

A Comprehensive Review on Role of Plant Tissue Culture in Ornamental Crops: Cultivation Factors, Applications and Future Aspects.

Abstract

Ornamental plants are grown largely for their artistic value, floriculturists must prioritize the proliferation and improvement of quality traits, as well as the production of unique diversity. Micropropagation, clonal reliability and conservation are all crucial factors to consider. Application of *in vitro* techniques in ornamental plant such as *in vitro* embryo rescue, somatic hybridization, *in vitro* pollination and *in vitro* ploidy manipulation but to enhance, techniques like as embryo rescue and somatic hybridization are commonly employed. The creation of synthetic seed allows for season-independent seed producing and long-term seed preservation. Many factors influence ornamental plant tissue culture, including plant genotype, explants type, and the physical environment (light, temperature, humidity, and CO₂), in addition to medium composition and growth regulators. We compiled and reviewed an overall update on cultivation factors, application procedures in ornamental plant tissue culture, *in vitro* plant enhancement approaches and future prospects in this study.

Keywords: Explants Type, Ornamentals, *In Vitro* Culture, Embryo Rescue, Hybridization, Temperature, Light.

Introduction

In general, the phrase "ornamental plant" or "ornamental" refers to plants that are grown primarily for their aesthetically pleasant traits such as shape, bark, leaves, flowers, fruit, or any combination. Globally, the feasible output of decorative plants is expanding [1]. The ornamental and floriculture sector in the United Kingdom is projected to be worth £2.1 billion in 2005, with overseas commerce of £60-75 billion. Its economic worth has skyrocketed over the previous two decades, and there is a strong likelihood of further expansion in both the local and international markets [2]. Tissue culture system in ornamental flowers like roses has been established [3,4,5,6]. Recently, in *in vitro* flower induction in roses was demonstrated [7,8]. Tissue culture techniques are used for micro propagation and the generation of pathogen-free plants [9]. Plant tissue culture has emerged as a promising method, forming the base of plant biotechnology. Growers want high-quality planting material to increase production. The effectiveness of the clonal propagation method is

dependent on a variety of parameters, including genotype, medium, plant growth regulators, and explants type, all of which should be experiential during the process [10]. Naphthalene Acetic Acid (NAA) and Benzyl Adenine (BA) are the most often utilized growth regulators for ornamental plant micropropagation via organogenesis, embryogenesis, and axillary proliferation. The high frequency of direct embryogenesis has been studied in thin layer cultures of hybrid seed geranium (*Pelargonium*). [11]. Plants raised through micropropagation are of uniform quality, pathogen free, and can be produced much more quickly, with new cultivars becoming commercially available within 2 to 3 years of development rather than the 5 to 10 years required for conventional propagation. They also produce uniformly superior seeds and have improved vigor and quality. Propagation via meristem has been identified as a mechanism of rapid regeneration in *Caladium* and the plants produced through this technology have a high export potential because they can be shipped internationally with few quarantine restrictions and have the potential to develop new cultivars of the species. Several studies have been conducted in recent years in order to produce semi-automatic systems that exploit the principle of development in temporary immersion with the goal of avoiding tissue hyper hydricity. Some systems of temporary immersion (TIS) have been employed on tropical plants [12,13] and fruit trees [14].

1. Application of *In vitro* Techniques in Ornamentals

Plant tissue culture is widely known for its ability to produce disease-free plantlets through clonal replication. *In vitro* cultivation provides several opportunities for modifying plant materials to increase their quality. For hybridization, *in vitro* procedures including as micropropagation, embryo rescue and somatic hybridization are utilized.

1.1. Plant Improvement by the Application of *In vitro* Embryo Rescue

Developing a viable plant from an embryo is referred to as embryo culture or embryo rescue (Figure 1). Hannig pioneered the embryo culture technique by growing mature embryos of a few Brassicaceae plants on sugar-supplemented salt media [15]. Dietrich revealed in 1924 that both mature and immature embryos may be saved. [16]. In 1925, the first interspecific hybridization was described in the perennial flax (*Linum perenne* L. x *Linum austriacum* L.) through embryo rescue from nonviable seeds [17]. Since its discovery, embryo rescue has been employed for interspecific hybridization in a wide range of crops, including floral, decorative, medicinal, and woody plants [18,19].

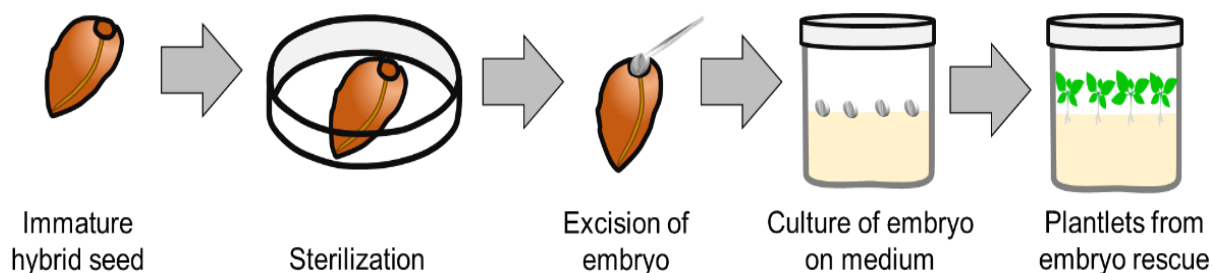


Figure 1. Process of embryo rescue from immature (or non-viable) seed after hybridization

It allows for the culture of the ovary, ovule, and embryo [20,21]. The success of embryo rescue depends on various factors, such as size and age of the embryo, intactness of embryo, excision procedure, sterilization, culture medium, supplementation in culture medium, light, temperature, etc. It has been used in crop improvement by intraspecific/interspecific/intergeneric hybrid development, haploid/double haploid production, overcoming embryo abortion, overcoming seed dormancy, overcoming self and cross-incompatibility, shortening the breeding cycle, propagating rare plants, etc. [22,23]. For example, breeding cycles were shortened by embryo rescue in rose [24], and lily [25]. Interspecific hybrids were developed in chrysanthemums by embryo rescue tolerant [26,27], salt-tolerant [28], aphid resistance [29], and heterotic [30,31] characteristics. A new flower shape and cold-tolerant intraspecific (*Campanula carpatica* 'White') and interspecific (*C. medium* and *C. formanekiana*) hybrid, respectively, were developed in bellflowers [32]. Interspecific hybrids, haploids, or double haploids were developed in rose [33], tulip [34], lisianthus [35], lily [36] and ornamental alliums [37,38]. Embryo rescue has been widely studied for crop improvement, while its current research has been reduced by the rapid evolution of advanced molecular breeding.

Furthermore, embryo rescue is commonly employed to overcome post-fertilization barriers in plants, although many ornamentals have pre-fertilization barriers that may be overcome by in-vitro pollination. Plant reproductive cells (stigma and anther) are separated and united under controlled circumstances to form a zygotic embryo with in-vitro pollination. The in-vitro approach has been used for blooming and pollination in several ornamentals [39,40].

