现代生物技术前沿

INTRODUCTION TO PLANT BIOTECHNOLOGY

H. S. 乔拉 著

植物生物技术

导论

(影印版)

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内容简介

近年来,植物生物技术有了飞速的发展,其应用领域益加广泛。本书是 Introduction to Plant Biotechnology 第二版的影印本,对植物的基因、基因组的组成,植物组织培养、重组 DNA 技术进行了全面翔实的阐述,与第一版相比,特别增加了体外突变发生、基因组学、生物信息学、基因转移方法和作物改良转基因技术等方面的内容。作者在生物技术和遗传学领域从事教学和研究 20 多年,在植物体外培养、基因转移、分子标记方面做出了杰出的成就,有丰富的经验,在写作本书时特别注重技术的应用,也提供了比较详细的实验室操作方案。

本书知识全面,内容丰富,集合了植物生物技术的基础知识和前沿进展,实用性强,适合相关学科的高年级本科生、研究生用作教材,也适合从事相关工作的科研技术人员参考使用。

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Preface to the Second Edition

Plant biotechnology has emerged as an exciting area of plant sciences as it can create unprecedented opportunities for the manipulation of biological systems. No branch of biology has developed at such a fast pace as biotechnology has. The scope of this textbook has been broadened in this revised edition keeping in view the new developments in the field of biotechnology, but the basic philosophy remains the same. Protocols are given in those chapters for which laboratory exercises are essential at the undergraduate level. Emphasis is placed on application of a technique, its contribution and impact on crop improvement.

Courses on biotechnology are offered at various levels of undergrad and grad. In the sections relating to plant tissue culture and organization of genetic material the contents have been suitably amended, wherever needed, but the basic aspects remain the same except for few additions. Plant tissue culture courses usually include history, laboratory organization, nutrition media, micropropagation, organ culture, cell suspension culture, haploid culture, somatic fusion, secondary metabolite production, somaclonal variation and cryopreservation. For a good understanding of recombinant DNA technology, organization of DNA in the genome has been expanded and a chapter on basic techniques involved in recombinant DNA technology has been added. Since understanding of plant tissue culture at the undergraduate level has become very critical for realizing the full potential of biotechnology, in almost all the chapters protocols have been given.

In the revised edition recombinant DNA technology part has been broadened to a large extent. Gene cloning is an important aspect in biotechnology and this aspect has been split into three chapters. A chapter on isolating plant genes has been added to give an overview of different gene isolation procedures at an introductory level. Since transposons are used in isolating plant genes, and, is one of the major approaches for tagging of genes, a separate chapter on transposons and gene tagging has been added. A chapter on *in vitro* mutagenesis has been introduced. This will enable students to learn how changes can be introduced in DNA, selecting them in the absence of phenotypic expression and studying the effects of these deliberate alterations in the DNA either *in vitro* or after reintroduction *in vivo*. Genomics and bioinformatics have been introduced in this edition. In these chapters both functional and structural genomics, proteomics, sequencing status of different organisms and DNA chip technology have been discussed.

Chapters on gene transfer methods and PCR have been suitably amended wherever some lacunae were there. Plant breeders strive to meet the challenge of increasing production by developing plants with higher yield, resistance to biotic and abiotic stresses, and for quality characteristics. The chapter on transgenics in crop improvement has been updated and I have tried to give suitable examples of transgenics developed for various characteristics. Also an overview of the impact of biotechnology on crop improvement in context of transgenics has been given.

The molecular markers and marker-assisted selection chapter has been expanded to give more information on some of the new molecular markers and on genetic fingerprinting. The scope of chapter on intellectual property rights has been broadened by giving basic information on various forms of

patenting, plant breeder's rights, biodiversity and some examples of patents, all being subjects which have generated debate.

The work was completed due to the grace of almighty God. I am highly indebted to God that He has given me strength and encouragement to complete this work and hope his gracious blessings will continue to be showered on me so that I can continue to improve this book.

I am highly indebted to reviewers of first edition of this book; that helped me immensely to improve this book. I am thankful to my well wishers, colleagues and innumerable students who have made great contributions directly or indirectly by giving suggestions for improvement in one way or the other for this revised edition. I would appreciate receiving your suggestions and criticism, covering any aspect relevant to this book.

I am thankful to my elders whose continuous support and inspirations led me to complete this work. I am fortunate in having a family which understands the preoccupation that goes with such projects. I am grateful to my wife and to my children, Komaljit and Jasmit, for their continued support and help.

March, 2002 H.S. Chawla

Preface to the First Edition

Plant biotechnology has emerged as an exciting area of plant sciences by creating unprecedented opportunities for the manipulation of biological systems. We are seeing the genes and genomes of a wide range of different organisms being manipulated by the use of new techniques for the benefit of man. One of the key techniques in genetic engineering is gene transfer, which encompasses a variety of methods for returning cloned genes to cells and to generate transgenic plants. Cell and tissue culture are the innovative breeding techniques applied to meet the increasing need for improved crop varieties. Tissue culture techniques can shorten the time and can lessen the labor and space requirements needed to produce a new variety. Cell and plant tissue culture and recombinant DNA technology constitutes an important aspect of plant biotechnology. Further, to understand gene technology it has become essential to understand the basic structure of gene and its organization in plant cell.

