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Editorial

Advancements in In Vitro Technology: A Comprehensive Exploration of Micropropagated Plants

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1. Introduction

In recent decades, the field of plant science has witnessed several breakthrough discoveries, particularly through the application of in vitro technology [1]. The term "micropropagation" refers to the process of producing numerous plants from small fragments of plant tissue. This technique has become helpful in addressing various challenges faced by traditional propagation methods, such as slow growth rates, plant sterility, susceptibility to diseases, and limited availability of planting material [2,3]. Moreover, in vitro propagation methods have improved plant breeding and conservation methods by providing efficient means by which to produce large quantities of genetically uniform plants under controlled conditions [4,5]. Today, in vitro technology covers a wide array of species and cultivars, ranging from economically important crops to endangered and rare plant species crucial for biodiversity conservation [6,7]. Nonetheless, the efficiency of micropropagation is influenced by several key factors, including the selection of an optimal nutrient medium, the precise balance and type of plant growth regulators, and the genetic makeup of the plant species studied. Additionally, environmental factors such as air temperature and light parameters play crucial roles in the success of micropropagation protocols [8]. Meticulous control of these factors is needed to achieve optimal outcomes.

The aim of this Special Issue is to provide a comprehensive overview of the latest advancements in in vitro technology research with a particular focus on micropropagation. This SI has captured the diversity of the studies that focus on the insights of micropropagation protocols, including the optimization of growth media, hormone supplementation, and the control of environmental factors, to achieve the highest success rates in plant regeneration. It also sheds light on the practical implications of in vitro techniques in agriculture, horticulture, and phytotherapy by means of polyploidization and virus elimination. The molecular, structural, and physiological aspects underlying the success of micropropagation that drive the efficient production of plants were discussed. By synthesizing recent research findings and highlighting key developments, this Special Issue serves as a valuable resource for researchers, educators, and practitioners searching for a deeper understanding of the potential offered by in vitro techniques in the realm of plant science.

2. Overview of Published Articles

Since the in vitro culture technique is most often used for the intensification of plant production, most of the articles published in this Special Issue focus on the micropropagation of ornamental, medicinal, and woody species. Basile et al. (contribution 1) performed research on *Cannabis sativa* subsp. *ruderalis* cv. "Finola", a dioecious Finnish cultivar with a remarkably short cultivation cycle of less than three months. The study aimed to establish an effective micropropagation protocol for in vitro multiplication, rooting, and ex vitro acclimatization, utilizing different explant sources: seed-derived in vitro explants and nodal segments with axillary buds. The optimal results were achieved by combining the Murashige and Skoog medium with sucrose, thidiazuron, and 1-naphthalenacetic acid for

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shoot proliferation, while rooting induction proved successful under red/blue lights on a medium enriched with sucrose and indol-3-butyric acid, ensuring a high survival rate of over 90% upon transplantation into a controlled greenhouse environment.

The study of Pourhassan et al. (contribution 2) focuses on the development of a comprehensive in vitro propagation protocol for the black-leaved Raven® ZZ plant (*Zamioculcas zamiifolia*), a drought-resistant ornamental foliage plant originating from Africa. The researchers employed meticulous disinfection techniques using sodium hypochlorite and mercury chloride to initiate an axenic culture. The optimized micropropagation method involved culturing leaf explants on Murashige and Skoog medium supplemented with 6-benzyladenine and/or α-naphthalene acetic acid, resulting in increased shoot and root production. The successful acclimatization of rooted plantlets in greenhouse conditions underscores the significance of this study as the first report on a complete micropropagation protocol for the black-leaved zamioculcas, offering valuable insights for the floricultural industry.

The article by Kaviani et al. (contribution 3) outlined an efficient protocol for the in vitro multiplication and root induction of narrow-leaf firethorn (*Pyracantha angustifolia*) using various basal culture media supplemented with 6-benzylaminopurine (BAP) and indole-3-butyric acid (IBA). Successful axillary bud disinfection, with a 70.23% success rate, was achieved on a Murashige and Skoog (MS) basal medium augmented with 0.5 mg·L^{-1} gibberellic acid (GA_3). The resulting micro-shoots, cultivated under optimized conditions, exhibited a high multiplication coefficient (2.4) on MS medium with 2.5 mg·L^{-1} BAP, followed by successful rooting in the presence of 1 mg·L^{-1} IBA. The acclimatization of rooted plantlets to greenhouse conditions demonstrated a notable 92.84% survival rate, highlighting the successful application of this protocol for the mass propagation of *P. angustifolia*.