1.2. Plant Improvement by Somatic Hybridization and *In vitro* Pollination

Somatic hybridization has been shown to be a significant source of genetic diversity, also known as somaclonal variation. Many somaclones are thought to be better hybrids. The donor-recipient approach and cytoplasm-protoplast fusion are the two most common strategies for producing somatic hybrids. In cytoplasm-protoplast fusion, protoplasts are allowed to fuse

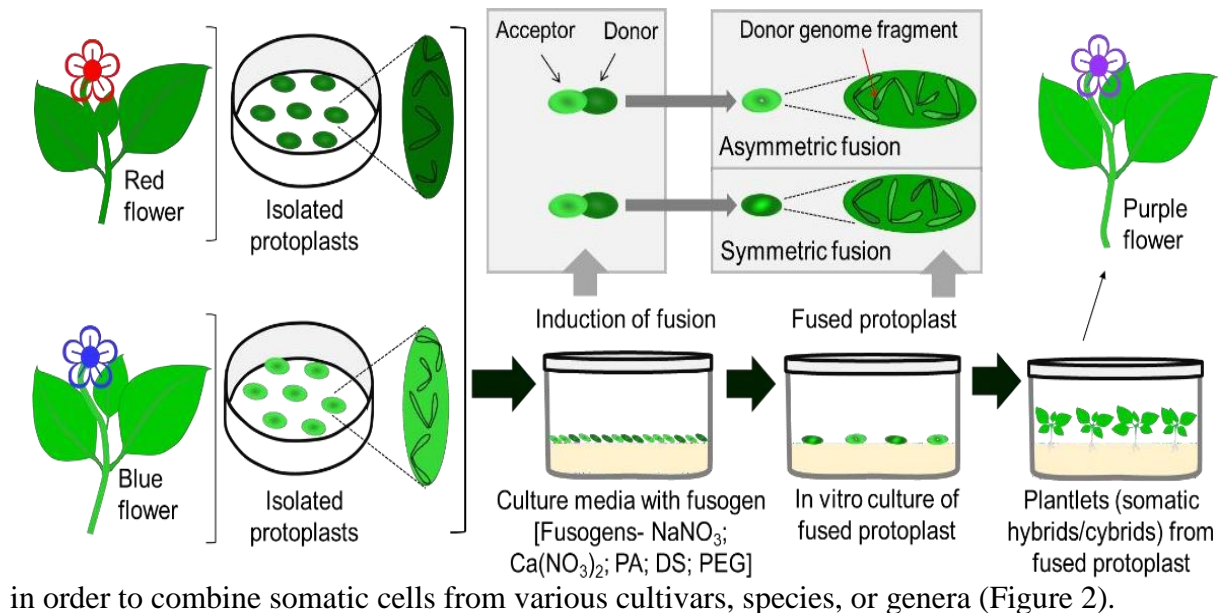


Figure 2. Illustration of somatic hybrid or cybrid development through protoplast fusion.

Here, NaNO_3 ; sodium nitrate, $\text{Ca}(\text{NO}_3)_2$; calcium nitrate, PA; polyvinyl alcohol, DS; dextran sulfate, polyethylene glycol (PEG).

Somatic hybridization occurs when the nuclear genome of one parent is combined with the mitochondrial and/or chloroplast genome of the other parent. The donor-recipient fusion approach, which transfers particular genes or chromosomes, is an alternative and better way of somatic incompatibility [41,42]. Fusogens are chemicals that are utilized for protoplast fusions. Common fusogens include sodium nitrate (NaNO_3), calcium nitrate (CaNO_3), dextran sulfate, polyvinyl alcohol, and polyethylene glycol [43]. Somatic hybridization through protoplast fusion can produce symmetric or asymmetric hybrids, known as somatic hybrids or cybrids (Fig.2). The first asymmetric hybrid was discovered by somatic hybridization of *Nicotiana tabacum* (tobacco) and *Petroselinum hortense* (parsley) [44,45]. Many wild plant species have important features, including disease and pathogen resistance, which can be transmitted to cultivated crop species. Somatic hybridization allows desired features to be transferred to boost yield, resistance, tolerance, and so on [46,47]. It enables

breeders to generate unique hybrids using an asexual technique rather than traditional breeding (Figure 2).

Somatic hybridization has been applied for the genetic improvements of various flowering and ornamentals, such as rose [48], Dendrobium [49], chrysanthemum [50], dianthus [51], gentian [52], iris [53] and Saintpaulia [54].

Somaclonal variants or somatic hybrids can be confirmed by morphological, biochemical, protein marker, cytogenetic, and molecular analyses. Restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), methylation-sensitive amplification polymorphism (MSAP), transposon-based marker systems, and Next-Generation Sequencing (NGS) have been applied for the validation of somatic hybrids at the molecular level in several ornamentals. Somaclonal variation is highly dependent on the PGRs [55]. The problems in separating protoplasts (mentioned in Section 3.2), creating unanticipated and useless variations, newly created variants that are not original, and so on are the fundamental limits of somatic hybridization [56].

1.3. Production of Synthetic Seeds

A synthetic seed or artificial seed is any encapsulated plant tissue, somatic embryos, or other micropropagules (Figure 3). Synthetic seeds have several advantages over natural seeds, including season-independent seed production, genetic uniformity, maintaining hybrid vigor, long-term storage capacity, rapid multiplication, freedom from vegetative and seed-borne pathogens, high-volume low-cost propagation, ensuring quality plant materials and shortening life cycles. [57,58]. In ornamentals, somatic embryos, nodal segments, and branch tips are commonly employed as explants for the generation of synthetic seeds, although callus is seldom used and PLBs are mostly used in orchids to produce synthetic seeds. Synthetic seeds have been generated in *Caladium bicolor* (caladium), *Eustoma grandiflorum* (lilianthus), *Pinus patula* (pine), *Genista monosperma* (bridal broom), *Hyoscyamus muticus* (Egyptian henbane), and *Clitoria ternatea* (bluepea or bluebellvine) from the somatic embryo; *Gypsophila paniculata* (gypsophila), *Saintpaulia ionantha* (saintpaulia), *Urginea altissima* (tall white squill), and *Taraxacum pieninicum* (Mniszek pieninski) from shoot tip; *Rosa damascena* f. *trigintipetala* (Damask rose), *Syringa vulgaris* (lilac), *Nerium oleander* (oleander), *Centella asiatica* (Asiatic pennywort), *Eclipta alba* (false daisy), *Erythrina variegata* (tiger's claw), *Photinia fraseri* (red tip photinia), *Ruta graveolens* (rue), *Salix*

tetrasperma (Indian willow) from axillary buds/nodes, *Anthurium andreanum* (anthurium) from callus, *Lilium longiflorum* (easter lily) from bulb, and different species of orchids from PLBs (*Cymbidium giganteum*, *Vanda coerulea*, *Geodorum densiflorum*, *Coelogyne breviscapa*, *Cremastra appendiculata*, *Flickingeria nodosa*, *Spathoglottis plicata*, etc.) [57,58].

In vitro synthetic seeds in ornamentals enable season-independent seed synthesis, long-term storage, and timely supply to growers. Concentrations of sucrose, sodium alginate (Na-alginate), and calcium chloride (CaCl₂) are critical for the synthesis of artificial seeds in ornamentals. Concentrations of 2-3% sucrose, 2-3% Na-alginate, and 50-100 mM CaCl₂ were discovered to be beneficial for synthetic seed formation in ornamentals [57,58].

Synthetic seeds have some limitations over the advantages: low efficient root systems, development of non-synchronous seeds from the somatic embryo (the most effective plant material for synthetic seed development), deviation from the normal structure, loss of embryogenic potential with time, etc. Synthetic seed technology can be used more effectively in the commercial ornamental plant propagation sector after resolving these limitations.

