Applied and Fundamental Aspects of

Plant Cell, Tissue, and Organ Culture

Edited by
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With 181 Figures

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Preface

Recent progress in the field of plant cell and tissue culture has made this area of research into one of the most dynamic and promising in experimental biology. In vitro cultures are now being used as tools for the study of various basic problems, not only in plant physiology, cell biology and genetics, but also in agriculture, forestry, horticulture and industry. The introduction and development of these techniques has allowed the study of problems previously inaccessible, and has turned the "dreams" of Haberlandt, White and Gautheret into realities.

Cell and tissue cultures have enabled us to increase our knowledge in many areas, including totipotency, differentiation, cell division, cell nutrition, metabolism, radiobiology and cell preservation. We are now able to cultivate cells in quantity, or as clones from single cells, to grow whole plants from isolated meristems, to induce callus or even single cells to develop into complete plants either by organogenesis or directly by embryogenesis in vitro. It is also possible to obtain plants of various levels of ploidy by tissue and endosperm culture, and to produce haploids by using refined embryo culture techniques after interspecific hybridization followed by chromosome elimination of one of the parents. These are only a few from a number of examples which prove the importance of plant cell and tissue culture techniques in research.

Successful work in fundamental research, while being stimulated by applied studies, has also provided the basis for these, and the study of plant tissue and cell culture is no exception to this generalization. For example, it is now possible to propagate plants of economic importance such as orchids and other ornamentals in large numbers by meristem culture or by other in vitro methods and by this means they can be freed from viruses. In plant breeding, embryo, ovary and ovule culture as well as in vitro pollination have been employed to overcome sterility and incompatibility. However, one of the main reasons for the recent increase in the use of plant organs or cells in culture has been the successful production of haploids from anthers or isolated microspores and of protoplasts from higher plant cells, and the recognition of the potential of these materials in genetics and plant breeding.

Haploid plants, especially when they can be produced in large numbers, are important to geneticists because, (a) mutants can be easily detected and, (b) homozygous plants can be obtained directly in a single generation. This material is now available and with anther cultures, or those of isolated microspores it is possible to produce haploids in large numbers from more than 20 species. Protoplasts of higher plant cells are potentially of equal importance as tools for genetic engineering and somatic hybridization. They can be produced by enzyme treatment in large numbers, they can be cultured, they will regenerate cell walls, and

divide and develop into haploid or diploid plants. Under appropriate conditions they fuse and the fusion products can be cultured; even the regeneration of somatic hybrids has been recently reported. Protoplasts can also take up genetic material contained in nuclei and chloroplasts as well as isolated DNA molecules. This provides the opportunity (a) to combine by fusion the genotypes of species which are sexually incompatible and (b) to introduce foreign genetic material such as organelles or DNA into the genome. Since both cultures of haploids and protoplasts can be manipulated by using the methods of microbial genetics it is understandable that these new developments have attracted the intense interest of geneticists and plant breeders.

This survey would be incomplete without a consideration of some of the difficulties inherent in the situation. Cell cultures are being used effectively in vegetative propagation and in the production of virus-free plants as well as in the investigation of secondary products. However, research into the production of haploids and the synthesis of somatic hybrids has not reached a comparable stage of development. The work on haploids has clearly shown that it is mostly microspores from a number of species of the Solanaceae, and some Gramineae, that can be induced either directly to undergo embryogenesis in vitro or indirectly through callus cultures to form plantlets. Similar, but not identical difficulties exist with cultured protoplasts. Despite the fact that the technical hurdles for the production and fusion of protoplasts have been surmounted there are only two reports of successful somatic hybrid formation and here the yields in terms of plantlet formation are far below 1%. Clearly, there are at present restrictions to the application of these techniques and until difficulties have been overcome further progress may be limited. In the case of haploids, conditions must be established for the routine culture of pollen from recalcitrant species and techniques worked out for the selection of induced variability. The problems are similar for the manipulation of protoplasts, more efficient methods must be developed for the growth and selection of hybrid cells and for the regeneration of plantlets from such cultures.

Considering the present situation with its background of success and of unsolved problems, we thought it essential to take a fresh look at the whole topic by producing a book covering the major lines of current research in the subject with the main emphasis on developments relevant to agriculture, forestry, horticulture and industry. This led to the selection of chapters on Regeneration of Plants, Vegetative Propagation and Cloning, Haploids, Cytology, Cytogenetics and Plant Breeding, Protoplasts, Somatic Hybridization and Genetic Engineering, Tissue Culture and Plant Pathology, and Cell Culture and Secondary Products. Some of these chapters are mainly concerned with established technological aspects, while others deal mainly with important theoretical aims and developments for the future. To the latter belong, for instance, articles on gene amplification, incompatibility, and to a lesser degree cell modification and cryobiology, all of which are in *status nascendi*.

The contributions to this book have been written by specialists from different fields and the attempt has been made to avoid unnecessary duplication. However, in certain places repetition does occur and where this may be of benefit to the

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reader it has been retained. It is hoped that the efforts of the authors and the editors will provide a book which will be a source of information on current methods, experimental achievements and ideas for a wide range of workers in various disciplines of pure and applied plant science as research workers teachers or students.

October, 1976

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Contents

Chapter I
Regeneration of Plants, Vegetative Propagation and Cloning

Chapter II Haploids

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Chapter VI
Cell Culture and Secondary Products

Chapter VII Miscellaneous

Chapter I

Regeneration of Plants, Vegetative Propagation and Cloning

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