissus samples was 400 for the cultivar 'Gloriosus' but only 20 and 23 for 'Magic Step' and 'Silver Bells', respectively. In *Hippeastrum*, Khaleel et al. [90] reported the level of pollen viability at 60–80% for nine hybrids and Marcinek et al. [87] found it to be 66–83% for three cultivars. Many cultivars obtained by crossing show reduced pollen viability or sterility. He et al. [91] proved this outcome for nine lily genotypes in which the percentage of germinating pollen 1 day after anthesis was 81% for *Lilium sulphureum*, 73.4–77.1% for

three hybrid cultivars and only 17.8% for the cultivar 'Tiny Padhye'. The pollen of the cultivar 'Jinghe' did not germinate at all.

From the point of view of classical *Hippeastrum* breeding, improving the storage of pollen grains at low temperatures may be helpful in crossing this genus with other species with different flowering dates. This eventuality is especially important for creating new cultivars by crossing genotypes with unique traits, then selecting and evaluating interesting duels. Hybrids selected in this way may differ in the timing and number of annual flowering cycles compared to the initial forms [84].

4. In Vitro Techniques for Breeding

Tissue culture techniques are used to overcome sexual barriers in intersectional or intergeneric crosses. The cut-style method and in vitro pollination are used to overcome pre-fertilisation limitation, while the post-fertilisation barriers focus on preventing embryo abortion, including embryo rescue, ovary slicing and ovule culture [92–94]. Recently, in vitro pollination combined with embryo rescue has been applied successfully in *Lilium* to obtain hybrids from crosses allotriploid Oriental × Trumpet (OTO) lilies with Oriental lily (OTO × OO) [95], *L. auratum* × *L. henryi* [96] and interploidy crosses of F_1 hybrids Longiflorum × Asiatic with autotetraploid Asiatic lily (LA × AAAA) [97]. Moreover, embryo and ovule cultures have been applied in tulips to overcome crossing barriers in interspecific hybrid resulted from crosses *Tulipa gesneriana* × *T. fosteriana* 'Red Emperor', *T. gesneriana* × *T. eichleri* 'Excelsa' and *T. gesneriana* × *T. greigii* [98].

The long process of vegetative propagation of new breeding clones or new cultivars of geophytes can be sped up by using the in vitro propagation method. Currently, efficient in vitro techniques are available for most flower bulbs. At the beginning of the 21st century, a new method of tulip micropropagation based on cyclic multiplication of adventitious shoots was developed, enabling the production of approximately 1000 microbulbs from a few bulbs over 2–3 years [14,99–101]. In this method, regeneration is obtained on fragments of flower stems isolated from bulbs. The method is based on cyclic shoot multiplication performed by using thidiazuron (TDZ) instead of other cytokinins, such as 6-benzylaminopurine (BAP) and *N*6-(-isopentyl)adenine (2iP), with sub-culture every 8 weeks. The shoots are induced by low-temperature treatment to form bulbs, which finally develop on a sucrose-rich medium at 20 °C. Bulbs are then dried for 6 weeks and rooted in vivo. The number of multiplication subcultures should be limited to 5–10 cycles to lower the risk of mutation [14]. In other studies, Maślanka and Bach attempted to regenerate tulip plants through organogenesis from vegetative bud explants–isolated from uncooled bulbs [102] and from seed-derived explants [103]. The initial results of further studies on tulip regeneration on fragments of flower stem isolated from cooled bulbs showed the possibility of using the aromatic cytokinin meta-topolin (mT), both at regeneration and shoot multiplication stages, instead of the commonly used TDZ and 2-iP [104].

The regeneration efficiency of tulips has been improved further using systems based on somatic embryogenesis (SE) [105–108]. Recently, Podwyszyńska and Marasek-Ciolakowska [109] described efficient in vitro regeneration systems of tulips based on the cyclic multiplication of the embryogenic callus. This method enabled the researcher to obtain on average 30–55 embryos able to form bulbs per 100 mg callus on a medium containing 0.01 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and TDZ or BAP alone or in combination, enriched with 100 mg L^{-1} proline.

Micropropagation is also a rapid and efficient method of propagation of *Hippeastrum* spp. In vitro propagation of this genus was started in the late 1970s [110] and has developed during the last two decades [111–113]. Many studies have been performed on *Hippeastrum* × *chmielii*, bred at Warsaw University of Life Sciences, Poland [114–117]. Because of environmental trends, it seems interesting to evaluate the addition of biostimulator Goteo to the media during micropropagation of *Hippeastrum* as an alternative to traditional plant growth regulators. The positive effect of Goteo on the increased number of regenerated bulblets as well as the increased root number and plantlet weight were observed

during the bulb formation stage in *H. hybridum* 'Double Roma' and *H.* × *chmielii* clone no. 18 [117]. In vitro tissue culture techniques have also found application in breeding to obtain and to improve cultivars [118]. In triploid plants, which may be self-sterile, fertilisation may not occur despite fully formed generative organs. Sometimes, however, an embryo can be artificially induced. However, it will not be capable of further development due to the presence of postzygotic barriers. For genotypes derived from triploid forms crossed with diploids, it is possible to sustain developing embryos by using an in vitro embryo culture, an approach that has also been confirmed for *Hippeastrum* [24,82,119].

Propagation of lilies by tissue cultures has been well reviewed by Kim and De Hertogh [120], Langens-Gerrits [121] and Bakhshaie et al. [94]. A myriad of studies concerning micropropagation of this genus have been performed. Currently, *Lilium* is the only bulbous taxon that is micropropagated on a significant production scale. Lilies are commonly propagated in Dutch and Polish labs [50]. Different plant fragments can be used as initial explants, but the bulb scales have been found to be the most responsive explants for direct and indirect organogenesis, plant regeneration and SE in *Lilium* [94]. The main disadvantage of bulb scales compared with the other explants is that they are difficult to decontaminate and to obtain aseptic cultures. Therefore, Bakhshaie et al. [94] suggested a two-step protocol: regenerating in vitro plantlets first from bulb scales or leaf segments and then using microscales of these aseptic bulblets (the transverse thin cell layer [tTCL] technique) for further multiplication. A crucial factor for improving the in vitro bulbils yield of lilies (and many other geophytes) is a high level of sucrose in the media, as high as 9% [122,123]. In the case of lilies propagated in vitro, Gabryszewska and Sochacki [124] probably published the first report concerning the effect of nitrogen salts and their interaction with sugar on the formation and growth of bulblets. They showed clearly that a high sucrose level and the nitrogen salts in the medium strongly promoted bulblet fresh weight of lilies. By contrast, a medium with a high sucrose-to-nitrogen ratio had an inhibitory effect on leaf formation. It is clear that also in *Lilium*, in vitro methods have been successfully developed for shortening breeding programmes (SE [125] and liquid media in bioreactors [126]). Bakhshaie et al. [125] succeeded in plant regeneration via SE from both roots and bulblet microscales derived from bulblets of *Lilium ledebourii* using the tTCL technique. Tang et al. [127] established a protocol for optimum callus induction and plant regeneration of *L. leucanthum* by using in vitro cultured leaves, petioles and small scales. There have been successful outcomes in genetic transformation studies using meristematic nodular callus (NOD) [128–131]. The main advantage of the NOD system is its high and continuous growth and regeneration efficiency.