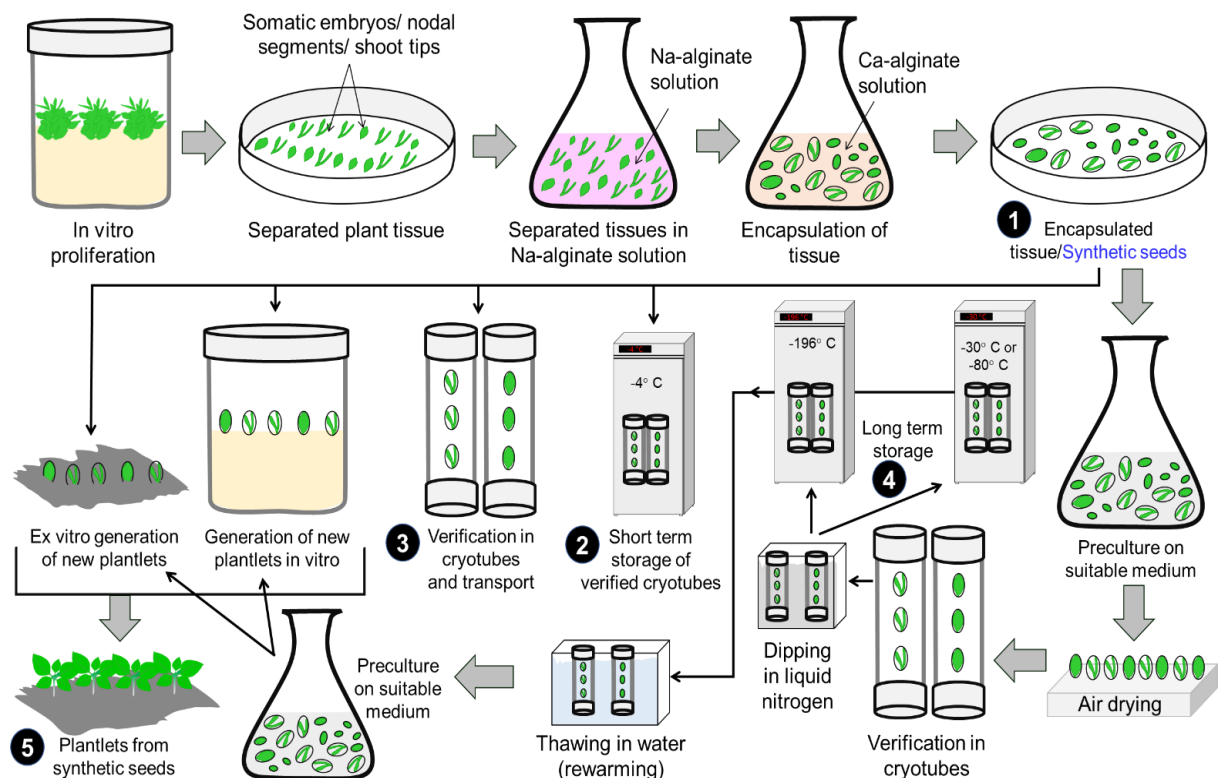


Figure 3. Production and application of synthetic seeds. The numbers in the figure represent the ending point of each step, such as the production of synthetic seeds (1), short-term storage of synthetic seeds (2), synthetic seeds for transportation (3), long-

term storage of synthetic seeds (4), and plantlet generation from synthetic seeds (5). Figure 6. Production and application of synthetic seeds. The numbers in the figure represent the ending point of each step, such as the production of synthetic seeds (1), short-term storage of synthetic seeds (2), synthetic seeds for transportation (3), long-term storage of synthetic seeds (4), and plantlet generation from synthetic seeds (5).

1.4. *In vitro* Ploidy Manipulation

In vitro ploidy modification is a method of promoting genetic diversity by raising or lowering the number of chromosomes (Figure 4). Polyploidy induction is employed for ornamental crop development and can extend breeding chances to enhance decorative features, environmental tolerances, and restore fertility in broad hybrids [59]. For chromosomal doubling, the most often utilized antimitotic drugs are colchicine and oryzalin [60]. Two ginger lily lines, *Hedychium gardnerianum* Shepard ex Ker Gawl. and *H. coronarium* J. Koenig, were employed for chromosomal doubling using colchicine or oryzalin, and the tetraploid ginger lily was effectively created [61]. Forty—eight tetraploids were developed in ornamental aroid plants using colchicine (*Caladium × hortulanum* Birdsey) that showed variation in leaf shape, color, and thickness compared to the wild type [62]. Tetraploid anise hyssop (*Agastache foeniculum* L.) was induced by the application of colchicine, which showed a wide range of variation compared to diploid plants in their morphophysiological characteristics [63]. Polyploid has also been inducted in *Dendrobium*, *Phalaenopsis*, *Epidendrum*, and *Odontioda* orchids by the application of oryzalin [64]. *In vitro*-generated polyploids of rose, lilies, phlox, petunia, bellflowers, rhododendron and other plants showed a wide range of phenotypic differences. Aside from antimitotic agents, ploidy modification is affected by species, explants kinds, antimitotic agent exposure technique,

