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# In Vitro Regeneration, Micropropagation and Germplasm Conservation of Horticultural Plants

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In vitro tissue culture technologies provide novel tools for improving plant production. Organogenesis and somatic embryogenesis are the two pathways for plant regeneration, and have been widely used for in vitro micropropagation and germplasm conservation of horticultural crops [1–5]. This Special Issue collects eleven publications, including nine research articles, one review and one essay article that address in vitro plant regeneration, micropropagation and germplasm conservation of horticultural crops. They have already attracted great interest as about 28,000 reads and more than 35 citations have been recorded.

In vitro tissue culture provides important approaches for both propagation and conservation of cultivated and wild species [6–10]. Working on a perennial herbaceous wild species, Basiri et al. [11] established an efficient micropropagation system for indirect shoot regeneration from root explants of Foxtail lily (*Eremurus spectabilis*). In vitro callus induction and indirect shoot regeneration were induced from root explants cultured on suitable culture media. The shoot development from callus was highly dependent on the saline formulation of the basal medium and the concentration of cytokinin. Regenerated plantlets were successfully rooted in vitro and re-established following the acclimatization process. The results of the present study are expected to contribute to in vitro propagation and ex situ conservation of this species. Furthermore, due to the medicinal properties of this wild species, this protocol has potential applications in the large-scale production of secondary metabolites under laboratory conditions.

In a similar study of indirect organogenesis, Tang et al. [12] successfully established a protocol for the propagation of *Agapanthus praecox* subsp. *orientalis* 'Big Blue'. A callus induction rate of 100% was achieved in root tips collected from tissue-cultured plants grown in a medium containing picloram, kinetin and naphthalene acetic acid (NAA). Adventitious shoots formed in the callus, and further developed into plantlets with roots in 90 days. About 93% of the plants were re-established after acclimatization. The authors found that the concentrations and types of plant growth regulators were crucial to enhance the process of callus and shoot regeneration. This study provided an effective tissue culture system for micropropagation of *A. praecox*, and would facilitate further practical applications for germplasm conservation and genetic improvement of this species.

Using flower buds as explants for micropropagation of *Rhododendron decorum*, Wu et al. [13] described a simple and efficient protocol for in vitro regeneration via indirect organogenesis. Effects of basal medium and plant growth regulators on the formation and proliferation of adventitious shoots and rooting were studied. The highest callus induction (95%) and shoot differentiation (91%) rates were achieved from explants grown on Wood Plant Medium (WPM) supplemented with thidiazuron and NAA. Shoots were successfully rooted in an auxin-enriched medium and more than 90% of plants survived acclimatization. The in vitro regeneration protocol optimized in this study has potential applications in the genetic improvements of this species.

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Lee and Chang [14] reported an efficient micropropagation procedure for red-fleshed 'Da Hong' pitaya (*Hylocereus polyrhizus*). To efficiently reduce bacterial and fungal infections, a common problem in traditional vegetative propagation, in vitro cultures were initiated from disinfected pitaya seeds. Robust and healthy plantlets were produced within eight weeks and successfully transplanted into the field without any signs of pathogenic infection. 'Da Hong' pitaya plantlets grown on media supplemented with activated charcoal (AC) exhibited increased growth and development of plantlets. Shoots were efficiently micropropagated when cultured on a culture medium supplemented with 200 mg/L AC and 0.10 mg/L NAA. Although spontaneous rooting occurred during shoot development, root density was almost two-fold higher in plantlets cultured in a medium supplemented with 0.20 mg/L NAA. This protocol supported the production of healthy seedlings in self-pollinating pitaya and should be tested for additional accessions.

The use of liquid media in bioreactors has potential applications to maximize micropropagation and facilitate automation [15–17]. The study of Gago et al. [18] used commercial RITA© bioreactors for the micropropagation of two local plum varieties of *Prunus domestica* from the northwest of Spain. The authors investigated the effect of light intensity, supplementation of $CO_2$-enriched air and sucrose on the proliferation, rooting and acclimation of the shoots produced in bioreactors. They found that plum shoots cultured in bioreactors under high light intensity and $CO_2$ enrichment grew and proliferated with 1 and 3% sucrose, but shoot growth was poor when cultured on the medium without sucrose. Successful rooting and acclimation were achieved regardless of sucrose presence in the culture medium in bioreactors, but a lower proportion of rootable shoots occurred when shoots were multiplied without sucrose. Comparing micropropagation produced in bioreactors with that in jars containing semisolid medium, the authors demonstrated that shoot multiplication was much more efficient in bioreactors than in jars. Shoots of both plums cultured in jars or bioreactors with 3% sucrose were successfully rooted irrespective of the culture system. The results of this research provided a novel approach for the massive propagation of plum trees and may provide new perspectives for the propagation of other related plant species.

