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Editorial

Application of Tissue Culture in Plant Reproduction

Elena Corredoira [1,*] and Rita L. Costa [2,*]

[1] Instituto de Investigaciones Agrobiológicas de Galicia (IIAG)-CSIC, Avd Vigo s/n, 15705 Santiago de Compostela, Spain
[2] Instituto Nacional de Investigação Agrária e Veterinária I.P., Avenida da República, Quinta do Marquês, 2780-159 Oeiras, Portugal
* Correspondence: elenac@iiag.csic.es (E.C.); rita.lcosta@iniav.pt (R.L.C.)

The increasing degradation of forests, together with a higher demand for wood and fruit, has led to the need for more efficient trees adapted to the current climatic conditions and, thus, to the need for genetic improvement programs [1]. Traditional methods of genetic improvement by itself are limited by the long reproductive cycle of woody species, as well as by the genetic complexity of many characteristics of interest, including tolerance to abiotic stress, diseases, and pests, and also the quality of forest products. Biotechnology provides exciting opportunities to improve the economic performance of trees by increasing their yield and quality and also speeding up traditional breeding programs, still important despite their difficulty and complexity. An efficient plant regeneration system involving in vitro culture is a prerequisite for the successful use of biotechnology in tree improvement programs, and micropropagation is the most reliable biotechnological method available for propagating woody species [2]. The papers in this special issue report different aspects of the use of biotechnological techniques based on in vitro culture to improve, conserve and propagate different woody species. The issue compiles nine original research papers and two reviews by 69 authors from several important groups involved in research on this topic.

Of the three micropropagation methods available at present (axillary bud proliferation, adventitious bud proliferation, and somatic embryogenesis (SE)), SE is widely recognized as the most efficient in the field of plant biotechnology. This technique is the main and most efficient system of regenerating any type of cell or tissue that has been genetically modified or cryoconserved [3]. Many studies have reported different aspects of the application of somatic embryogenesis in woody plants, either to induce somatic embryos or to produce genotypes that display tolerance to biotic or abiotic stress. For example, Salaj et al. [4] described SE induction in immature zygotic embryos, the long-term maintenance of embryogenic tissue in vitro or by cryopreservation and also the maturation of somatic embryos in an economically important conifer species, *Abies alba* Mill. These researchers obtained induction frequencies ranging from 0.83% to 13.33%, although the different cytokinins evaluated did not have significantly different effects. Cotyledonary somatic embryos were developed on maturation medium with ABA (10 mg·L^{-1}) and with PEG-4000 (7.5%). Embryos were successfully recovered in seven cell lines tested after storage in liquid nitrogen for one year, with regrowth frequencies ranging from 81.1% to 100%.

Regarding the application of SE to improve tolerance/resistance to biotic stress, Martínez et al. [5] used SE to propagate holm oak plantlets selected for their tolerance to *Phytophthora cinnamomi*. Initially, axillary shoot cultures were established from tolerant plants, and these cultures were used to provide the shoot tips used as the initial explants for SE induction. Somatic embryos and/or nodular embryogenic structures were obtained on induction medium with or without indole-acetic acid (4 mg L^{-1}) in two out the three genotypes evaluated, and induction rates ranged between 2% and 4%. Similarly, Edesi et al. [6] studied the SE-propagation ability of elite Norway spruce material carrying root

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rot resistance traits. To achieve this goal, these researchers investigated the presence of the root rot resistance locus *PaLAR3B* in 80 Finnish progeny-tested Norway spruce plus-trees used for SE-plant production, as well as in 241 SE lines (genotypes) derived from these trees. The researchers concluded that the root rot resistance locus *PaLAR3B* is successfully delivered from elite Norway spruce parent trees to their SE progeny through the SE-propagation method and there is no trade-off between root rot resistance locus *PaLAR3B* and somatic embryo production ability. McGuigan et al. [7] used SE in combination with genetic transformation to confer resistance to *Cryphonectria parasitica* and *P. cinnamomi* in American chestnut. Somatic embryos were successfully transformed with a detoxifying enzyme, oxalate oxidase, to enhance blight tolerance, or with the Cast_Gnk2-like gene, which encodes for an antifungal protein, to be tested for putative tolerance to *P. cinnamomi*. These researchers compared three selection methods (on semi-solid medium in Petri plates, in liquid medium in RITA® temporary immersion bioreactors (Sigma Aldrich, St. Louis, MO, USA), and in liquid medium in We Vitro containers (Magenta®, We Vitro Inc., Guelph, ON, Canada)), but did not find any differences between them.

Regarding the application of SE to improve tolerance/resistance to abiotic stress, two studies have investigated the combined application of SE and priming to generate plants with greater tolerance to hydric stress. Priming is based on the idea that plants can store information from stressful conditions at early embryogenic stages and then acquire memory to respond more efficiently to future environmental constraints [8]. Marques do Nascimento et al. [9] showed that the production of embryogenic masses of *P. radiata* and *P. halepensis* is not affected by the high temperatures applied during maturation. In addition, these authors observed that plants obtained from embryogenic masses of *P. radiata* are more resistant to high temperature and drought. Pereira et al. [10] characterized the primed embryogenic masses of *P. halepensis*, analyzing both the phytohormones involved in the success of the SE process as well as the cytological characterization of embryogenic cultures. These authors suggested that cytokinins may potentially act as regulators of stress–response processes during the initial steps of SE.

Micropropagation through the organogenic route involves axillary shoot proliferation and induction of adventitious buds or caulogenesis. A study conducted by Yu et al. [11] investigated the effects of hormones and epigenetic regulation on callus and adventitious bud induction in *Fraxinus mandshurica*. These authors reported that the addition of the DNA demethylation agent 5-azacytidine and the histone deacetylase inhibitor trichostatin A increased the frequency of adventitious bud induction by 17.78% relative to the control. Micropropagation by axillary budding is also a powerful technology for large production of new improved genotypes, selected from breeding programs, enabling the production of quality plants that respond well in field conditions. In this regard, Fernandes et al. [12] reported a three-step protocol for the production of several hybrid genotypes selected from a breeding program implemented for disease resistance of chestnut to root rot caused by *Phytophthora cinnamomi*.

Two review papers have considered the application of biotechnological methods in economically important genera such as *Quercus* and *Eucalyptus*. In the first of these, Ballesteros and Pritchard [13] provided a detailed perspective of how major cryobiotechnological methods can be used for the ex situ conservation of *Quercus* species, which are key species in functioning landscapes. These authors highlighted the recent advances made in the cryoconservation of pollen and zygotic embryos. In the second review paper, Abiri et al. [14] summarized the most important physiological and molecular aspects of *Eucalyptus* micropropagation and identified the bottlenecks hampering the establishment of efficient micropropagation protocols at the industrial level.

Finally, in vitro cell culture is potentially useful for producing bioactive secondary metabolites in plants. In this respect, Fan et al. [15] explored the biochemical process involved in betulin production, a valuable metabolite with antiviral, antibacterial, and antitumor properties, in white birch cell suspension cultures treated with putrescine.

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