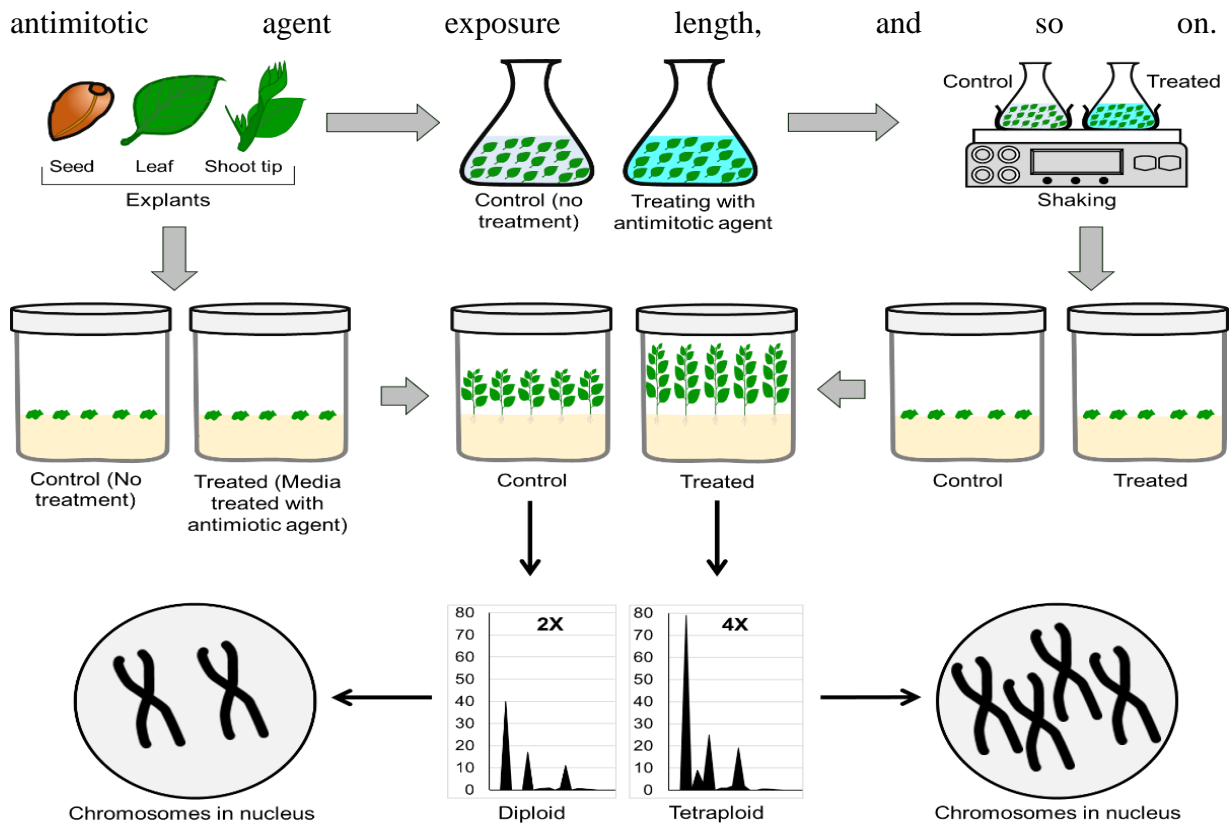
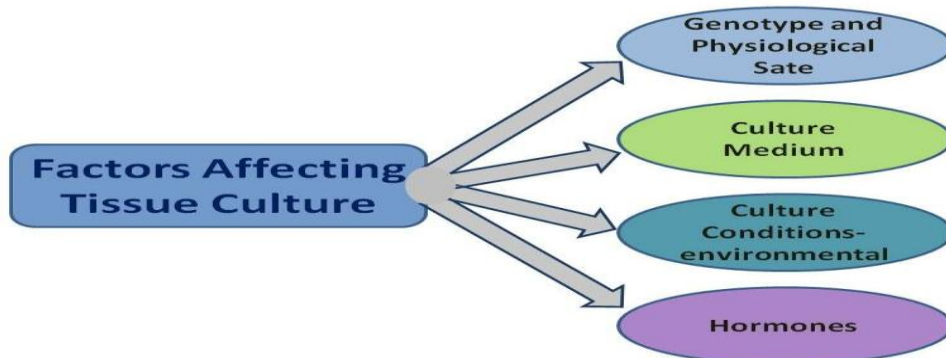


Figure 4. *In vitro* chromosome doubling (ploidy manipulation) for genetic diversification

2. Factors Affecting on Tissue culture techniques



2.1. Genotype

Genotype is one of the most important factors affecting tissue culture [65]. Genotypic differences between six genotypes of *Primula vulgaris* were achieved in callus induction rate, type of callus, root formation during the callus phase, and shoot regeneration rate [66]. Shen *et al.* (2008) found significant differences in callus and shoot formation from leaf explants among four *Dieffenbachia* cultivars [67]. Also, Gheisari and Miri (2017) observed that

hormonal requirement for callus induction and direct bulblet regeneration of two lisianthus varieties was different.

2.1.1. Source of explants

Explants source, both *in vitro* and *in vivo*, is also vital for regeneration. *In vitro* explants is thought to be the best method for organogenesis. Cactus plants grown outdoors or in greenhouses can be utilized as explants sources to generate *in vitro* cultures; *in vitro* plants raised from seeds can also be used as starting material for cactus micropropagation [68].

2.1.2. Type of explants

The precise selection of explants material can have a significant impact on tissue culture success [69, 70]. This might be owing to the varying levels of endogenous hormones found in various plant sections. Explants types such as leaf, petiole, hypocotyl, epicotyl, embryo, internode, and root had a substantial impact on plant tissue culture [71,72]. Bulb scale segments are the most commonly utilized explants source for *in vitro* multiplication of geophytes ornamental plants such as *Fritillaria imperialis*, *Hyacinthus orientalis* and *Polianthes tuberosa*. Explants supplies such as ovary, flower stalk, leaf stalk, and mature seeds are also utilised for bulb growth [73]. Petal explants of *P. tuberosa* was implemented to initiate globular and heart somatic embryos that after 3 weeks developed as torpedo and cotyledonary embryos. Axillary branching using axillary bud and stem node, as in other plants, is the majority common explants type utilized for direct shoot propagation of *Dieffenbachia* [74]. The majority of the strategies established for *in vitro* multiplication of *Phalaenopsis* include seedling propagation or cultured the dormant buds located at the base of the inflorescence. The most widely utilized explants for regeneration is the leaf [75]. The greatest rate of regeneration was seen in chrysanthemum cv. Borami leaf explants [76].

2.1.3. Orientation of explants

Orientation of mother plant in the culture medium also affects the shoot proliferation and regeneration effectiveness. In general, regeneration efficiency is higher in horizontal position as compared to vertical due to little contact of explants to medium in vertical position. The effect of explants location in the multiplication of *Dieffenbachia* was evaluated. The highest number of shoots was obtained with sub-apical segments placed vertically [77].

2.2. Medium Factors

2.2.1. Media

The type of tissue culture medium selected depends upon the species to be cultured. Some species are sensitive to high salts or have different requirements for macro- and micronutrients [78]. Even tissues from different areas of a plant may have differing growing needs. For micropropagation, many basal media such as White, Nitsch & Nitsch, and B5 (Gamborg) have been used, but the most generally used culture medium is (MS medium), since it includes all of the required nutrients for *in vitro* growth. In comparison to many other formulations, it is classed as a high salt medium, with high quantities of nitrogen, potassium, and several micronutrients, notably boron and manganese. However, due to the high salt concentration, this nutrient solution is not always appropriate for *in vitro* plant growth and development. B5 medium proved to be suitable for direct shoot regeneration of lisianthus. Also, the use of dilute media formulations has sometimes promoted better formation of shoot and especially roots, since high concentration of salts may inhibit root growth, even in presence of auxins in the culture medium. Phalaenopsis orchid explants' capacity to create shoots. They concluded that 12MS culture medium provided optimal axillary shoot multiplication and seedling development [79, 80].

2.2.2. Carbon source

Carbohydrate is a fundamental component of any nutritional medium, and its inclusion is required for *in vitro* culture growth and development [81]. Sucrose is by far the most often utilized carbon source for a variety of reasons. It is inexpensive, widely accessible, autoclavable, and easily digested by plants. MS medium supplemented with 0.1 mg/l NAA + 0.1 mg/l BA and 60 g/l sucrose was shown to be better for *Lilium ledebourii* bulblet regeneration. Other carbohydrates, such as glucose, maltose, and galactose, as well as the sugar-alcohols glycerol and sorbitol (particularly in the Rosaceae family), can be employed. *Primula* sp. seed germination rate and percentage are greater in MS medium containing 10 g/l sucrose than in glucose [82].

2.2.3. Complex organic compounds

These are a group of undefined supplements such as casein hydrolysate, coconut milk, orange juice, tomato juice, grape juice, pineapple juice, banana puree, etc. These compounds are often used when no other combination of known defined components produces the desired growth or development. Some of them are used as organic sources of nitrogen such as casein hydrolysate, peptone, tryptone, and malt extract. These mixtures are very complex and

contain vitamins as well as amino acids. The highest seed germination percentage, PLB development and seedling growth of *Phalaenopsis* obtained with MS or ½MS media containing 100 mg/l coconut water and 1-2 g/l peptone [83]. Polyamines, particularly spermine and spermidine, are sometimes beneficial for somatic embryogenesis and direct regeneration.