Sage is a plant genus of medicinal and ornamental significance. The study by Papafotiou et al. (contribution 4) aimed to develop efficient micropropagation protocols for five Mediterranean sage species: *Salvia fruticosa*, *S. officinalis*, *S. ringens*, *S. tomentosa*, and *S. pomifera* ssp. *pomifera*. Through systematic experimentation with various growth regulators, the research identified optimal conditions for shoot multiplication, with *S. officinalis* demonstrating the highest efficiency among the studied species. Despite encountering hyperhydricity challenges, successful rooting and subsequent acclimatization were achieved, providing essential protocols for the propagation of these sage species, valuable in both pharmaceutical and floriculture industries.

Ficus carica, or common fig, is a highly nutritional fruit, well-known for its medicinal and economic values. The study of Ling et al. (contribution 5) aimed to establish an efficient protocol for the mass propagation of *F. carica* cv. "Violette de Solliès". The researchers optimized shoot induction (15.2 shoots per explant) using 6-benzylaminopurine in Murashige and Skoog medium and achieved robust rooting (93.33%) with indole-3-butyric acid in Woody Plant Medium. The resulting plantlets exhibited genetic and morphological stability over six subculture cycles, and successful acclimatization in biochar soil with a 100% survival rate further supports the efficiency of this protocol for commercial propagation of uniform and true-to-type *F. carica* "Violette de Solliès" plant stocks.

Haida et al. (contribution 6) focused on the endangered *Curcuma caesia*, commonly known as Kali Haldi or black turmeric, addressing its decline in natural habitats due to overharvesting for various purposes, including pharmaceutical demands. By employing plant tissue culture techniques, the researchers established an effective protocol for the propagation of *C. caesia*, emphasizing the optimization of shoot induction, multiplication, and rooting stages. The results demonstrated the suitability of the MSB5 medium for shoot induction, a combination of 6-benzylaminopurine and indole-3-butyric acid for enhanced shoot multiplication, and the full-strength MSB5 medium supplemented with indole-3-acetic acid for successful rooting, ultimately providing a valuable protocol for large-scale raw material production, conservation, or bioactive compound extraction.

The next research published in this SI by Clapa et al. (contribution 7) utilized micropropagation in enhancing the large-scale production of blackberry (*Rubus fruticosus*) plant material. The research aimed to assess the proliferative capacity of blackberry in vitro by comparing wheat starch-gelled culture medium to the conventional agar-gelled medium, while also evaluating the genetic fidelity between the proliferated shoots and their parent plants. Results revealed that wheat starch-gelled culture medium significantly outperformed agar-gelled medium in terms of shoot proliferation and length in all tested blackberry cultivars, and the genetic uniformity of the micropropagated shoots was confirmed through molecular markers.

The study by Khatoon et al. (contribution 8) addressed the longstanding challenge of in vitro propagation of olive plants (*Olea euorpea*), characterized by their woody nature and susceptibility to culture oxidation. Focused on optimizing shoot induction and proliferation protocols, the research investigates the effects of different concentrations of 6-benzylaminopurine (BAP) and pre-cooling regimes on various olive cultivars. The results highlight significant improvements in morphological, physiological, and biochemical attributes, with the 48 h pre-cooling and 2.5 $mg \cdot L^{-1}$ BAP concentration interaction yielding the most favorable results, demonstrating the potential for enhanced olive cultivation through in vitro techniques.

The next published article, that by Faisal et al. (contribution 9), focused on the in vitro regeneration of *Plectranthus amboinicus*, a perennial plant with aromatic leaves and health benefits. Using thidiazuron treatment, the authors achieved optimal shoot regeneration from nodal segments, with the highest number and length of shoots. The in vitro-regenerated plants had higher contents of phenolic, tannin, and flavonoid compounds, along with enhanced antioxidant activity compared to ex-vitro plants. Flow cytometry analysis confirmed the nuclear genome stability of the in vitro-propagated plants. This efficient in vitro multiplication method not only enhances availability but also provides insights into the genetic and phytochemical properties of *P. amboinicus*, contributing to its preservation and sustainable use.