Also working on propagating plant material in liquid media by using temporal immersion systems, Pérez-Caselles et al. [19] developed an effective micropropagation protocol for the apricot cultivars 'Canino' and 'Mirlo Rojo'. The authors also investigated the effect of the application of silver nanoparticles (AgNPs) on the development of in vitro cultures and their penetration into plant tissue. The addition of AgNPs enhanced the overall plant growth of apricot cultivars. Moreover, the elimination of calcium chloride in the culture medium increased (23-fold) the AgNPs' penetration into the plant tissue without any detrimental effect on the micropropagation of apricot cultivars. Their focus on the increased silver intake of plant tissues will facilitate further investigation on the virucidal activity of AgNPs in plant disease management. Therefore, this study provided a basis for further applications of AgNPs against pathogens in tissue-cultured apricot plants in bioreactors.

In addition to mass propagation and the production of healthy plant materials, tissue culture technology facilitates the safe exchange of plant material within and across countries [20–24]. Li et al. [25] proposed an interesting in vitro incubation system for the long-distance shipping and exchange of plant germplasm based on slow growth in a vacuum-sealed microplate. Potato and ginger were used as model crops to optimize the protocol, which was later applied to sweet potato. The effects of light regime, temperature, iron concentration, plant growth retardants and package types on plant viability were assessed. Cultures were safely transported across thousands of kilometers within China without package or sample damage. Plantlets were recovered and genetic fidelity was confirmed. This protocol is valuable for the safe movement and distribution of tissue-cultured plant germplasm.

Genetic improvements in plant breeding are dependent upon the availability of and easy access to plant genetic resources [6,21,26,27]. In vitro culture technologies have been widely used to establish medium-term (in vitro conservation) and long-term (cryopreserva-

tion) preservation methods for the germplasms of horticultural plants [28–31]. Effective cryopreservation procedures have been identified for cryopreserving seeds, pollen, cell cultures, dormant buds and shoot tips [32–36]. Đordević et al. [37] reported a successful conservation of plum pollen, in which pollen was harvested from flowers in the late balloon stage, adjusted to a moisture content between 6.1 and 6.8% (dry weight basis), and stored in darkness at 4, −20, −80 and −196 °C). Pollen that was stored in sub-zero temperatures continued to have stable viability after 12 months, while a temperature of 4 °C was only suitable for short-term storage of up to 3 months in all tested genotypes. This study provided an easy and practical method to conserve plum pollen for up to one year but also a cheap alternative for short-term storage.

Aiming at the establishment of an efficient procedure for maintaining specific gene combinations of citrus and pineapple cultivars, Ozkaya et al. [38] and Villalobos-Olivera et al. [39] developed shoot tip cryopreservation methods using droplet-vitrification. Ozkaya et al. [38] focused on critical points pre- (pretreatment of donor plants, preculture and dehydration conditions) and post-freeze (recovery medium) in cryogenic procedures for enhancing shoot tip recovery of four citrus cultivars. They evaluated different strategies for improving the cryotolerance of shoot tips to vitrification solutions and investigated recovery media formulations to further increase post-cryopreservation recovery. Villalobos-Olivera et al. [39] investigated the morpho-anatomical and physiological characteristics of cryo-derived pineapple plants after acclimatization. Their study showed that acclimatized pineapple plantlets obtained from cryopreservation had comparable development to conventionally micropropagated and non-cryopreserved plants after 45 days of growth in the greenhouse. These efficient procedures provide valuable information on the use of droplet-vitrification cryopreservation for setting up cryobanks of citrus and pineapple plants.

Micrografting has been widely used to produce virus-free plants and for the formation of whole plants, particularly when shoots (scions) have difficulty forming adventitious roots [40–42]. In a comprehensive review, Wang et al. [43] addressed the application of micrografting to improved micropropagation of horticultural species in the 21st century and discussed factors affecting the success of micrografting. The practical aspects and applications of in vitro micrografting discussed in this review paper should attract the attention of readers and support basic and applied research, as well as the implementation of in vitro micrografting within tissue culture laboratories.

In conclusion, the papers collected in this Special Issue provide a representative and valuable collection of the applications of the in vitro tissue culture technologies used for horticultural species. The Special Issue also prospects for future studies on the application of developed technologies. We hope that the information described in this Special Issue will promote further research and practical implementation of biotechnologies for crop improvement and germplasm conservation.

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