2.3. Culture Conditions and environment factors

2.3.1. Gas exchange and relative humidity

The culture vessel is typically a closed system, although depending on the kind of vessel, the closure, and how firmly they are sealed together, some gas exchange may occur. The vessels' sealing must allow for enough ventilation to prevent severe ethylene buildup and CO₂ depletion. The use of tightly closed containers that impede gas exchange may have a detrimental impact on proper plant growth and development during *in vitro* culture. Several studies have demonstrated the benefits of utilizing closures with filters or vented containers, which enable gas exchange and hence increase photosynthetic capability, multiplication rate, and plant survival following transfer to *ex vitro* settings [84].

The relative humidity within the culture vessels is typically quite high, resulting in a poorly established epicuticular wax layer and dysfunctional plantlet stomata. As a result, various methods for reducing relative air humidity inside the vessel have been tested, such as opening culture containers for a few days prior to acclimatization, using special closures that facilitate water loss, or cooling container bottoms, which increases condensation of water vapors on the gel surface. Furthermore, relative humidity management during *in vitro* acclimation is an important role in improving the morphological characteristics of plantlets when transplanted *in vivo* [85].

2.3.2. Light

The three features of light, which influence *in vitro* growth characteristics such as stem elongation, leaf size and plant anatomy, are wavelength, flux density and photoperiod. Tapingkae investigated the effects of light quality and quantity on growth and development of *Anigozanthos bicolor* and *Zieria fraseria* grown *in vitro*. Three white light intensities of 40, 80 and 200 $\mu\text{mol m}^{-2}\text{s}^{-1}$; light of five spectral quantities: white (390-760 nm), blue (450-492 nm), green (492-550 nm), yellow (550-588 nm) and red (647-770 nm); and five light-dark cycles: 4L2D, 6L6D, 12L12D, 16L8D and 24L were applied. Total rhizome number and dry

weight of *A. bicolor* plantlets after 6 weeks of growth was positively affected by high light intensities of 80 to 200 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and short light-dark cycle of 4L2D. The light spectrum was not critical for rhizome production in *A. bicolor*. Shoot dry weight of *Z. fraseria* was highest in plantlets grown under 200 $\mu\text{mol m}^{-2}\text{s}^{-1}$ irradiance. Shoot length was positively affected by blue light. Light cycle had no effect on the productivity but shorter photoperiods reduced shoot length. The Red (R) and Blue (B) LED light on the *in vitro* multiplication of *Rosa* sp. were used and responses compared with explants grown under 16/8 h (light/dark) fluorescent light. Shoots under RB LED (16:4) showed the greatest growth and elongation [86].

Several studies found that light promoted root development and shoot growth, while darkness promoted root formation. *Lisianthus* calli grown in the light (1200 lux) had a higher fresh weight and were green, but calli cultivated in the shade were yellow. The breakdown of endogenous IAA causes the diminished rooting in the presence of light. Shoots from proliferating cultures were sometimes moved to root induction media with auxin for 4-7 days in the dark before being switched to the same medium without auxin and incubated under light for root elongation.

2.3.3. Temperature

Temperature influences several physiological activities, including respiration and photosynthesis. The most frequent temperature range for cultivation has been 20°C to 27°C, however ideal temperatures vary greatly depending on genotype. Prior to *in vivo* transplanting, air temperature is stated as a significant element in determining *in vitro* growth and development of *Phalaenopsis* plantlets. Plantlets of *Phalaenopsis* acclimatized to varied air temperatures (15, 25, and 35 °C) were transplanted immediately to *in vivo* habitats for 14 days. Plantlets acclimatized to low air temperature retained higher levels of chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoid content than those acclimatized to high temperature.

2.4. Plant growth regulators (PGRs)

PGRs regulate and govern the beginning and growth of shoots and roots on explants and embryos, as well as cell division and expansion. Plant growth regulators are classified into various groups, including cytokinin, auxins, gibberellins, ethylene, and abscisic acid. The production of adventitious shoots and roots frequently need a balance of auxin and cytokinin. High amounts of auxin relative to cytokinin increased root development, whereas high levels

of cytokinin compared to auxin stimulated shoot formation. In lisianthus and gladioli, for example, maximal callus induction was observed on MS medium supplemented with 2 and 10 mg/l NAA, respectively [87], whereas the highest shoot regeneration was achieved with MS medium containing 5 mg/l BA+0.1 mg/l NAA and 4 mg/l Kin+0.5 mg/l NAA (19, 39). Similar results have been obtained for callus induction of fritillary and bulblet regeneration of hyacinth. The balance of growth regulators depends on the objective of the cultivation *in vitro* (as e.g., shoot, root, callus or suspension culture) and on the micropropagation phase considered (initiation, multiplication or rooting) [88]

3. Future aspects

Tissue culture techniques have been adjusted in recent decades to promote plant growth, biological activity, transformation and secondary metabolite synthesis due to development and a desire to grow on a large scale. A considerable advancement in strategies has been sought to deal with the problem of low concentrations of secondary metabolites in entire plants. The sterile plantlets will solve the contamination problem and shorten the sterilizing procedure. Secondary metabolites and medicinally relevant chemicals have found *in vitro* propagation to be quite effective for selective metabolite formation.

In recent years, researchers have begun to examine ornamentals at the molecular level, including genetic modification, utilizing *in vitro* technologies [89]. Agrobacterium tumefaciens-mediated transformation has been used to create transgenic ornamental species in around 40 genera [90], but only a few ornamentals, such as Phalaenopsis and petunia, have acceptable and effective transformation strategies. Many genes and transcriptions are involved in the *in vitro* organogenic callus, shoot, root, somatic embryos, and PLBs, and their transcriptions are also controlled by the exogenous administration of various growth regulators [91].

Conclusion

The role of plant tissue culture in ornamental crops has been significant, offering various benefits, applications, and promising future prospects. Plant tissue culture has revolutionized the cultivation of ornamental crops by providing a reliable and rapid means of propagation. It enables mass production of genetically identical plants, reducing the time and space required for traditional methods like seed germination or cutting propagation. Tissue culture techniques allow for the selection and propagation of desirable traits in ornamental plants, such as colour variations, disease resistance, and novel shapes. Conservation plays a

crucial role in the conservation of endangered and rare ornamental plant species by preserving genetic diversity and enabling their reintroduction into the wild. Tissue culture can be used to eliminate pathogens from ornamental plants, creating disease-free stock for growers and maintaining the health of plant collection. Some ornamental plants are valuable for their secondary metabolites, and tissue culture can be used for the controlled production of these compounds. Integration with genomics and molecular techniques will enable the precise manipulation of ornamental plant genomes, leading to the creation of unique and superior cultivars. Advances in automation and bioreactor technology will enhance the scalability and efficiency of ornamental crop production through tissue culture. The ornamental plant market continues to grow, and tissue culture will play a vital role in meeting the demand for new and improved varieties. Tissue culture can contribute to the development of environmentally sustainable practices by reducing the need for chemical treatments and the environmental impact associated with traditional cultivation methods.

In conclusion, plant tissue culture has emerged as a fundamental tool in the ornamental crop industry, providing solutions to various challenges and offering opportunities for innovation. As technology and research in this field continue to advance, we can anticipate an even more prominent role for tissue culture in the cultivation and conservation of ornamental plants.