The first work on micropropagation of narcissus was conducted independently in several research centres in the 1970s and early 1980s in the United Kingdom, Canada, Japan, and Israel. Preparation of bulbs from which initial explants will be taken involves obtaining an aseptic and regenerable explant. To increase the multiplication rate of shoots, Chow et al. [132] developed a method of 'severe cutting', and other authors have employed the mini-twin-scales method, which is based on re-planting mini-twin scales obtained from bulbs in vitro onto the initiation medium [133]. A significant increase in propagation rate was obtained by extending the culture initiation period to 24 weeks and successively cutting off regenerating shoots from the initial explant [134]. To obtain bulblets, shoots are transferred to a new medium without growth regulators (or with a low concentration), with the addition of activated carbon and increased sucrose levels [135]. A positive effect of cold treatment of bulbs on growth initiation after planting from in vitro conditions has also been shown. Sochacki [136] performed additional research on *Narcissus* micropropagation. The experiments showed a positive effect of supplementation of medium with organic substances on the number of shoots in several cultivars tested. In recent years, increasing research has been performed on the intensification of the multiplication factor by propagating narcissi on liquid media and in bioreactors [137–140]. Large-scale liquid cultures in bioreactors can be used for micropropagation via both or-

ganogenesis or SE; however, the problem of malformed tissues and organs in liquid culture often hinders ex vitro transplanting [141]. Using the temporary immersion technique, which protects the culture from oxidative stress, has proved to be efficient for commercial micropropagation of *Narcissus* via organogenesis or proliferation of the embryogenic tissue [138,139,142]. During the last two decades, SE has also been developed for *Narcissus* [143–146]. Sage et al. [143] produced somatic embryos from bulb and shoot culture leaf explants of *N. pseudonarcissus* 'Golden Harvest' and 'St. Keverne' on media with a range of 2,4-D and BAP concentrations. 'Golden Harvest' callus has been transformed with an engineered *Agrobacterium tumefaciens* strain with a binary vector containing the green fluorescent protein (GFP) gene, and 'St. Keverne' scapes have been transformed with a wild type *A. tumefaciens* strain [144]. Transformation may be used for the introduction of desirable genes to *Narcissus* cultivars, but this approach is ethically and legislatively problematic in many countries.

5. Polyploidisation for Crop Improvement

In the breeding and development of ornamental geophytes, polyploidisation plays a remarkable role because it can affect the emergence of new distinctive characteristics [147,148]. Currently, the major ornamental bulbous crops include polyploids in their commercial assortment [149]. According to Ramanna et al. [66], among ornamental geophytes, there is a tendency to replace diploids with polyploid cultivars. This trend has been especially visible in *Narcissus* and *Lilium*. At present, nearly 75% of *Narcissus* cultivars are tetraploids while the diploids and triploids amount to only about 12% each [69]. Similarly, in *Lilium*, most of the modern inter-sectional cultivars are triploids, but also several commercial cultivars are aneuploids [150–152]. In *Hippeastrum*, most of the cultivars with single and double flowers available on the market are tetraploid genotypes characterised by large flowers–for example, 'Pink Surprise', 'Rapido', 'Apple Blossom' and 'Cherry Nymph' [153]. Tulips are an exception from this group of ornamentals: diploid cultivars maintain a leading position on the market. The triploid ($2n = 3x = 36$) and tetraploid ($2n = 4x = 48$) tulips mostly belong to the DH cultivars and have yielded a significant percentage of the market assortment of the late last millennium [154].

Many neopolyploids originated spontaneously among ornamental bulbous crops through the functioning of numerically unreduced (*2n*) gametes (meiotic doubling). For example, in *Tulipa*, the triploid DH cultivars such as 'Apeldoorn', 'Pink Impression', 'Yellow Dover', 'Kouki' and the tetraploid 'Tender Beauty' were the result of spontaneous polyploidisation [79,155–157].

The occurrence of *2n* gametes has been reported in species of *Narcissus*, [69] cultivars and interspecific hybrids of *Tulipa* [79,158,159] and in *Lilium* distant F_1 hybrids such as Longiflorum × Asiatic (LA) and Oriental × Asiatic (OA) [96,160–164]. The selection of the parents producing *2n* gametes allows manipulation of ploidy level of the offspring. In *Lilium* and *Tulipa*, most of the progenies that have resulted from the use of unreduced gametes are triploid. According to Zhang et al. [152], allotriploid breeding may be a future trend for new cultivars in *Lilium*. For example, the triploid sexual progenies (AOA) were obtained from backcrossing F_1 OA hybrids producing *2n* gametes with an Asiatic cultivar [163,165], whereas allotriploid and allotetraploids were produced as a result of unilateral and bilateral sexual polyploidisation by backcrossing F_1 hybrids of LA and OA to Asiatic parents (AA) [166]. Odd-allotetraploid lilies representing the LAAA genome can breed from LA × AAAA interploidy crossing [97] in which the maternal form provides *2n* eggs. Similarly, Marasek-Ciolakowska et al. has reported a low yield of polyploid tulips in 2x × 2x crosses involving *2n* pollen [149,159,167]. Cytological analysis of sexual polyploid progenies has shown that the use of *2n* gametes can induce intergenomic recombination in interspecific hybrids [164–166,168–172], which plays an essential role in introgression of desired traits [173]. For example, in *Lilium* triploid progenies with intergenomic recombination were produced when OA hybrids producing functional 2*n* gametes were backcrossed with diploid Asiatic cultivar [165].

The ploidy level of the progeny can be manipulated depending upon the ploidy level of the parental forms. For example, polyploids of tulips and lilies were obtained as a result of interploidy crosses (4x × 2x or 4x × 2x [150,174]). Triploid tulip cultivars such as 'Lady Margot' and 'Sunny Child' resulted from crossing diploid cultivars with tetraploids [155]. Autotriploid (AAA) and allotriploid (AOA, LAA, LLO) lilies and triploid DH tulips could be used as a maternal parent [95,149,150,159,167,175,176].

