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In Vitro Embryogenesis in Higher Plants

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Cover image: Somatic embryogenesis in sweet orange (Citrus sinensis (L.) Osbeck). Photo of Maria Antonietta Germanà

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Dedication

To Emanuela,
Maria Luisa,
Antonio and
Gabriele,

the best experiments of my life

Maria Antonietta Germanà

To my beloved children
Matilde and Tommaso

Maurizio Lambardi

Foreword

I was fortunate to start my research career in plant tissue culture in the 1970s when this field of research was expanding rapidly. The next few decades witnessed an exponential growth in knowledge, understanding, and application of many tissue culture protocols to a wide range of plant species. Then followed a period in the 1990s and turn of the century when plant tissue culture research was neglected. Many of the leading researchers of the era such as Toshio Murashige, Pierre Debergh, and Walter Preil retired. Postgraduate students and young researchers now wanted to work in the new field of biotechnology. For a time, plant tissue culture was becoming the "forgotten art" even though it underpinned new biotechnologies such as plant transformation. However, some scientists continued to work on plant tissue culture and applied new molecular genetic techniques, such as gene identification, function, and expression, to an understanding of basic plant pathways such as embryogenesis. It has been encouraging for me, as I now reach retirement, to see the next generation of experienced plant tissue culturists now filling the ranks of the experts who have gone before. Maurizio Lambardi and Maria Antonietta Germanà are two of those scientists who are renowned for their research on plant tissue culture. I have known Maurizio both through his research and his contribution to the International Society for Horticultural Science in his role as Chair of the Commission Molecular Biology and In Vitro Culture. Maurizio is both an accomplished researcher and a genuine person who is passionate about his field of research. Maria Antonietta Germanà is an experienced researcher in gametic and somatic embryogenesis in fruit crops. I recommend them as leaders in their field and ideal authors of this book on embryogenesis.

When I first started working on plant tissue culture in the early 1970s, very little was known about embryogenesis. Why species had a predetermined genetic bias to regenerate from callus by embryogenic or organogenic pathways was a mystery. Of the species that were easy to tissue culture, why was carrot embryogenic and tobacco organogenic? In the 1980s, one of my Ph.D. supervisors advised me not to work on embryogenesis because it appeared to depend on "phases of the moon." The message was that experimental results were inconsistent because of our lack of understanding; thus it was not recommended as a topic for students who were facing a deadline and needed reliable and repeatable results. However, our knowledge of embryogenesis has been greatly expanded in recent years. This book represents a detailed overview of the current status of research on embryogenesis and the advances that have been made by researchers who have worked on biotechnology and in vitro culture. Thus the book contains chapters on "Recent advances on genetic and physiological bases of in vitro somatic embryo formation," "A central role of mitochondria for stress-induced somatic embryogenesis;" "…What can we learn from proteomics?," "Genome-wide approaches and recent insights," and "Microspore embryogenesis." There are chapters on somatic embryogenesis in a range of horticultural species, and an excellent series of protocols for embryogenesis from a range of explants.

I would recommend this book to students, researchers, and those who have an interest in plant tissue culture, and to those who may not realize the importance of knowledge of this "forgotten art."

President of the International Society for Horticultural Science (ISHS)
Leuven, Belgium

Roderick Drew

Preface

Embryogenesis in higher plants, one of the different routes of morphogenesis of the plant kingdom, is a fascinating example of cellular totipotency. In fact, different kinds of plant cells (somatic, gametic, nucellar, and fertilized egg cells) are able to regenerate, in nature or in vitro, an entire organism through the formation of a somatic, gametic, or zygotic embryo, a bipolar structure without vascular connection with the surrounding tissue. In vitro somatic, gametic, and zygotic embryogenesis, apomixis, and secondary embryogenesis are actually valuable tools to support plant breeding, propagation, and conservation, with relevant implications to agriculture, forestry, horticulture, and preservation of plant genetic resources. Advances in plant biotechnology, and particularly in tissue culture, led in time to a better understanding of the physiological and biochemical bases regulating the process of plant embryogenesis, and to the establishment of more and more efficient protocols of in vitro embryo induction, maturation, and conversion to plant. Moreover, the recent molecular, genomic, and proteomic studies have produced additional valuable contributions to the comprehension of the in vitro embryogenic developmental process.

The intent of the book is to present an overview of recent advances, innovative applications, and future prospects of in vitro embryogenesis in higher plants by means of topical reviews and stepwise protocols of selected species. With this goal, the book has been divided into five parts. *Part I* contains reviews on general topics (microspore, zygotic and somatic embryogenesis, in vitro and in vivo asexual embryogenesis, advances on the genetic, physiological, and proteomic knowledge of somatic embryo formation, role of programmed cell death and mitochondria in somatic embryogenesis, and innovation in the use of bioreactors). The remaining part of the book contains stepwise protocols on somatic embryogenesis in selected horticultural plants (*Part II*) and forest trees (*Part III*), on gametic embryogenesis (*Part IV*), and on some pivotal topics (*Part V*), such as the detection of epigenetic modifications during microspore embryogenesis, the in vitro embryogenesis and plant regeneration from isolated zygotes, the synthetic seed production, the induction and maturation of somatic embryos, and the cryostorage of embryogenic cultures. Some useful "Notes," a peculiarity of the series "Methods in Molecular Biology," complete all the stepwise chapters, with additional information directly coming from the authors' valuable daily experience in the tissue culture laboratory.

We are extremely grateful to all the authors for providing such excellent contributions, coming from their remarkable expertise on the different aspects of in vitro plant embryogenesis. It is our hope that this book will be a useful source of information and ideas for plant tissue culturists, cell biologists, embryologists, horticulturists, and operators of commercial nurseries. It is also our hope that it will attract students and young scientists toward the fascinating world of in vitro embryogenesis in higher plants.

Palermo, Italy — *Maria Antonietta Germanà*
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