References

1. Jain SM. Feeding the world with induced mutations and biotechnology Proceedings International Nuclear Conference -Global trends and Perspectives. Seminar 1: agriculture and bioscience. Bangi Malaysia MINT. 2002;6(2) 1–14.
2. Frankenberger EA, Hasegawa PM and Tigchelaar EC. Influence of environmental and developmental state on the shooting capacity of Cordyline genotypes. *Plant Physiology* 1981; 102(4) 221-232.
3. Hsia CN and Korban SS. Organogenesis and somatic embryogenesis in callus cultures of *Rosa hybrida* and *Rosa chinensis minima*. *Plant Cell Tissue and Organ Culture*. 1996; 44(10) 1-6.

4. Kintzios S, Manos C and Makri O. Somatic embryogenesis from mature leaves of rose (*Rosa sp.*). *Plant Cell Reports*. 1999; 18(6) 467-472.
5. Ibrahim R and Debergh PC. Factors controlling high efficiency of adventitious bud formation and plant regeneration from *in vitro* leaf explants of roses (*Rosa hybrida*). *Scientia Horticulturae*. 2001; 88(2) 41-44.
6. Kim CK, Chung JD, Jee SO and Oh JY. Somatic embryogenesis from *in vitro* grown leaf explants of *Rosa hybrida* L. *African Journal of Plant Biotechnology*. 2003; 5(3) 169-172.
7. Rout GR, Mohapatra A and Mohan Jain S. Tissue culture of ornamental pot plant: A critical review on present scenario and future prospect. *Biotechnological Advances*. 2006; 24(6) 531-560.
8. Wang GY, Yuan MF and Hong Y. *In vitro* flower induction in roses. *In vitro Cellular and Developmental Biology-Plant*. 2002; 38(5) 513-518.
9. Vu NH, Anh PH and Nhut DT. The role of sucrose and different cytokinins in the *in vitro* floral morphogenesis of rose (hybrid tea) cv. 'First Prize'. *Plant Cell Tissue and Organ Culture*. 2006; 87(1) 315-320.
10. Kaviani B, Hesar AA and Kharabian-Masouley A. *In vitro* propagation of *Matthiola incana* (Brassicaceae) - an ornamental plant. *Plant Omics Journal*. 2011; 4(7) 435-440.
11. Gill R, Gerrath J and Saxena PK. High-frequency direct embryogenesis in thin layer cultures of hybrid seed geranium (*Pelargonium*). *Canadian Journal of Botany*. 1992; 71(6) 408-413.
12. Alvard D, Cote F and Teisson C. Comparison of methods of liquid culture for banana micropropagation. *Plant Cell Tissue and Organ Culture*. 1993; 43(1) 55-60.

13. Lorenzo JC, Gonzales BL, Escalona M, Teisson C, Espinosa P and Borroto C. Sugarcane shoots formation in an improved temporary immersion system. *Plant Cell Tissue and Organ Culture*. 1998; 54(1) 197-200.
14. Damiano C, La Starza SR, Monticelli S, Gentile A, Caboni E and Frattarelli A. 1 st Symp. Liquid Systems for *in vitro* Propagation of Plants. 2002; (As, 29/5-2/6) 140-141.
15. Hannig E. Zur physiologie pflanzlicher embryonen. I. Ueber die cultur von cruciferen-embryonen ausserhalb des embryosacks. *Bot. Ztg.* 1904; 62, 45–80.
16. Dieterich K. U'ber kultur von Eembryonen ausserhalb des samens. *Flora* 1924, 117, 379–417.
17. Laibach F. Das taubwerden von bastardsamen und die kunstliche Aufzucht fruh absterbender bastardembryonen. *Z. Bot.* 1925; 17, 417–459.
18. Raghavan V. One hundred years of zygotic embryo culture investigations. *Vitr. Cell Dev. Biol. Plant.* 2003; 39, 437–442.
19. Marasek-Ciolakowska A, Nishikawa T, Shea DJ, Okazaki K. Breeding of lilies and tulips-Interspecific hybridization and genetic background. *Breed. Sci.* 2018; 68, 35–52.
20. Sharma DR, Kaur R, Kumar K. Embryo rescue in plants: A review. *Euphytica* 1996; 89, 325–337.
21. Deng Y, Teng N, Chen S, Guan Z, Song A, Chang Q. Reproductive barriers in the intergeneric hybridization between *Chrysanthemum grandiflorum* (Ramat.) Kitam. and *Ajania przewalskii* Poljak. (Asteraceae). *Euphytica* 2010; 174, 41–50.
22. Sahijram L, Rao BM. Hybrid embryo rescue in crop improvement. In *Plant Biology and Biotechnology*; Bahadur, B., Venkat Rajam, M., Sahijram, L., Krishnamurthy, K., Eds.; Springer: New Delhi, India, 2015; Chapter 18, pp. 363–384

23. Pramanik K, Sahoo JP, Mohapatra PP, Acharya LK, Jena C. Insights into the embryo rescue—A modern in-vitro crop improvement approach in horticulture. *Plant Cell Biotechnol. Mol. Biol.* 2021; 22, 20–33.
24. Caser M, Dente F, Ghione GG, Mansuino A, Giovannini A., Scariot V. Shortening of selection time of *Rosa hybrida* by *in vitro* culture of isolated embryos and immature seeds. *Propag. Ornament. Plants* 2014; 14, 139–144.
25. Yuan MS, Wu MC, Shii CT. Shortening breeding cycles of spider lilies (*Lycoris spp.*) through embryo culture and dikaryotype hybridization between *Lycoris aurea* and “a” karyotype species. *Acta Hort.* 2003; 620, 345–352.
26. Sun CQ, Chen FD, Teng NJ, Liu ZL, Fang WM, Hou XL. Factors affecting seed set in the crosses between *Dendranthema grandiflorum* (Ramat.) Kitamura and its wild species. *Euphytica* 2009; 171, 181–192.
27. Sun CQ, Chen FD, Teng NJ, Liu ZL, Fang WM, Hou XL. Interspecific hybrids between *Chrysanthemum grandiflorum* (Ramat.) Kitamura and *C. indicum* (L.) Des Moul. and their drought tolerance evaluation. *Euphytica* 2010; 174, 51–60.
28. Zhu WY, Jiang JF, Chen SM, Wang L, Xu LL, Wang HB, Li PL, Guan ZY, Chen FD. Intergeneric hybrid between *Chrysanthemum × morifolium* and *Artemisia japonica* achieved via embryo rescue shows salt tolerance. *Euphytica* 2013; 191, 109–119.
29. Deng YM, Chen SM, Lu AM, Chen FD, Tang FP, Guan ZY, Teng NJ. Production and characterisation of the intergeneric hybrids between *Dendranthema morifolium* and *Artemisia vulgaris* exhibiting enhanced resistance to chrysanthemum aphid (*Macrosiphoniella sanbourni*). *Planta* 2010; 231, 693–703.
30. Cheng X, Chen S, Chen F, Deng Y, Fang W, Tang F, Liu Z, Shao W. Creating novel chrysanthemum germplasm via interspecific hybridization and backcrossing. *Euphytica* 2011; 177, 45–53.