In tulips produced by crossing triploid DH with diploids producing *2n* pollen, Marasek-Ciolakowska et al. [149] received aneuploids, tetraploids and a few pentaploid genotypes. Similarly, in *Lilium*, Zhou et al. [95] successfully crossed allotriploid OTO lilies with OO lilies to produce aneuploid progenies. There are also examples of the use of triploids as a pollen donor. It has been demonstrated cytogenetically that certain triploid hybrids can produce aneuploid and euploid (x, 2x and 3x) gametes [169,170,177]. In DH tulips, most progenies resulted from 2x × 3x hybridisation were triploid with the exception of a few aneuploids (3x + 1 and 3x − 1) [159], whereas Okazaki and Nishimura [174] reported that, in the 2x × 3x crosses, over 90% of the progenies were diploids and rare genotypes (7.4%) were aneuploids. In *Lilium*, crosses of the triploids with diploids and tetraploids produced aneuploid or near diploid and pentaploid progenies, respectively [178]. Aneuploid lily cultivars obtained from interploidy crosses (3x × 2x or 3x × 4x) may be a good source of variation in morphological, ecological and physiological characteristics [175].

5.1. Induction of 2n Gamete Formation

Because the spontaneous production of *2n* gametes by species and distant hybrids is usually low, numerous attempts have been made recently to induce the formation of these gametes (Table 1). Lokker et al. [179] successfully stimulated the production of *2n* pollen in 2% of the sterile *Lilium* OA hybrids using heat-shock treatment. Wu et al. [180] induced diploid female gametes by treating young flower buds of Oriental cultivars 'Con Amore' and 'Acapulco' with different concentrations of colchicine. When treated plants with diploid eggs were used in crosses with *n* pollen, the triploid F_1 progenies were obtained. Caffeine (0.3%) injection to the flower bud has successfully induced fertile *2n* gametes in F_1 interspecific OA hybrids [168]. They were subsequently crossed both as male and female parents to Asiatic hybrids and all of the obtained BC_1 progenies were triploids. The formation of *2n* gametes has also been induced by nitrous oxide (N_2O) gas treatment for 24–48 h at 6 atm pressure of the flower buds in tulips [147,181,182] and lilies [183–187]. The optimal meiotic stage of gametogenesis at which to induce *2n* pollen by N_2O treatment is metaphase I [147,185,187]. Kitamura et al. [188] showed that N_2O gas acts as a meiotic doubling agent by inhibiting microtubule polymerisation, a phenomenon that stops chromosome movement towards both poles during anaphase. Certain polyploids have been obtained in *Lilium* and *Tulipa* using *2n* gametes induced by N_2O treatment [181–185,189]. Triploid and tetraploid progenies were obtained from the crosses of N_2O-treated OA hybrids to Asiatic parent [184]. N_2O treated plants can be used for hybridisation both as male and female parents [184]. In lilies, treatment with N_2O has been also applied to overcome sterility of OA and Oriental × Trumpet (OF) F_1 hybrids [184,187,189]. Sato et al. [186] demonstrated that treatment of *L.* × *formolongi* hort. plants with N_2O gas for 2 h at 6 atm 13 days after pollination can induce *4n* zygotic embryo.

Table 1. Reports on the inducing factor of unreduced gamete formation and their application for meiotic polyploidisation in selected ornamental bulbous crop.

Method of *2n* Gamete Induction	Crop	Species/Cultivars	Explant/Treatment/Hybridisation	Ploidy Level of Progeny Plants	References
Nitrous oxide (N_2O) gas	*Lilium*	Oriental × Asiatic (OA) hybrids	Flower buds 5–10 mm in length; 6 bars (6×10^5 Pa) *, for 24 and 48 h; Formation of *2n* pollen and *2n* egg cells	Crosses both as male and female: AA × OA and OA × AA; triploids, tetraploids plants	[183,184]
		Asiatic hybrids	Flower buds at different meiotic stages; 6 atm (6.08×10^5 Pa); 24 h	Tetraploid cultivars were pollinated with the N_2O-treated pollen; tetraploid offspring	[185]
		Asiatic hybrid 'Regata' and *Lilium longiflorum* 'Hinomoto'	Flower buds (17–22 mm); 6 atm (6.08×10^5 Pa); 24 h	-	[188]
		Lilium × *formolongi*	Induction of 2*n* pollen: flower buds (19–23 mm); 6 atm (6.08×10^5 Pa); 24 h Induction of 4*n* embryo: plants treated with N_2O 13 days after the pollination; 72 h; 6 atm (6.08×10^5 Pa)	Tetraploid seedlings developed from zygotic embryo treated with N_2O gas	[186]
		Asiatic and Oriental hybrids; Longiflorum × Asiatic (LA) hybrids	Flower buds (1–10 mm); 6 atm (6.08×10^5 Pa); 48 h;	Backcrossing the N_2O-treated pollen to *Lilium* × *formolongi*; triploid BC_1 plants	[189]
		Oriental × Trumpet (OT) 'Nymph', 'Gluhwein', 'Yelloween' and 'Shocking'	Flower buds; prophase I–metaphase I stage of meiosis; 600 kPa (6×10^5 Pa); 48 h	-	[187]
	Tulipa	*Tulipa gesneriana*, and *Tulipa fosteriana* cultivars	Bulbs 6 atm (6.08×10^5 Pa); 24 or 48 h; treated plants produced a mixture of *n*, 2*n* and aneuploid pollen	Low triploid formation in crosses with the N_2O-treated pollen	[181]
Colchicine	*Lilium*	Oriental cultivars 'Acapulco' and 'Con Amore'	Flower buds/0.02–0.2% colchicine injection for 72 h	Crosses of mutated cultivars (*2n* eggs) with *n* pollen of 'Acapulco', 'Con Amore'; diploid, triploid and aneuploid progenies	[187]
Caffeine	*Lilium*	OA hybrids	Flower buds of 20–23 mm and 34–37 mm in length; 0.3% caffeine injection	F_1 OA hybrid backcrossed with Asiatic (A × OA; OA × A); triploid progenies	[168]
Interspecific hybridisation	*Lilium*	Longiflorum-Rubellum (LR) hybrids	BC_1 progeny plants were obtained from back-crossing amphidiploid LLRR with *L. longiflorum*; BC_1 plants were pollinated with tetraploid (LLLL) *L. longiflorum*	Triploid BC_1 LLR hybrids; aneuploid BC_2 LLLR hybrids	[190]
		OA hybrids	Selection of *2n* gametes producing genotypes and backcrossing with Asiatic cultivar	3x and 4x AOA hybrids	[162,163]
		LA and OA hybrids	BC_1 progeny plants were obtained from LA × AA, AA × LA and AA × OA crosses; F_2 LA hybrids were obtained from LA × LA crosses	Allotriploid BC_1 LA and OA hybrids (unilateral sexual polyploidisation); allotetraploid F_2 LA progenies, (bilateral sexual polyploidisation)	[166]
		LA and OA hybrids	BC_1 progeny plants were obtained from LA × AA and AA × OA crosses.	Allotriploid BC_1 LA and OA hybrids with numerous recombinant chromosomes	[171]

