

Review Article

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, explant 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: eExplant Type, Ornamentals, Jin Vitro Ceulture, 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 £2.1billion in 2005, with overseas commerce of £60-75billion. Its economic worth has sky rocketed 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

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demonstrated [7,8]. Tissue culture techniques are used for ~~micropropagation~~ 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 ~~explant~~ explants type, all of which should be experiential during the process [10]. Naphthalene Aacetic acid (NAA) and benzyl~~a~~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~~ 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 hyperhydricity. Some systems of ~~Temporary Immersion (TIS)~~ Temporary Immersion (TIS) have been employed on tropical plants [12, 13] and fruit ~~trees~~ 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 to modify in-plant material to increase their quality. For hybridization, *in vitro* procedures including 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 Brassica *cea* plant species on sugar-supplemented salt media [15]. Dietrich revealed in 1924 that both mature and immature embryos may

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besaved.[16].In1925,the firstinterspecific hybridization was described in the perennial flax (*Linum perenne* L. x *Linum austriacum* L.) through embryo rescue from non-viable seeds [17]. Since its discovery, embryo rescue has been employed for interspecific hybridization in a wider range of crops, including floral, decorative, medicinal, and woody plants [18,19].

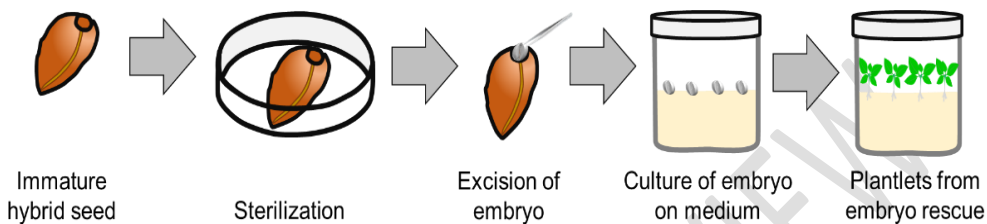


Figure 1. Process of embryo rescue from immature (or non-viable) seed after hybridization

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/intergenerichybrid 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 within-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

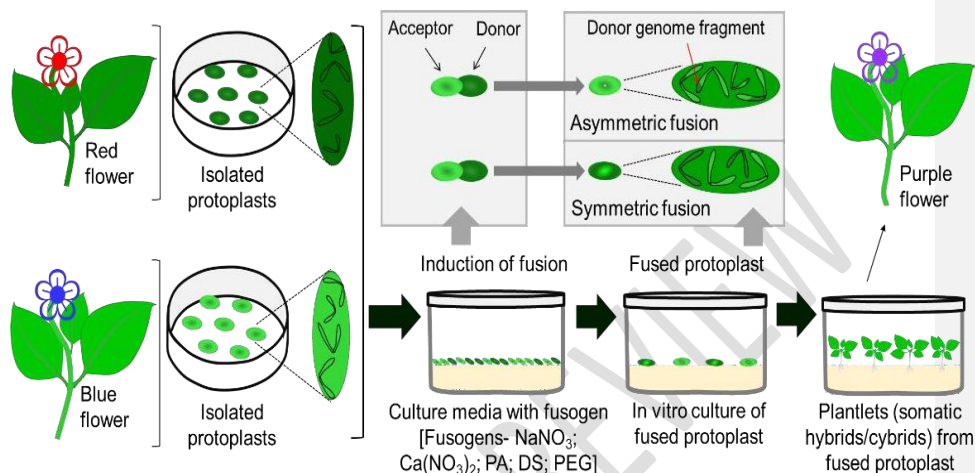


Figure 2. Illustration of somatic hybrid or cybrid development through protoplast fusion. Here, NaNO_3 ; sodium nitrate, $\text{Ca}(\text{NO}_3)_2$; calcium nitrate, PA; polyvinyl alcohol, DS; dextran sulfate, polyethylene glycol (PEG).

Somatic hybridization 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 in order to combine somatic cells from various cultivars, species, or genera (Figure 2).

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 pr

roducesymmetricorasymmetrichybrids,knownassomatichybridsorcybrids(Fig.2).ThefirstasymmetrichybridwasdiscoveredbysomatichybridizationofNicotianatabacum(tobacco)andPetroselinumhortense(parsley)[44,45].Manywildplantspecieshaveimportantfeatures,includingdiseaseandpathogenresistance,whichcanbetransmittedtocultivatedcropspecies.Somatichybridizationallowsdesiredfeaturestobetransferredtoboostyield,resistance,tolerance,andsoon[46,47].Itenablesbreederstogenerateuniquehybridsusinganasexualtechniqueratherthantraditionalbreeding(Figure2).

Somatichybridizationhasbeenappliedforthegeneticimprovementsofvariousfloweringandornamentals,suchasrose[48],Dendrobium[49],chrysanthemum[50],dianthus[51],gentian[52],iris[53]andSaintpaulia[54].