31. Tang F, Chen F, Chen S, Teng, N, Fang W. Intergeneric hybridization and relationship of genera within the tribe Anthemideae Cass. *Dendranthema crissum* kitam. × *Crossostephium chinense* (L.) Makino). *Euphytica* 2009; 169, 133–140.
32. Röper AC, Lütken H, Hegelund JN, Petersen KK, Christensen B, Müller R. Effect of different ovule isolation times on the embryo development of *Campanula* hybrids. *Acta Hort.* 2012; 953, 161–166.
33. Holeman DJ. Simple Embryo Culture for Plant Breeders: A Manual of Technique for the Extraction and In-Vitro Germination of Mature Plant Embryos with Emphasis on the Rose, 1st ed.; Rose Hybridizers Association: Hartford, CT, USA, 2009; pp. 1–34.
34. Puangkrit T, Nontaswatsri C. Inter subgeneric hybridization between *Paracurcuma* and *Eucurcuma* via embryo rescue. *Acta Hort.* 2014; 1025, 37–42.
35. Wang Q, Zhang Y, Kawabata S, Li Y. Double fertilization and embryogenesis of *Eustoma grandiflorum*. *J. Jap. Soc. Hort. Sci.* 2011; 80, 351–357.
36. Marasek-Ciolakowska A, Sochacki D, Marciniak P. Breeding aspects of selected ornamental bulbous crops. *Agronomy* 2021; 11, 1709.
37. Nomura Y, Maeda M, Tsuchiya T, Makara K. Efficient production of interspecific hybrids between *Allium chinense* and edible *Allium spp.* through ovary culture and pollen storage. *Breed. Sci.* 1994; 44, 151–155.
38. Dubouzet JG, Arisumi KI, Takeomi E, Maeda M, Sakata Y. Studies on the development of new ornamental *Allium* through interspecific hybridization III. Hybridization of autumn-flowering species through pull-style pollination, cut flower culture and embryo rescue. *Mem. Fac. Agric. Kagoshima Univ.* 1994; 30, 35–42.
39. Zulkarnain Z, Tapingkae T, Taji A. Applications of *in vitro* techniques in plant breeding. In *Advances in Plant Breeding Strategies: Breeding, Biotechnology and*

Molecular Tools; Al-Khayri, J.M., Jain, S.M., Johnson, D.V., Eds.; Springer: Cham, Switzerland, 2015; Chapter 10, pp. 293–328.

40. Murthy KSR, Kondamudi R, Rao PVC, Pullaiah T. *In vitro* flowering—A review. *J. Agric. Technol.* 2012, 8, 1517–1536.
41. Yamashita Y, Terada R, Nishibayashi S, Shimamoto K. Asymmetric somatic hybrids of Brassica: Partial transfer of *B. campestris* genome into *B. oleracea* by cell fusion. *Theor. Appl. Genet.* 1989; 77, 189–194.
42. Trick H, Zelcer A, Bates GW. Chromosome elimination in asymmetric somatic hybrids: Effect of gamma dose and time in culture. *Theor. Appl. Genet.* 1994; 88, 965–972.
43. Anthony P, Marchant R, Blackhall NW, Power JB, Davey MR. Chemical fusion of protoplasts. In *Plant Tissue Culture Manual*; Lindsey, K., Ed.; Springer: Berlin, Germany, 1995; Chapter 1, pp. 1–15.
44. Smith HH, Kao KN, Combatti NC. Interspecific hybridization by protoplast fusion in *Nicotiana*. Confirmation and extension. *J. Hered.* 1976; 67, 123–128.
45. Dudits D, Fejer O, Hadlaczky G, Koncz C, Lazar GB, Horvath G. Intergeneric gene transfer mediated by protoplast fusion. *Mol. Gen. Genet.* 1980; 179, 283–288.
46. Wang J, Jiang J, Wang Y. Protoplast fusion for crop improvement and breeding in China. *Plant Cell Tissue Organ Cult.* 2013; 112, 131–142.
47. Ranghoo-Sanmukhiya VM. Somaclonal variation and methods used for its detection. In *Propagation and Genetic Manipulation of Plants*; Siddique, I., Ed.; Springer: Singapore, 2021; Chapter 1, pp. 1–18.
48. Pati PK, Sharma M, Ahuja PS. Rose protoplast isolation and culture and heterokaryon selection by immobilization in extra thin alginate film. *Protoplasma.* 2008; 233, 165–171.

49. Kanchanapoom K, Jantaro S, Rakchad D. Isolation and fusion of protoplasts from mesophyll cells of *Dendrobium pompadour*. *ScienceAsia*. 2001; 27, 29–34.
50. Furuta H, Shinoyama H, Nomura Y, Maeda M, Makara K. Production of intergeneric somatic hybrids of chrysanthemum [*Dendranthema × grandiflorum* (Ramat.) Kitamura] and wormwood (*Artemisia sieversiana* JF Ehrh. ex. Willd) with rust (*Puccinia horiana Henning*) resistance by electrofusion of protoplasts. *Plant Sci*. 2004; 166, 695–702.
51. Nakano M, Mii M. Somatic hybridization between *Dianthus chinensis* and *D. barbatus* through protoplast fusion. *Theor. Appl. Genet*. 1993; 86, 1–5.
52. Tomiczak K, Sliwinska E, Rybczy nski JJ. Protoplast fusion in the genus *Gentiana*: Genomic composition and genetic stability of somatic hybrids between *Gentiana kurroo* Royle and *G. cruciata* L. *Plant Cell Tissue Organ Cult*. 2017; 131, 1–14.
53. Shimizu K, Miyabe Y, Nagaike H, Yabuya T, Adachi T. Production of somatic hybrid plants between *Iris ensata* Thunb. and *I. germanica*. *Euphytica* 1999; 107, 105–113.
54. Afkhani-Sarvestani R, Serek M, Winkelmann T. Protoplast isolation and culture from *Streptocarpus*, followed by fusion with *Saintpaulia ionantha* protoplasts. *Europ. J. Hort. Sci*. 2012; 77, S249–S260.
55. Chin CK, Lee ZH, Mubbarakh SA, Antony JJJ, Chew BL, Subramaniam S. Effects of plant growth regulators and activated charcoal on somaclonal variations of protocorm-like bodies (PLBs) of *Dendrobium Sabin Blue* orchid. *Biocatal. Agric. Biotechnol*. 2019; 22, 101426.
56. Ranghoo-Sanmukhiya VM. Somaclonal variation and methods used for its detection. In *Propagation and Genetic Manipulation of Plants*; Siddique, I., Ed.; Springer: Singapore, 2021; Chapter 1, pp. 1–18.