Tulipa	Martagon × Asiatic (MA); OT hybrids	BC_1 progenies were obtained from MA × AA and OT × OO crosses; BC_2 progenies of triploid OOT × OO hybrids	Diploid, triploid and aneuploid BC_1 progenies of the OT hybrids: aneuploid BC_2 progenies of triploid OOT hybrids; triploid and aneuploid BC_1 progenies of the MA hybrids	[191]
	Lilium auratum × *Lilum henryi* (AuH)	Selection of *2n* gametes producing genotypes and backcrossing with Oriental hybrids	3x Oriental–Auratum–Henryi (OAuH) hybrids	[96]
	LA hybrids	Interploidy cross LA × AAAA; in which LA hybrid produced *2n* eggs	Odd-allotetraploids LAAA hybrid	[97,192]
	T. gesneriana × *T. fosteriana* (Darwin hybrids)	F_1, BC_1 and BC_2 progenies of Darwin hybrids obtained by backcrossing to *T. gesneriana*	Diploid and tetraploid BC_1; diploid and aneuploid BC_2 hybrids	[167]
	Darwin hybrids	*2n* gamete producing F_1 Darwin hybrids were crossed with diploid and triploid *T. gesneriana* cultivars	Diploids and triploids from 2x × 2x (*2n*); tetraploid and pentaploids from 3x × 2x (*2n*) crosses; triploids and aneuploids from 2x × 3x crosses	[149,159]

* Conversions to SI units performed by the authors of this review article.

5.2. In Vitro Ploidy Manipulation

Another strategy for producing polyploids is chromosome doubling using an antimitotic agent inhibiting spindle formation and chromosome division during mitosis. The most frequently used antimitotic agents in bulbous ornamentals is colchicine [148,180,193–196], oryzalin [148,194,196–200], surflan [201] or trifluralin [148]. Chromosome doubling is predominately used to avoid crossing barriers [202], to restore the fertility of F_1 hybrids [203] and to improve the characteristics of ornamental plants [194,204–206]. Successful chromosome doubling has been achieved in species and interspecific hybrids mainly in *Lilium* and *Tulipa*, and in limited cases in *Narcissus*. In *Lilium*, both autotetraploids, such as LLLL, AAAA and TTTT, and allotetraploids, such as LALA, OAOA, LOLO, LLTT and OTOT, have been induced [154,207–210]. Table 2 shows an overview of the application of mitotic polyploidisation in the last decade in selected bulbous flowers. Induced chromosome doubling could enhance quality characteristics. In *Lilium*, for example, induced tetraploids had stronger stems [211], more leaves and more branches, greater plant height and stem length, and produced a wider bulb scale [194], larger flowers and thicker leaves [212]. The whole genome duplication may significantly affect gene expression, which can lead to increased production of secondary metabolites and change the tolerance to environmental stresses and the crop development, but the effect varies among species and the method of polyploidy induction [213]. Cao et al. [214] studied the effects of polyploidisation with colchicine on cellular, photochemical and photosynthetic characteristic of *Lilium* Formolongi × Oriental (FO) hybrids. The leaves of tetraploid plants had a thicker epidermis and spongy mesophyll tissue and showed more and thicker thylakoid lamellae and higher chlorophyll and carotenoid contents compared with diploid progenitors. The doubling of the cell genome may also have disadvantages. Several negative effects of polyploidy were observed in *Tulipa*: tetraploids had smaller flowers and leaves, lower pollen fertility and were characterised by higher fragility of the scape compared with their diploid progenitors [148]. Tetraploid genotypes of *L. rosthornii* Diels induced by colchicine (0.05% for 36 h) and oryzalin (0.01% for 24 h) had similar morphology and growth traits as diploid progenitors [196]. The major drawback of mitotic polyploidisation is the absence of intergenomic recombination due to autosynthetic chromosome pairing [163]. The absence of intergenomic recombination was observed in BC_1 and BC_2 progenies derived from allotetraploid Longiflorum × Rubellum hybrid (LLRR) [161]. This has also been observed in progenies from crossing an allotriploid Longiflorum × Oriental hybrid (LLO) with an allotetraploid Longiflorum × Trumpet hybrid (LLTT), both derived from

somatic chromosome doubling [171]. *Rhodophiala montana*, a species that is closely related to *Hippeastrum* spp., succeeded in achieving chromosome duplication on the medium with colchicine [193], but this treatment reduced the survival rate of microbulbs by 20% and their ability to produce shoots and roots by 73% and 30%, respectively, compared with the medium without colchicine.

Table 2. Recent reports on mitotic polyploidisation in selected ornamental bulbous crops.

Genus	Species/Cultivars	Explant	Method (Agent, Concentration, Time of Treatment)	New Characteristics	References
Lilium	Asiatic lily (*Lilium hybrida* L. 'Pollyanna'	Bulb scales segments	Oryzalin: 30–200 μM for 2–6 h (0.001%, 0.003%, 0.005%, 0.007% or 0.01%) for 2, 4 or 6 h	Delayed rooting, shorter roots, shorter leaves	[199]
	Lilium pumilum, *Lilium sargentiae*, *Lilium tsingtauense*		Colchicine, 0.02% or 0.04%, and oryzalin, 0.006% or 0.01%, for 24 or 48 h	Thicker and shorter leaves, fewer stomata per leaf area unit	[210]
	Lilium martagon var. *album*	Bulb scales segments	Colchicine: 0.5 (0.05%) * or 1.0 (0.1%) g L^{-1} for 4 h Oryzalin: 10 and 100 mg L^{-1} (0.001% and 0.01%, respectively) for 4 h Oryzalin: 0.5 and 5.0 mg L^{-1} (0.00005% and 0.0005%, respectively) exposure on medium for 16 weeks Trifluralin: 0.5 or 5.0 mg L^{-1} (0.00005% and 0.0005%, respectively) exposure on medium for 16 weeks	-	[215]
	Lilium davidii var. *unicolor* Salisb	Tissue culture bulb	Colchicine: 0.03%, 0.05% or 0.08% for 32, 40, or 48 h Oryzalin: 0.002%, 0.005%, 0.008% or 0.01% for 3, 6, 9, 12 or 24 h	Larger flower, thicker leaves, lower stomatal density, larger guard cells	[212]
	Lilium × *formolongi* × Oriental hybrid	Basal scale segments	Colchicine: 1.25 (0.004%) or 2.50 (0.008%) mM for 18, 24 or 36 h	Thicker epidermal and spongy tissue, more and thicker thylakoid lamellae, higher chlorophyll and carotenoid contents, Higher net photosynthetic rate (Pn) and maximum net photosynthetic rate (Pmax)	[195] (polyploidy induction) [214] (polyploid analysis)
	Lilium distichum Nakai, *Lilium cernuum* Komar	Somatic embryos	Colchicine: 0.01%, 0.05% or 0.1%; *v*/*v* for 24, 48 or 72 h	More leaves, broader leaves, larger stomata, higher chlorophyll content	[209]
	Lilium regale	Bulb scales	Colchicine: 0.01%, 0.05% or 0.1%; *v*/*v* for 6, 12 or 24 h	Increased length of stomata and chloroplast number of guard cell, lower stomata number per mm^2	[216]
	Asiatic lily ('Petit Brigitte', 'Orange Pixie', 'Black Bird', 'Pollyanna')	Bulb scales	Oryzalin: 0.001%, 0.002%, 0.003% or 0.005% for 4 h	-	[207]
	Lilium rosthorinii 'Diels'	Germinated seeds	Colchicine: 0.025–01% for 12–36 h Oryzalin: 0.005–0.02% for 12–36 h	Larger leaves, higher germination rate of bulblets	[196]
	Lilium davidii var. *unicolor*	Bulb scales	Colchicine: 0.025%, 0.05% or 0.1% (*w*/*v*) for 24 h	Fewer leaves, greater leaf width, lower stomata density and longer guard cell length	[217]
Narcissus	12 cultivars of *N.* × *poetaz*	Twin scales	Colchicine: 0.1% for 8 h	-	[218]
Tulipa	'Fringed Black', breeding clones	Flower stems	Oryzalin; amiprophos methyl (AMP): 5 (0.0005%) or 10 (0.001%) mg L^{-1} 7 or 14 days	-	[200]