Somaclonalvariantsorsomatichybrids 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 markers 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 soon are the fundamental limits of somatic hybridization [56].

1.3. Production of Synthetic Seeds

Asyntheticseedorartificialseedisanencapsulatedplanttissue,somaticembryos,orothermicropropagules(Figure3).Syntheticseedshaveseveraladvantagesovernaturalseeds,includingseason-independentseedproduction,geneticuniformity,maintaininghybridvigor,long-termstoragecapacity,rapidmultiplication,freedomfromvegetativeandseed-borne pathogens, high-volume low-cost propagation, ensuring quality plant materials and shortening lifecycles. [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* (lishianthus), *Pinus spatula* (pine), *Genista monosperma* (bridal broom), *Hyoscyamus muticus* (Egyptian henbane), and *Clitoria ternatea* (blue pea or blue bell vine) from the somatic embryo; *Gypsophila paniculata* (gypsophila), *Saintpaulia ionantha* (saintpaulia), *Urginea altissima* (tall white squill), and *Taraxacum pinnatum* (Mniszek pinnatum) from shoot tip; *Rosadamasceana triflora* (D

amaskrose), *Syringavulgaris* (lilac), *Neriumoleander* (oleander), *Centellaasiatica* (Asiatic pennywort), *Ecliptaalba* (falsedaisy), *Erythrinavariegata* (tiger's claw), *Photiniafraseri* (redtip photinia), *Rutagraveolens* (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*, *Flickingerianodosa*, *Spathoglottis plicata*, etc.) [57,58].

In vitro synthetic seeds in ornamentals enable season-independent seeds 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 embryonic potential with time, etc. Synthetic seed technology can be used more effectively in the commercial ornamental plant propagation sector after resolving these limitations.

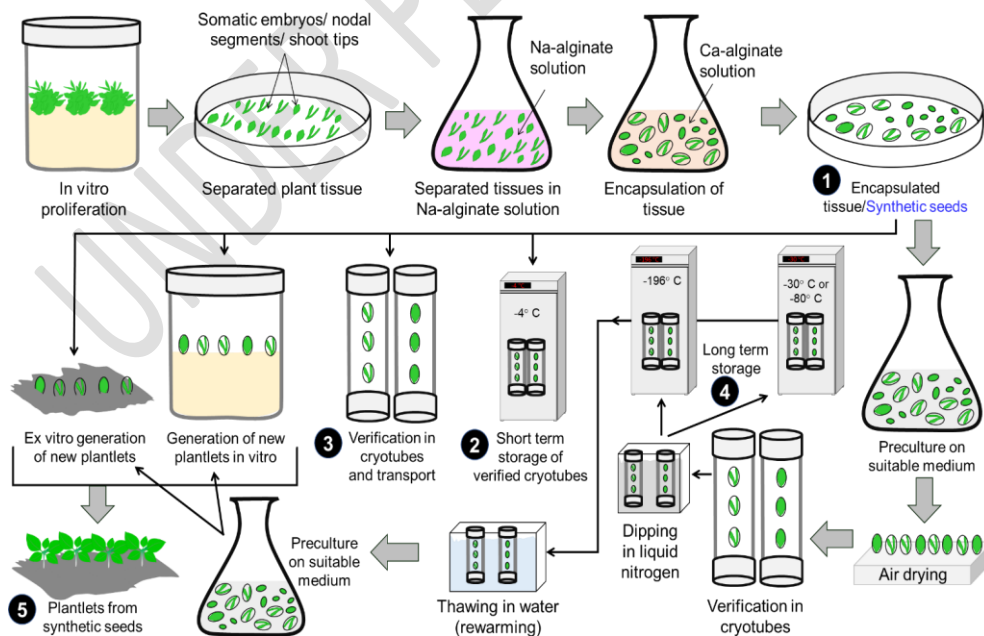


Figure 3. Production and application of synthetic seeds. The numbers in the figure represent the ending point of each step, such as the production of synthetic seeds (1), short-term storage of synthetic seeds (2), synthetic seeds for transportation (3), long-term storage of synthetic seeds (4), and plantlet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

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 wider range of variation compared to diploid plants in their morphophysiological characteristics [63]. Polyploid has also been induced 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 show a wider range of phenotypic differences. Aside from antimitotic agents, ploidy modification is affected by species, explant kinds, antimitotic agent exposure technique, antimitotic agent exposure length, a