Alongside micropropagation and large-scale production of plants, in vitro cultures can also be used for breeding purposes. Contribution 10 by Jin et al. addressed the importance of transforming the commercial cultivar "YX4" of *Gypsophila paniculata*, a popular cut flower, to produce blue flowers. By successfully introducing the flavonoid 3′5′-hydroxylase gene (PgF3′5′H) from *Platycodon grandiflorus*, the research achieved a transformation efficiency of 13.5%. The optimized protocol, involving shoot apex without meristem, thidiazuron supplementation, and a specific Agrobacterium-mediated transformation system, not only enhances the ornamental value of *G. paniculata* but also provides a valuable tool for studying genes associated with important ornamental traits in this commercially significant species.

The artificial polyploidization of *Ajuga reptans*—a medicinal species, involving genome doubling—was investigated by Navrátilová et al. (contribution 11) to assess its impact on the composition and quantity of biologically active substances from the glycoside and phytoecdysone families. The study revealed increased levels of *trans*-teupolioside, *trans*-verbascoside, and 20-hydroxyecdysone in the aboveground parts of the tetraploid lines compared to diploids. These findings suggest the potential use of Ajuga tetraploids in breeding programs to augment the production of substances with medicinal and industrial applications in pharmaceuticals, cosmetics, and food production, along with potential botanical pesticide effects.

Elimination of viruses is another practical application of plant tissue culture. Viruses have a significant economic impact on agriculture, particularly potato virus M (PVM) and potato virus S (PVS), which collectively cause over 50% of annual potato tuber yield losses. The research by Kereša et al. (contribution 12) aimed to eliminate PVM and PVS from the traditional Croatian potato (*Solanum tuberosum*) cultivar "Brinjak", valued for its economic profitability and genetic potential, to enhance its productivity. For this purpose, the authors used chemotherapy via the application of ribavirin at various concentrations. Despite a 33% success rate in eliminating PVS, ribavirin failed to eradicate PVM, leading

to more severe disease symptoms, adversely affecting photochemistry and multispectral parameters, and ultimately reducing the yield of tubers in plants with mixed infections (PVM + PVS) compared to those with a single PVM infection.

Finally, Koufan et al. (contribution 13) prepared a review article on Caper (*Capparis spinosa*), a shrubby plant species known for its difficulty in vegetative propagation and seed germination. Recognizing the limitations in seed germination, root induction from stem cuttings, and plant hardening, the study highlights the potential of tissue culture as a promising alternative for the clonal propagation of caper plants. This comprehensive review outlines various micropropagation methods, including in vitro seed germination, propagation via nodal segmentation, and adventitious organogenesis, highlighting their role in overcoming obstacles and an approach to the large-scale propagation and genetic improvement of caper plants.

3. Conclusions

Micropropagation has emerged as a powerful tool, offering significant advantages in terms of rapid multiplication, disease-free propagation, breeding, and conservation of plant genetic resources. The compilation of diverse studies presented in this Special Issue underlines the pivotal role of in vitro culture techniques, particularly micropropagation, in the advancement of plant production across various species. The presented research on ornamental, medicinal, vegetable, and woody plants, such as *Ajuga reptans*, *Cannabis sativa*, *Capparis spinosa*, *Curcuma caesia*, *Ficus carica*, *Gypsophila paniculate*, *Olea euorpea*, *Plectranthus amboinicus*, *Pyracantha angustifolia*, *Rubus fruticosus*, *Solanum tuberosum*, *Zamioculcas zamiifolia*, and *Salvia* species, demonstrate the versatility and applicability of tissue culture methods. These studies provide efficient protocols for shoot proliferation, rooting, and acclimatization, paving the way for large-scale production, genetic improvement, and conservation efforts. Overall, these contributions collectively contribute valuable insights into the optimization of in vitro techniques for plant propagation, offering practical solutions for challenges in agriculture and horticulture.

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