'Victor', 'Fringed Black' and breeding clone Pol-D 32	In vitro adventitious shoot cultures	Colchicine, 200 mg L^{-1} (0.02%); oryzalin; 5 mg L^{-1} (0.0005%); amiprophos methyl, 15 mg L^{-1} (0.0015%); or trifluralin, 100 mg L^{-1} (0.01%)	Smaller flower, shorter flower scapes, reduced leaf width, longer stomata, larger pollen grain diameter, lower pollen fertility	[148]

* Conversions to SI units performed by the authors of this review article.

6. Modern Molecular Cytogenetic Techniques

The breeding process of *Lilium*, *Tulipa* and *Narcissus* has been strongly facilitated by using cytogenetic techniques based on DNA–DNA hybridisation. Genomic in situ hybridisation (GISH) has been used to clarify how many cultivars form and the genome composition of the allopolyploid species [219–221]. An excellent example of this are studies in *Narcissus* in which GISH was used to elucidate the origin of the hybrid species *N. obsoletus* (2*n* = 4x = 30). This analysis confirmed that *N. serotinus* L. and *N. elegans* (Haw.) Spach are the parents of this allopolyploid species [68]. GISH and molecular markers (nucleotide binding site [NBS] profiling) have also been used to clarify the origin of allotriploid narcissus cultivar 'Tête-à-Tête' (2*n* = 3x = 24 + B) [222]. It was possible to prove that 'Tête-á-Tête' comprises two genomes of *N. cyclamineus* (2*n* = 2x = 14) and one genome of *N. tazetta* (2*n* = 2x = 20) together with a B chromosome [222]. GISH has been used to clarify the origin of triploid DH tulips that were spontaneously obtained by interspecific crosses between Darwin tulips and *T. fosteriana* [79,156]. Using GISH, researchers have demonstrated that the triploid DH have two copies of the *T. gesneriana* genome and one copy of the *T. fosteriana* genome, indicating that *T. gesneriana* has supplied unreduced gametes during triploid cultivar formation [79,156]. This molecular cytogenetic technique has played an important role in introgression breeding, enabling researchers to detect the presence of translocations between parental genomes and to monitor the inheritance of recombinant chromosomes to backcross progenies [149,157,167].

GISH has been used extensively to recognise the three genomes of *Lilium viz*., Asiatic (A), Longiflorum (L) and Oriental (O), and to study the recombinant chromosomes in the backcross progenies of LA and OA interspecific hybrids [95,162–166,169,170,172,178]. For example, Khan et al. [169,170] showed the presence of extensive intergenomic recombination among the chromosomes of diploid and triploid BC progenies of LA hybrids. Similarly, GISH has been used to assess the extent of intergenomic recombination in backcross progenies of DH tulips [149,157,167]. It was revealed that most BC_1 and BC_2 DH hybrids with the recombinant chromosomes were diploids, which proved that introgression in tulips is possible at the diploid level [149,157,167]. Moreover, several F_1 DH hybrids were found to produce both *n* and 2*n* gametes. This finding provided unique opportunities to generate polyploid as well as diploid BC_1 progenies from backcrossing F_1 DH hybrids to *T. gesneriana* parents. In these studies, GISH analysis enabled the researchers to trace the mode of origin of polyploid tulips and the role of 2*n* gametes in polyploidisation [79,157].

Fluorescence in situ hybridisation (FISH) is another cytomolecular technique applied in ornamental geophytes; it enables mapping of specific repetitive or single-copy sequences on chromosomes. FISH with the 5S and 45S ribosomal DNA (rDNA) sequences provided chromosomal markers, which has improved chromosome identification [160,223–225]. This method has been successfully used for verification of hybrids in *Lilium* [226,227] and *Tulipa* [157,223]. FISH mapping ribosomal RNA genes has been used to study genetic variation among species of *Lilium* [111,228–232] and to study the presence of karyotype rearrangements in long term micro-propagated tulips in the presence of TDZ [233]. FISH with the 5S and 45S rDNA sequences has been also used to clarify the process of triploid and tetraploid cultivars formation in *Narcissus* [225]. Zeng et al. [225] identified five genomes—A, B, C, D and E—among the *Narcissus* cultivars based on the number and localisation of rDNA loci on chromosomes. Their investigation confirmed that most of the analysed tetraploid *Narcissus* cultivars (including 'Queen's Day', 'Pink Charm', 'Stadium', 'Mount Hood', 'Eline', 'Accent', 'Dutch Master', 'Flower Parade', 'Replete', 'Las

Vegas', 'Flower Surprise', 'Ice Follies' and 'Easter Born') are autotetraploid ($2n = 4x = 28$ = EEEE) obtained from chromosome doubling, whereas 'Pink Parasol' is allotetraploid (2n = 4x = 30 = CCDD) [225]. The triploid cultivar Chinese Narcissus 'Jinzhanyintai' is autotriploid ($2n = 3x = 30$ = AAA) caused by unilateral sexual polyploidisation. The results will be valuable to explain the crossing-compatibility to guide breeding of narcissus. In *Hippeastrum*, to the best of our knowledge, no molecular cytogenetic work based on DNA–DNA hybridisation has been reported.