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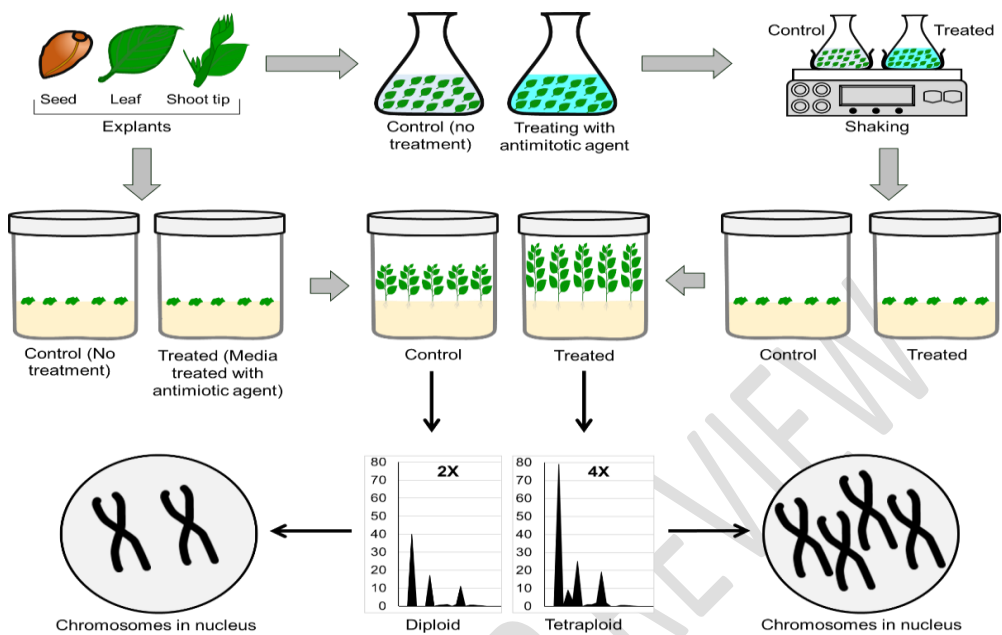


Figure 4. In vitro chromosome doubling (ploidy manipulation) for genetic diversification

2. Factors Affecting on Tissue culture techniques

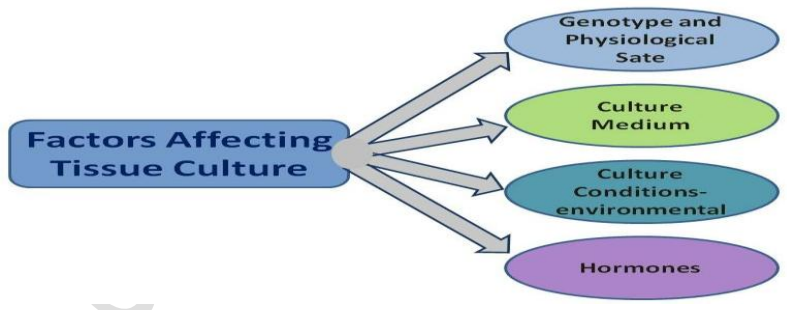


Fig 5. Factors Affecting on Tissue culture techniques

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 bulb regeneration of two *Iris* varieties was different.

2.1.1. Source of explant

Explant source, both *in vitro* and *in vivo*, is also vital for regeneration. *In vitro* explant is thought to be the best method for organogenesis. Cactus plants grown outdoors or in greenhouses can be utilized as explant 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 explant

The precise selection of explant 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. Explant types such as leaf, petiole, hypocotyl, epicotyl, embryo, internode, and root have a substantial impact on plant tissue culture [71, 72]. Bulb scale segments are the most commonly utilized explant source for *in vitro* multiplication of geophytes or ornamental plants such as *Fritillaria imperialis*, *Hyacinthus orientalis*, and *Polianthes tuberosa*. Explant supplies such as ovary, flower stalk, leaf stalk, and mature seeds are also utilized for bulb growth [73]. Petal explant 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 explant 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 explant 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 explant

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 explant to medium in vertical position. The effect of explant 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 *Lilium*. Also, the use of diluted media formulations has sometimes promoted better formation of shoots and especially roots, since high concentration of salts may inhibit root growth, even in presence of auxin in the culture medium. *Phalaenopsis* orchid explants' capacity to create shoots. They concluded that 1/2 MS 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 IBA and 60 g/l sucrose was shown to be better for *Lilium* ledebourii bulb 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 1/2 MS 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 build-up 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 vapor 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. Tapirkae investigated the effects of light quality and quantity on growth and development of *Anigozanthos bicolor* and *Zieria afraseri* 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. fraseri* was highest in plantlets grown under 200 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

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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 *Rosasp.* were used and responses compared with explant grown under 16/8h (light/dark) fluorescent light. Shoots under RBLED (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 calligrown* in the light (1200 lux) had a higher fresh weight and we regreen, 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 a high level 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* (e.g., shoot

ot, 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 metabolites 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 tissue culture 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 transcription factors are involved in the in vitro organogenic callus, shoot, root, somatic embryos, and PLBs, and their transcription factors are also controlled by the exogenous administration of various growth regulators [91].

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