Courses on biotechnology are offered at various levels of undergraduate and graduate studies in various departments of Botany, Genetics, Plant Breeding, Horticulture, Plant Pathology, Entomology, Plant Science, Biotechnology and Bioscience. A good understanding of genetic engineering and plant tissue culture at the undergraduate level has become very critical for realizing the full potential of biotechnology. There are a number of books which deal with specialized aspects of plant tissue culture, cloning of genes and genome organization, but, I expect it will be useful to have a book describing the basic aspects of gene and genome organization in plant cells, basic tissue culture techniques and the fundamentals of cloning, gene transfer approaches and molecular markers. Basic tissue culture courses usually include history of the technique, laboratory organization, nutrition media, micropropagation, organ culture, cell suspension culture, anther culture, somatic fusion, secondary metabolite production and cryopreservation. I have given information on variability generated by tissue culture as somaclonal variation in one of the chapters. Gene cloning, gene transfer techniques, genome mapping and molecular markers have been described in relation to plants. A chapter on intellectual property rights has been included to give basic information on various aspects of patenting, copyright and plant breeders right.

Plant breeders are striving to meet the challenge of increased production by developing plants with higher yield, resistance to pests, diseases and weeds and tolerance to various abiotic stresses. I have tried to give suitable examples of transgenics developed for various characters in one of the chapters so that a student is aware of the impact of biotechnology on crop improvement. In most of the chapters protocols for conducting laboratory exercises have been given. A very important point is that in most chapters emphasis is placed on application of a technique and its contribution and impact on crop improvement.

I would very much appreciate receiving your suggestions, criticism and research contributions (as reprints) which relate to different aspects relevant to this book. It will be most helpful during the penetration of a revised edition. Please mention the errors you find with page numbers and describe mistakes. I'll highly appreciate your assistance in this regard.

I am thankful to Mr. Vijay Upadhaya and Mr. Fahim for their patience and agreeing to my suggestions for making diagrams. I am thankful to innumerable students who have made great contributions in one way or the other during the preparation of manuscript.

I am thankful to my elders whose continuous support and inspiration led me to complete this work. I am fortunate in having a family which understands the preoccupation that goes with such projects. I am grateful to my wife and to my children, Komaljit and Jasmit for their perseverance and help.

March, 2000

H.S. Chawla

Abbreviations

2,4-Dichlorophenoxyacetic acid

IAA Indole-3-acetic acid
IBA Indole-3-butyric acid
NAA Naphthaleneacetic acid
PCPA p-Chlorophenoxyacetic acid

BAP or BA Benzylamino purine or benzyladenine

2iP 2-Isopentyladenine

Kin Kinetin Zea Zeatin

ABA Abscisic acid

A.t. Agrobacterium tumefaciens

B₅ Gamborg's medium
DMSO Dimethylsulfoxide
GA Gibberellic acid
kb (p) Kilobase (pairs)

LS Linsmaier and Skoog medium

Mb Megabase Mdal Magadalton

MS Murashige and Skoog medium

ng Nanogram

PAGE Polyacrylamide gel electrophoresis

pg Picogram

Ri Root inducing plasmid of Agrobacterium rhizogenes

SH Schenk and Hildebrandt medium

Ti Tumor inducing plasmid of Agrobacterium tumefaciens

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Embryogenesis

Ultra violet sterilization Maintenance of aseptic conditions Alcohol sterilization Flame sterilization Sterilization of explant Chemical sterilization **Protocols** Sterilization of seeds Sterilization of buds, leaf, stem, roots, tubers, scales, etc. Sterilization of tissue for immature embryos, ovules and flower buds for anther culture TYPES OF CULTURE 27 Cytodifferentiation Organogenic differentiation Types of culture Seed culture Embryo culture Mature embryo culture Immature embryo culture/ embryo rescue Applications of embryo culture Callus culture Organ culture Nucellus culture Application Endosperm culture **Application** Cell culture Protoplast culture **Protocols** Protocol for seed germination (Nicotiana) Protocol for embryo culture (Cereals - wheat, maize, barley rice, etc.) Protocol for embryo culture (Legumes - green gram, black gram, french bean, soybean, etc.) Protocol for callus induction (Nicotiana tabacum) Protocol for callus induction (Cereals - wheat, rice, maize, barley, etc.) **MICROPROPAGATION** 39 Axillary bud proliferation approach Meristem and shoot tip culture **Bud** culture Single node culture Axillary bud method Organogenesis Organogenesis via callus formation Direct adventitious organ formation

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Microsatellite directed PCR: anchored primers