7. Molecular Breeding

In conventional breeding, polymerase chain reaction (PCR)-based markers have become extremely useful tools enabling the fast verification of interspecific hybrids at the early stage of development. Random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) have been applied to confirm hybrid status in *Lilium* and *Narcissus* [234,235]. These markers have also been used successfully to assess the genetic fidelity of in vitro propagated tulips regenerated from somatic tissues of the 'Blue Parrot' cultivar [236]. In *Lilium*, the genetic stability of regenerants of the Oriental hybrid 'Siberia' was confirmed with amplified fragment length polymorphisms (AFLP) and ISSR markers [237]. In *Narcissus*, several molecular marker technologies, such as RAPD, short sequence repeats (SSR), AFLP and NBS markers, have been used to identify genetic diversity, population genetics and cultivars [222,238–240]. Tang et al. [241] used single nucleotide polymorphism (SNP) markers to assess the genetic diversity of 72 tulip accession numbers. The authors showed clear separation of the genomes representing *T. gesneriana* and *T. fosteriana*, but there was a relatively small variation in SNPs among cultivars representing the *T. gesneriana* genome.

Due to progress in the development of molecular technologies for mapping and sequencing DNA, molecular data including different coding and intergenic regions in the chloroplast genome have become available for phylogenetic studies. Chloroplast DNA (cpDNA) and nuclear DNA (ribosomal gene spacers) have been used successfully for plant systematic studies in *Lilium* and *Tulipa* [242–244]. Yanagisawa et al. [244] reported the relatedness of many species and cultivated tulips using coding regions of *trnL* and *matK* and intergenic spacer (IGS) region of *trnT-L* in chloroplast. Pourkhaloee et al. [245] used expressed sequence tag–simple sequence repeats (EST-SSRs), which are genic microsatellite markers, to study the genetic diversity and relationships among 280 individuals of 36 wild and cultivated tulip accession numbers from Iran and the Netherlands. Recently, researchers used plastid genome sequences of four Tulip species for comparative genomics and to study phylogenetic of 23 Liliaceae plastid genomes [246].

The breeding and the introgression of traits of interest from wild species to the assortment can be enhanced by molecular-assisted breeding (MAB), which can facilitate both the selection of parental forms for breeding and selection in progeny. For MAB, high-density linkage maps can be constructed using several molecular marker techniques [247,248]. In *Lilium*, well-saturated linkage maps that cover 89% of the lily genome were developed using AFLP, diversity arrays technology (DArT) markers and NBS profiling for two lily populations [249]. These genetic maps were used for mapping major genes and quantitative trait loci for several ornamental traits (flower colour, flower spots, antherless phenotype and flower direction) and resistances to *Fusarium oxysporum* and *Lily mottle virus* (LMoV). Six putative quantitative trait loci (QTLs) were identified for *Fusarium* resistance [249]. Moreover, the maps were saturated with SNP markers and EST-SSRs [34,250]. Recently, comprehensive linkage maps using SSR, SNP, AFLP and NBS profiling were constructed in *Tulipa* and six putative *Fusarium*-resistance QTLs were identified [251].

Genome Editing to Improve Ornamental Plants

In recent years, new plant breeding techniques (NPBTs) such as cisgenesis and genome editing technologies have been developed to assist breeders to improve important characteristic that are difficult to change via classical breeding techniques [252–254]. Genome editing technologies, particularly clustered regularly interspaced short palindromic repeats (CRISPR), allow researchers to modify DNA at precisely specified points in the plant genome. Thus far, NPBTs have been used in ornamental plants to increase resistance and to modify morphological and physiological traits such as flowering induction, flower colour, size and fragrance [254–256]. More recently, Yan et al. [257] reported the first application of CRISPR-associated protein 9 (CRISPR/Cas9) technology to *Lilium.* Transforming *L. pumilum* and *L. longiflorum* with a CRISPR/Cas9 construct targeting gene encoding phytoene desaturase (PDS) resulted in an albino phenotype. Leeggangers et al. [36] studied the role of phosphatidyl ethanolamine-binding protein (PEBP) genes and their role in flowering time control in *T. gesneriana* and *L. longiflorum*. Advances in the field of genome editing have great potential for further genetic improvement of ornamental bulbous crops and to shorten breeding programmes.

8. Breeding Strategies and Trends and Cultivar News

8.1. Hippeastrum

Breeding programmes of the genus *Hippeastrum* are dictated by market demand for original flowers. The resulting cultivars are divided into nine horticultural classification groups based on flower diversity, shape and size [258]. From the registration data of new cultivars provided by the Dutch Royal General Bulb Growers' Association (KAVB) and analysis of the years 2015–2019, in which 113 new cultivars were registered, we can conclude that this is a response to market demand. In *Hippeastrum* breeding, a great emphasis is placed on obtaining cultivars with large flowers [44,259]. That is why the largest number of registered cultivars (43) belongs to the Galaxy group (Figure 2a), the flowers of which are more than 16 cm in diameter, followed by the Diamond group (Figure 2b), characterised by medium-sized, single flowers of 12–16 cm in diameter; 28 cultivars were registered in this group. Small, single flowers with a diameter of less than 12 cm comprise the Colibri group. Only 10 cultivars were registered in this group between 2015 and 2019. The detailed number of registered cultivars from each group are shown in Figure 3a. Not only single flowers, but also full flowered cultivars are popular. The group Double Galaxy (Figure 2c) has large, double flowers, with a diameter of more than 16 cm. Twenty-three cultivars were registered in this group. In the remaining groups, only single cultivars were registered. Most cultivars are from the Netherlands and the United States, but there are also cultivars from India, China and Japan [260–264]. Selected cultivar novelties from each group are: Galaxy group, 'Red Reality', 'White Queen'; Diamond group, 'Shazam', 'Tierra'; Colibri group, 'Sleeping Beauty', 'Caetano'; Double Galaxy group, 'Canton Lady', 'Gypsy Girl'; Double Diamond group, 'Pink Lotus'; Butterfly group, 'Wild Amazone', 'Summer Breeze'; Trumpet group, 'Antoinette', 'Cygnet' [262–264]. Novelties of *Hippeastrum* presented on the International Plant Fair (*Internationale Pflanzenmesse* [IMP]) Essen 2020, Germany, are shown in Figure 2d.

Figure 2. Novelties of *Hippeastrum* cultivars. (**a**) Cultivar 'Pierrot' from the Galaxy Group presented by Dutch Breeding Company Fa. Gebr van Velden at Keukenhof Exhibition, the Netherlands, 2018; (**b**) breeding clone 0004-5 as result of further crossing of *Hippeastrum* × *chmielii* at Warsaw University of Life Sciences, Poland, preliminary classified as the Galaxy type; (**c**) breeding clone 0021-9 as result of further crossing of *Hippeastrum* × *chmielii* at Warsaw University of Life Sciences, Poland, preliminary classified as the Diamond type; (**d**) cultivar 'Pink Glory' belongs to the Double Galaxy Group, presented by Dutch Breeding Company Floralia at the Keukenhof Exhibition 2018; (**e**) cultivar novelties for 2020 presented by Brasbonitas Amaryllis–Kebol at IPM Essen, Germany, 2020.

