Molecular Methods of Plant Analysis

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Genetic Transformation of Plants

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Preface

Molecular Methods of Plant Analysis

Concept of the Series

The powerful recombinant DNA technology and related developments have had an enormous impact on molecular biology. Any treatment of plant analysis must make use of these new methods. Developments have been so fast and the methods so powerful that the editors of *Modern Methods of Plant Analysis* have now decided to rename the series *Molecular Methods of Plant Analysis*. This will not change the general aims of the series, but best describes the thrust and content of the series as we go forward into the new millennium. This does not mean that all chapters a priori deal only with the methods of molecular biology, but rather that these methods are to be found in many chapters together with the more traditional methods of analysis which have seen recent advances. The numbering of the volumes of the series therefore continues on from 20, which is the most recently published volume under the title *Modern Methods of Plant Analysis*.

As indicated for previous volumes, the methods to be found in *Molecular Methods of Plant Analysis* are described critically, with hints as to their limitations, references to original papers and authors being given, and the chapters written so that there is little need to consult other texts to carry out the methods of analysis described. All authors have been chosen because of their special experience in handling plant material and/or their expertise with the methods described. The volumes of the series published up to now fall into three groups: Volumes 1–5 and Volume 11 dealing with some basic principles of methods, Volumes 6, 7, 8, 10, 14, 16, 18 and 20 being a group determined by the raw plant material being analysed, and a third group comprising Volumes 9, 12, 13, 15, 17 and 19 which are separated from the other volumes in that the class of substances being analysed, is indicated in the volume title. Volume 21 and future volumes of *Molecular Methods of Plant Analysis* will continue in a similar vein but will include more chapters involved with the methods of molecular biology.

Development of the Series

The handbook, *Modern Methods of Plant Analysis*, was first introduced in 1954, and was immediately successful, seven volumes appearing between 1956 and 1964. This first series was initiated by Michael Tracey of Rothamsted and Karl Paech of Tübingen. The so-called *New Series of Modern Methods of Plant Analysis*, Volumes 1–20, began in 1985 and has been edited by Paech's successor, H.F. Linskens of Nijmegen, The Netherlands, and John F. Jackson of Adelaide, South Australia. These same editors have now teamed up with a third, Ross B. Inman of Madison, Wisconsin, USA, to produce the renamed series *Molecular Methods of Plant Analysis*. As before, the editors are convinced that there is a real need for a collection of reliable, up-to-date methods of plant analysis covering large areas of applied biology ranging from agricultural and horticultural enterprises to pharmaceutical and technical organizations concerned with material of plant origin.

Future volumes will include Various Aspects of Plant Genomics.

Volume 23: *Genetic Transformation of Plants*

This third volume in the molecular series deals with the topic of genetic transformation of plants. Most would view genetic transformation as a means of bringing about plant improvement, however, it can be a useful tool in analysing the function of plant genes. To this end, the present volume focuses on genetic transformation of a range of plants by a range of methods, a multiplicity of methods being necessary as some plants are more difficult to transform than others.

Since in genetic transformation we are dealing with biotechnological innovation, this volume begins with a chapter on "Biotechnology, Genetic Manipulation and Intellectual Property Rights". It is beyond dispute that property rights apply to the products of biological research, and there is no doubt that in the "developed" world DNA sequences and cells of plant or animal origin can be patented. This first chapter then explores these property rights, be they physical or intellectual, and how they effect the use to which the transformed plant is put and the right to reproduce it.

The following chapter describes the many methods used to carry out plant transformation, beginning with *Agrobacterium rhizogenes*-mediated transformation, which leads to "hairy root" syndrome. This is particularly useful in analysing the interaction between roots and soil organisms or chemical compounds. Thus, promoter-trapping strategies using hairy roots have been utilized to identify genes that form nodules, while hairy roots have also been used to study the interaction between roots and nematodes. Analysis of responses to such chemicals as fungicides, nematicides and herbicides can also utilize the hairy root condition. The next chapter deals with *Agrobacterium tumefaciens*-mediated transformation of whole plants of *Petunia hybrida*, in this case, by a

suspension of *Agrobacterium* cells applied directly to the flower stigma at pollination. *Allium* species have well developed sulphur and carbohydrate biochemical pathways which need to be thoroughly investigated. However, *Allium* has proven to be very difficult to transform; a chapter on *Allium* transformation is therefore included in the belief that it will assist in the analysis of these pathways and identify which are important for normal physiology and which are crucial for the unique neutraceutical qualities ascribed to garlic and onions. Similarly, barley has proven difficult to transform, thus an electroporation method is described in this volume for barley.

Sorghum, like barley, proved difficult to transform at first. A chapter therefore follows on transformation of sorghum using *Agrobacterium tumefaciens*. Polyethylene glycol (PEG) was amongst the first gene transfer systems to be used for successful integration of foreign genes into plant cells. A chapter is included in this volume describing effective production of transgenic sunflower by a PEG-mediated transformation system. However, a large number of sunflower protoplasts need to be used to ensure a significant number of transformed plants. Sunflower exhibits considerable sexual incompatibility between crop and wild species, which limits access to the genetic pool for gene analysis (or plant improvement), and so transformation provides an alternative approach. The last few chapters of this book deal with particle bombardment and WHISKERS-mediated methods of transformation. Norway spruce transformation can be carried out by particle bombardment of embryonic cultures or pollen; the method is important in developing better or new qualities of wood. The author also discusses the considerable problems associated with gene flow by pollen following spruce transformation. A chapter follows on WHISKERS transformation of embryonic maize suspension cultures leading to regeneration into fertile transgenic plants. A subsequent chapter deals with genetic transformation of soybean with biolistics. The latter involves bombardment of proliferative embryonic cultures with DNA coated on 1-μm diameter particles followed by selection and plant regeneration. Both spruce and soybean biolistic transformation methods described above utilized gold particles coated with DNA, although tungsten particles were used in the past. It seems that tungsten, unlike gold, causes considerable DNA damage including DNA strand scissions and inhibition of cell differentiation; these and other genotoxic effects of tungsten particles are assessed in the final chapter.

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