57. Qahtan AA, Abdel-Salam EM, Alatar AA, Wang QC, Faisal M. An introduction to synthetic seeds: Production, techniques, and applications. In Synthetic Seeds; Faisal, M., Alatar, A.A., Eds.; Springer: Cham, Switzerland, 2019; Chapter 1, pp. 1–20.
58. Maqsood M, Khusrau M, Mujib A, Kaloo ZA. Synthetic seed technology in some ornamental and medicinal plants: An overview. In Propagation and Genetic Manipulation of Plants; Siddique, I., Ed.; Springer: Singapore, 2021; Chapter 2, pp. 19–31.
59. Touchell DH, Palmer IE, Ranney TG. *In vitro* ploidy manipulation for crop improvement. *Front. Plant Sci.* 2020; 11, 722.
60. Dhooghe E, van Laere K, Eeckhaut T, Leus L, van Huylenbroeck J. Mitotic chromosome doubling of plant tissues in vitro. *Plant Cell Tissue Organ Cult.* 2011; 104, 359–373.
61. Habibi M, Shukurova MK, Watanabe KN. Testing two chromosome doubling agents for *in vitro* tetraploid induction on ginger lilies, *Hedychium gardnerianum* Shepard ex Ker Gawl and *Hedychium coronarium* J. Koenig. *Vitr. Cell Dev. Biol. Plant* 2022; 58, 489–497.
62. Cai X, Cao Z, Xu S, Deng Z. Induction, regeneration and characterization of tetraploids and variants in ‘Tapestry’ caladium. *Plant Cell Tissue Organ Cult.* 2015; 120, 689–700.
63. Talebi SF, Saharkhiz MJ, Kermani MJ, Sharafi Y, Raouf Fard F. Effect of different antimetabolic agents on polyploid induction of anise hyssop (*Agastache foeniculum* L.). *Caryologia* 2017; 70, 184–193.
64. Miguel TP, Leonhardt KW. *In vitro* polyploid induction of orchids using oryzalin. *Sci. Hortic.* 2011; 130, 314–319.

65. Kumar N, Reddy. *In vitro* plant propagation: a review. *Journal of Forest Science*. 2011; 27(2), 61-72.
66. Schween G, Schwenkel HG. Effect of genotype on callus induction, shoot regeneration, and phenotypic stability of regenerated plants in the greenhouse of *Primula ssp.* *Plant Cell, Tissue and Organ Culture*. 2003; 72: 53-61.
67. Shen X, Kane ME, Chen J. Effects of genotype, explants source, and plant growth regulators on indirect shoot organogenesis in *Dieffenbachia* cultivars. *In vitro Cellular & Developmental Biology – Plant*, 2008; 44(4), 282-288.
68. Pérez-Molphe-Balch E, Santos-Díaz MS, Ramírez-Malagón R, Ochoa-Alejo N. Tissue culture of ornamental cacti. *Scientia Agricola* 2015; 72(6), 540-561.
69. Gheisari M, Miri SM. Effect of growth regulators on regeneration of hyacinth cv. Blue Jacket. National Conference on New Concepts in Agriculture, Tehran, Iran.
70. Soheili Sh, Miri SM, Ghazijahani N. Effect of explants type and growth regulators on callus induction of hyssop (*Hyssopus officinalis*). M.Sc Thesis of Horticulture, Islamic Azad University-Karaj Branch.
71. Rezaei Nazari M, Miri SM, Ghazijahani N. High frequency somatic embryogenesis in *Hypersicum perforatum*. 3rd International Conference on Research in Science and Technology, Berlin, Germany. 2016
72. Rezaei Nazari M, Miri SM, Ghazijahani N. Somatic embryogenesis in *Hypersicum perforatum* by using petiole as explants . 3rd International Conference on Research in Engineering, Science and Technology, Batumi, Georgia. 2018
73. Habibi MM, Miri SM, Mirmasumi SM. Effects of benzyladenine (BA) and naphthalene acetic acid (NAA) on production bulblet two locally mass fritillary. 2nd National Conference on New Concepts in Agriculture, Saveh, Iran. 2013.

74. Elsheikh, A.M., Daffalla, H.M., and Khalfala, M.M., (2013). *In vitro* micropropagation of the ornamental plant Dieffenbachia- A review. *Universal Journal of Plant Science*, 1(3): 91-99.
75. Mousavi ES, Behbehani M, Hadavi E, Miri SM. Callus induction and plant regeneration in lisianthus (*Eustoma grandiflorum*). *Trakia Journal of Sciences*. 2012; 10(1), 22-25.
76. Lim KB, Kwon SJ, Lee SI, Hwang YJ, Naing AH. Influence of genotype, explants source, and gelling agent on *in vitro* shoot regeneration of chrysanthemum. *Horticulture, Environment, and Biotechnology*. 2012; 53(4), 329-335.
77. Mogollon JN. Effect of three cytokinins and the location of the explants *in vitro* multiplication of the *Dieffenbachia maculata* Schott. cv. Sublime. The 46th Annual Meeting of the Interamerican Society for Tropical Horticulture, Miami, USA. 2000.
78. Abdali dehdezi A, Azadi P, Miri SM. Study on regeneration of tuberose (*Polianthes tuberose*) by *in vitro* culture. 2013; M.Sc Thesis of Horticulture, Islamic Azad University-Karaj Branch.
79. Abbaszadeh SM, Miri SM, Naderi R. Optimization of asymbiotic germination of Phalaenopsis orchid in *in vitro* conditions. International Conference on Agricultural Science, Medicinal Plants and Traditional Medicine. 2018.
80. Soleimani V, Mohammadi A, Miri SM. Study of *in vitro* propagation of Phalaenopsis orchid. 2014; M.Sc Thesis of Horticultural Science, Islamic Azad University-Karaj Branch.
81. Trigiano RN, Gray DJ. *Plant Tissue Culture*. 2011; CRC Press.
82. Rafiee M, Naderi R, Miri SM. Disinfection and germination of primrose (*Primula sp.*) seeds. 4th International Conference on Applied Research in Chemistry Science and Biology, Tehran, Iran. 2017.

83. Abbaszadeh SM, Miri SM, Naderi R. Optimization of *in vitro* seed germination of *Phalaenopsis* orchid. 2018; M.Sc Thesis of Horticultural Science, Islamic Azad University-Karaj Branch.
84. Miri SM. Effect of IAA, IBA and NAA auxins on *in vitro* rooting of M.9 and M.26. *Cellular and Molecular Plant Biology Journal*. 2018; 12(4), 15-23.
85. Bayanati M, Davoodi D, Jafarkhani Kermani M. Effect of agar and different culture media on the micropropagation of *Rosa hybrida* cv. 'Black Baccara'. *Journal of Ornamental Plants*. 2015; 5(2), 75-81.
86. Azadi P, Khosh-Khui M. Micropropagation of *Lilium ledebourii* (Baker) Boiss as affected by plant growth regulator, sucrose concentration, harvesting season and cold treatments. *Electronic Journal of Biotechnology*. 2007; 10(4).
87. Zivyar S, Miri SM, Rahimi Meydani A. The effect of plant growth regulator of BAP and NAA on callus formation from corm explants of *Gladiolus*. 2014; 1st National Ornamental Congress.
88. Kumar N, Reddy MP. *In vitro* plant propagation: a review. *Journal of Forest Science*. 2011; 27(2), 61-72.
89. Giovannini A, Laura M, Nesi B, Savona M, Cardì T. Genes and genome editing tools for breeding desirable phenotypes in ornamentals. *Plant Cell Rep*. 2021; 40, 461–478.
90. Koetle MJ, Finniea JF, Balázssab E, Staden JV. A review on factors affecting the *Agrobacterium*-mediated genetic transformation in ornamental monocotyledonous geophytes. *S. Afr. J. Bot*. 2015; 98, 37–44.
91. Bull T, Michelmore R. Molecular determinants of *in vitro* plant regeneration: Prospects for enhanced manipulation of lettuce (*Lactuca sativa* L.). *Front. Plant Sci*. 2022; 13, 1211.