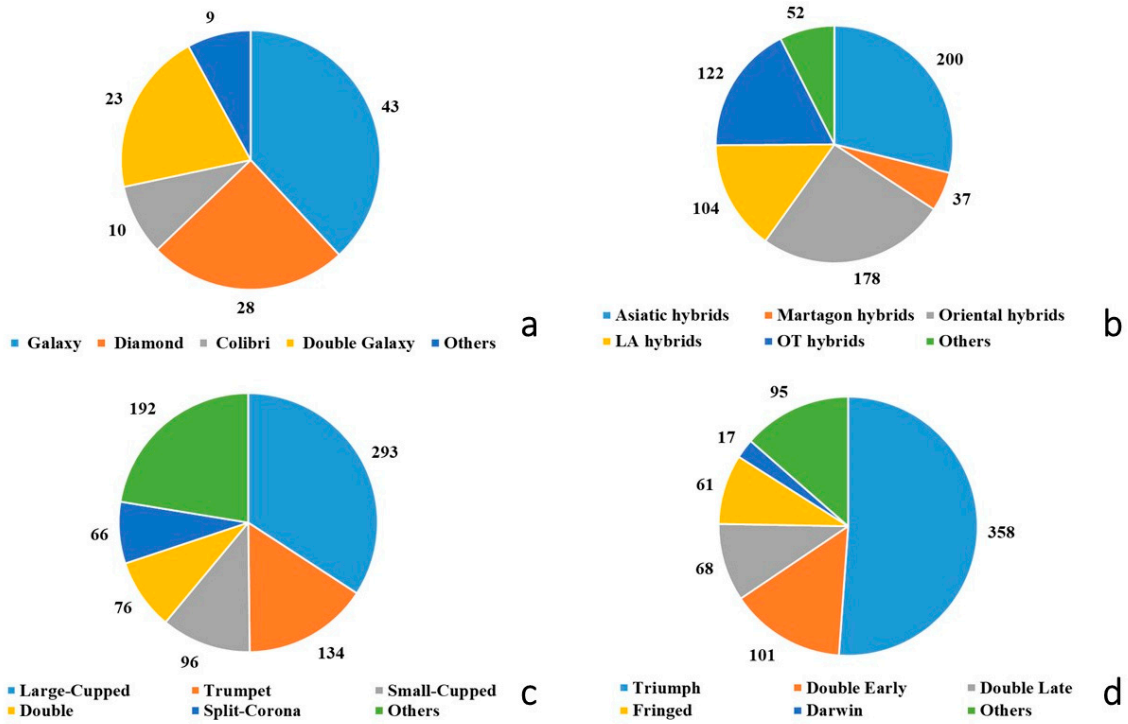

Figure 3. Number of cultivars in the different groups of the international horticultural classification, specific to each botanical genus, registered in authorised international registers during the last 5 years: (**a**) *Hippeastrum* (2015–2019) in the Dutch Royal General Bulb Growers' Association (KAVB), Hillegom, the Netherlands; (**b**) *Lilium* (mid-2014 to mid-2018) in the Royal Horticultural Society (RHS), London, United Kingdom; (**c**) *Narcissus* (mid-2015 to mid-2020) in the RHS, London, United Kingdom; (**d**) *Tulipa* (2015–2019) in the KAVB, Hillegom, the Netherlands (the pie charts represent the authors' elaboration).

8.2. Lilium

Lily cultivars are classified in nine divisions, according to the international horticultural classification [265]. Lily divisions of great economic importance are the Asiatic hybrids (Division I) and Oriental hybrids (Division VII), but they have been bred and grown less and less, and their place has been taken by the LA and LO interdivisional hybrids (Division VIII), respectively. In Division VIII, we find also: AT (Asiapets) hybrids, which have resulted from the crossing of Asiatic and trumpet hybrids; LT (Longipet) hybrids, which have resulted from longiflorum and trumpet hybrids; OA hybrids, which have resulted from oriental and Asiatic hybrids; and OT (Orienpet) hybrids, which have resulted from oriental and trumpet hybrids [265]. The number of registered OT cultivars (122 of all 693, almost 18%) in the last 5 years (mid-2014 to mid-2018) in the Royal Horticultural Society (RHS) (London, United Kingdom) register [266,267] supports the suggestions of van Tuyl et al. [80] from 10 years ago that Oriental hybrids will be partially replaced by OTs. As far as decorative values are concerned, lily breeding trends are directed—apart from attractive and unusual colours and the size of individual flowers—towards cultivars with a delicate scent dedicated to cut flower production (Asiatics and LA) and towards tall cultivars (so-called tree lilies; OT) for garden cultivation. OT lilies reach a height of over 2 m and have the best features of their parents: increased resistance to spring frosts and drought, thick and rigid stems and showy flowers (15–25 cm in diameter), often with a rather strong sweet scent. Most OT hybrids are triploid and have been developed by backcrossing with one of the parents, comprising a genome composition of OOT, which has been confirmed by GISH for tetraploid OT hybrid 'Stentor', resulting in 36 Oriental and 12 Trumpet lily chromosomes with two genomic recombinations [221]. Examples of new OT and LA hybrids with a duplicated number of chromosomes from either parent are: 'Hongxing' VIII (LAA), bred at the Beijing University of Agriculture in 2015, and 'Pink App' VIII (OOT), bred at Testcentrum voor Siergewassen B.V., the Netherlands [266]. A strong trend in lily breeding is to create cultivars with double flowers (Figure 4a,b) and cultivars dedicated for pot cultivation (Figure 4c,d). A detailed number of registered cultivars from each group are shown in Figure 3b. New lily cultivars have been bred predominantly by Dutch companies (481 cultivars during the period mid-2014 to mid-2018), followed by Chinese companies (95). Cultivars have also been bred in Canada (26), Poland (20), Australia (16) and Russia (16) [266,267].

Figure 4. Cultivar novelties of lilies. Double lilies: (**a**) 'Polar Star' and (**b**) 'Diantha' of the Dutch company C. Steenvoorden, presented at IMP Essen, Germany, 2020. Cultivars for pot production: (**c**) dwarf oriental 'Magny Course' and (**d**) Lily Looks series bred by Mak Breeding B.V., the Netherlands.

