PREFACE

Plant micropropagation is one of the most classical and widespread biotechnological tools used around the world. Undoubtedly, this technique brought quite important advances to the authors' knowledge about morphological, physiological and developmental patterns of plants, to the progress of genetic breeding and to the establishment of the genetic engineering, among others.

This technique is based on the "in vitro farming" of plant fragments in an axenic environment, where all nutrients needed are kindly provided to the cultivated plant, which is maintained under controlled temperature and light-darkness periods. Although it can be considered an established technique, several advances have been integrated to the routines of micropropagation laboratories each year, incorporated to the productive sector and to the academy.

In Micropropagation: Methods and Effects, the authors aimed to shortly present some of these advances, as well as practical results of using this biotechnology towards the conservation of plant genetic resources. In seven chapters, methods employed for the micropropagation of different plant species, from agricultural cultures to tree species of the Brazilian Atlantic Forest and advances in techniques for explants disinfection and for analysis of stomata complexes, as well as the progresses related to the micropropagation of apple and the use of bioreactors are presented.

The seven chapters of this book will contribute towards incorporating new ideas to the daily effort of each reader, reaching the basic requests of students, professors, researchers and even non-specialized readers.

Chapter 1 - Micropropagation is an efficient technique that provides vast applications in most varied fields of agriculture, such as in plant propagation, genetic engineering, secondary metabolites production and molecular farming. In micropropagation technique, browning explants (i.e., due to phenolic oxidation), hyperhydricity and contamination are some serious challenges. New advances in the biotechnology field involve the overcoming of these problems and the developing of new methods and techniques to *in vitro* cultivation and propagation. During the last decade, molecular farming had emerged as a novel system for the production of secondary metabolites. Cell suspension, hairy root and calli proliferation has been used as a source to obtain a great amount of phenolic compounds. While the genetic transformation has succeed to overcome the classic limitations of genetic breeding. Therefore, this chapter describes the advances and techniques in the field of plant biotechnology, which can play a significant role in plant production by micropropagation.

Chapter 2 - Micropropagation is a technique of tissue culture suitable for application in the production of seedlings for environmental projects, such as restoration of degraded areas, reforestation or formation of *ex situ* collections. The authors' research group has already studied some woody species from Atlantic Rainforest in relation to micropropagation at its various steps, like the establishment and superficial disinfestation, multiplication, rhizogenesis and *in vitro* and *ex vitro* acclimatization. This chapter present and discuss some steps of micropropagation in four important native species from the Brazilian Atlantic Rainforest: *Peltophorum dubium* (Sprengel) Taubert (Fabaceae), *Eugenia involucrata* De Candolle (Myrtaceae), *Luehea divaricata* Martius et Zuccarini (Malvaceae) and *Handroanthus chrysotrichus* (Mart. ex DC.) J. Mattos (Bignoniaceae).

Chapter 3 - *In vitro* propagation is a technique of plant tissue culture that aims the large-scale cultivation of free of contaminants plants. These contaminants may be bacterial or fungal in nature, which in excess can

lead the explant to death. On the other hand, the need of plant-microbe interaction for *ex vitro* survival of plants is also a crucial issue. Therefore, it is necessary to exclude the main contaminants in the explants, but not all the plant endosymbionts. New methodologies have arisen aiming to improve this technique, and one of such innovations is the use of nanoparticles in disinfestation of explants.

Chapter 4 - Sweet potato (*Ipomoea batatas* L. [Lam.]) is a species of social and nutritional importance, especially to food security. The occurrence of viruses can reduce productivity, causing the degeneration of plants. The meristem culture can to solve this problem through the clonal cleaning method. However, the development of automation for *in vitro* cultivation is necessary to increase the plant production and reduce costs. In this chapter, the authors' research group proposes the cultivation of sweet potatoes in temporary immersion bioreactors (TIBs). A simple method for the establishment and multiplication of sweet potato in bioreactors was developed. Additional studies are being carried to optimize multiplication.

Chapter 5 - The functionality of stomatal complexes of *in vitro* propagated plants is a crucial issue towards successful micropropagation enterprises. The evaluation of the stomatal complexes based on light microscopy is the most effective alternative to determine the functionality of those structures. In this chapter the authors discuss the aspects related to the light microscopic analysis and the statistical computations related to the shape of the stomatal complexes of plants from the Myrtaceae family.

Chapter 6 - A continuous progress with the researches and the technological development has been observed in apple production in South of Brazil, turning it into one of the main agricultural activities in the region. Improvements in apple production, such as in fruit quality, yield, and disease resistance, have generally achieved through conventional crossbreeding. However, F_1 individuals containing the desirable traits of both parents are not obtained because apples are highly heterozygous and have long juvenile period. Propagation through the formation of adventitious shoots or adventitious somatic embryos, based on explants originating from somatic tissues can occur directly from tissues of the

excised explants without previously formed callus (direct organogenesis or embryogenesis) or indirectly, when shoots or embryos regenerate on previously formed callus or in cell culture (indirect organogenesis or embryogenesis). More challenging goals go beyond the scope of immediate application in the development of seedlings and renewal. These objectives concern the fruitful improvement programs, with emphasis on some *in vitro* cultivation techniques, such as embryogenesis, embryo rescue, somatic hybridization and transformation, identification and conservation of germplasm *in vitro*. The biotechnologies associated to the techniques of tissue culture present great use potential for the improvement of elite germplasm and they allow, among other, the capture and fixation of genetic gains starting from superior genotypes. The discussion of this review is about new technologies and strategies of *in vitro* propagation of apple towards genetic of the species.

Chapter 7 - The Plant Tissue Culture has fundamental importance for forestry breeding programs. Several techniques for the clonal propagation of superior genotypes and plant germplasm conservation are used. In addition, tissue culture is often indispensable in certain stages of some techniques of improvement by allowing the regeneration, multiplication or maintenance of plants. The use of molecular markers associated with tissue culture has been very important in the evaluation of genetic stability during *in vitro* culture and should become laboratory routine in the coming years for to increase the reliability about genetic homogeneity of cloned material and certify the quality of clonal plants. In this chapter, the authors will contribute to knowledge of the use of molecular markers to analyze genetic stability in tissue culture, especially during the micropropagation, somatic embryogenesis, and germplasm conservation.