8.3. Narcissus

For a large number of narcissi (daffodils) cultivars and botanical forms, the international horticultural classification has been elaborated. The newest, established by the RHS, consists of 13 groups, and the last International Daffodil Register & Classified List of the RHS contains 26,000 names of genotypes [268]. At present, almost all cultivars are triploid and tetraploid [268], which explains the lack of need for artificial polyploidisation of narcissus cultivars. According to analysis of the RHS register of daffodils, 857 new cultivars were registered during the last 5 years (mid-2015 to mid-2020) [269–273], predominantly large-cupped daffodil cultivars (293, 34.2%), followed by Trumpet cultivars (134, i.e., almost 16%). The third and fourth positions belong to small-cupped cultivars (96, 11.2%) and double daffodil cultivars (76, 8.9%). The final new cultivar division in the top five include the closed by split corona daffodil cultivars, with 66 novelties (7.7%). Detailed number of registered cultivars from each group are shown in Figure 3c. Most narcissus have white or yellow flowers, but the most sought-after colours are pink and red. This is also the direction in which the breeding of new cultivars is heading. During the last 5 years, 96 and 167 new cultivars, with red or pink mid-zone or rim of the corona, respectively, were registered. In addition, two cultivars with both colours of the corona—'Retro Rose' (2W-PPR) and 'Valley Secret' (2 W-PRR), bred by Collin Crotty from New Zealand—were registered in 2015 and 2017, respectively [274]. An important breeding goal in *Narcissus* is to achieve disease and pest resistance. Based on a British programme investigating the genetic basis of resistance to basal rot caused by *F. oxysporum* f. sp. *narcissi* [275], new lines and cultivars have been obtained [276,277]. The leading country in narcissus breeding is the United Kingdom, but many cultivars have also come from New Zealand,

the Netherlands and the United States. According to the Database of the American Daffodil Society [278], 402 cultivars were bred in the United Kingdom, 218 in New Zealand, 144 in the United States, 123 in the Netherlands and 58 in Australia during the last 5 years (2016–2020). Narcissus breeding has also been conducted in Poland, resulting in six cultivars, but they have only been registered only in this country. Several breeding clones crossed in the National Institute of Horticultural Research in Skierniewice, Poland, are currently propagated in vitro to obtain more bulbs for further evaluation (Figure 5a–d). One hundred ninety-eight cultivars were bred in Latvia at the end of the 20th century and the beginning of the 21st century, but none after 2010 [279].

Figure 5. Breeding clones of *Narcissus* (daffodils) crossed at the Research Institute of Horticulture in Skierniewice, Poland, currently propagated in vitro to obtain more bulbs for further evaluation: (**a**) 8–97; (**b**) 7–97; (**c**) 34–97; (**d**) 10–97.

8.4. Tulipa

Each year, many new tulip cultivars are created around the world. Over the past 5 years (2015–2019), between 123 and 155 new cultivars have been added annually to the international tulip cultivar register maintained by the KAVB [260–264]. In total, this register has been enriched by more than 700 cultivars during that period. Most were produced by Dutch breeding companies. However, tulip breeding is performed on a smaller scale in other countries. For example, in 2015, six cultivars bred in France and two in Latvia were registered; in 2016, four in France, two in Latvia, two in China and one in Japan were registered; and in 2019, three in China and three in Latvia were registered. Due to the variety of tulip forms and the huge number of cultivars, an international horticultural classification has been introduced. The latest classification divides tulips into 15 groups [155]. However, progress in breeding required the creation of a new group, coronet tulips, in 2018 (Figure 6a) [280,281]. The first cultivar entered in the Dutch KAVB register classified in the Crown group was 'Crown of Negrita' [279], although as early as 1949 G. Baltus registered the cultivar 'Picture', with laterally compressed petals creating the spout at

their tip [280]. Beginning in 1992, more cultivars with the 'Picture' type flower shape began to emerge, but until 2017, they had been registered as Single Late or Triumph. The newly registered cultivars belong predominantly to the Triumph Group (358 cultivars during the past 5 years, or 50.6%), which embraces cultivars perfect for forcing and with a long vase life. However, increasing numbers of new cultivars from the Double Early Group (Figure 6b), the Double Late Group (101 and 68, respectively) and the Fringed Group (61) have been noticed. Cultivars of the Double Fringed Group are also more popular (Figure 6c). Conversely, only 17 cultivars of DH—popular at the end of the last century—have been registered during the last 5 years [260–264]. The number of registered cultivars from each group is shown in Figure 3d. Since the Dutch tulipomania in the mid-1700s to the present day, it has been every breeder's dream to create a cultivar with black flowers. In 2012, the Polish cultivar 'Fringed Black', which is one of the darkest cultivars available in the entire world to date, was registered by the KAVB [15]. Further breeding with the objective of unusual flower shape has led to create more elongated flowers, similar in shape to *Curcuma* or artichoke flowers (Figure 6d) or green malformed flowers as cultivar 'Little Queen' (Figure 6e). Apart from decorative values of flowers, suitability for forcing and long vase life, resistance breeding has become important in tulips. *Tulip breaking virus* (TBV), *Fusarium* and *Botrytis* are the most important disease-causing agents and, therefore, are the main targets for resistance breeding [281]. For these purposes, both interspecific crossing supported by cytogenetic studies and gene mapping studies are used, an endeavour that has allowed the detection of six different QTL loci for resistance to *Fusarium* and single *locus* for resistance to TBV [281].

Figure 6. Novelties of *Tulipa* cultivars presented at the Keukenhof exhibition, the Netherlands, 2018: (**a**) cultivar 'Elegant Crown', an example of the new Coronet Group; (**b**) 'Perfect Love', from the Double Early Group; (**c**) 'Brest', a double fringed cultivar (officially registered in the Fringed Group); (**d**) cultivar 'Artichoke', with a new flower shape, officially registered as Viridiflora; (**e**) 'Little Queen', from the Double Early Group.

9. Concluding Remarks and Future Prospects

The breeding of ornamental geophytes cannot progress without the support of scientific research. Research is focused on shortening the breeding period, including shortening the juvenile phase and improving in vitro propagation methods. There has been notable development in techniques for early selection of desirable traits among seedlings, which has also led to the acceleration of breeding work. The last two decades have seen further progress in resistance breeding based on increasingly broader and deeper cytogenetic and molecular studies. Not only ex situ gene banks, but the natural genetic resources of ornamental geophytes are increasingly appreciated. Hence, there is a continuous search for new traits, including resistance, in wild species.

We see the further progress in breeding of new cultivars of geophytes in using NPBTs such as cisgenesis and genome editing technologies. Rapidly advancing climate change necessitates the breeding of cultivars resistant to biotic and abiotic stresses, especially those better adapted to production in regions with warm climates. Because we are talking about ornamental plants, features such as a long vase life, new flower shapes and colours or new inflorescence structure will always remain the most important features of new cultivars. Conversely, for the medicinal use of geophytes (such as *Narcissus*), breeding for characteristics such as alkaloid or essential oil content will be required.

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