现代生物技术前沿

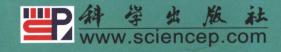
INTRODUCTION
TO PLANT
BIOTECHNOLOGY

H. S. 乔拉 著

植物生物技术

导论

(影印版)



现代生物技术前沿

INTRODUCTION
TO PLANT
BIOTECHNOLOGY

64年美国合作。哲学国家 如用指加坡及口温用的为政党和自然合作。这些经历,是全国联节

H. S. 乔拉 著

植物生物技术

一 College To 字图

斜 学 出 版 社 北 京

THE REPORT OF SECTION

TOWN TRAINING AWAY THE STATE OF THE STATE OF

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。

图书在版编目(CIP)数据

植物生物技术导论/乔拉(Chawla, H.S.)著.一影印本.一北京:科学出版社,2004.3 (现代生物技术前沿)

ISBN 7-03-012799-4

Ⅰ,植… Ⅱ,乔… Ⅲ,植物-生物技术-英文 Ⅳ,Q94-33

中国版本图书馆 CIP 数据核字(2004)第 007444 号

责任编辑:莫结胜/责任印制:安春生/封面设计:王 浩 陈 敬

备举业股业 出版

北京东黄城根北街16号 邮政编码:100717 http://www.sciencep.com

新春即刷厂 印刷

科学出版社发行 各地新华书店经销

*

2004年3月第 一 版 开本:787×1092 1/16 2004年3月第一次印刷 印张:35

印数:1-3 000 字数:830 000

定价: 72.00 元

(如有印装质量问题,我社负责调换〈新欣〉)

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-D 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

Contents

Preface to the Second Edition Preface to the First Edition Abbreviations

PART I: PLANT TISSUE CULTURE

1.	INTRODUCTION New technologies Origin of biotechnology History	3
2	LABORATORY ORGANIZATION Washing facility General laboratory and media preparation area Transfer area Culturing facilities Light units Green houses Laboratory and personal safety	10
3.	NUTRITION MEDIUM Facilities and equipment Units for solution preparation Media composition Inorganic nutrients Carbon and energy source Vitamins Growth regulators Organic supplements Gelling agent pH Protocol: General methodology for medium preparation	14
4.	STERILIZATION TECHNIQUES Preparation of sterile media, containers and small instruments Steam sterilization Units of energy and pressure Dry sterilization Filter sterilization	23

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

5. TYPES OF CULTURE

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 6.

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

Embryogenesis

27

Advantages of micropropagation

Problems associated with micropropagation

Protocols

Meristem and node culture of potato (Solanum tuberosum)

Proliferation of axillary buds (Strawberry- Fragaria chiloensis)

Organogenesis - adventitious shoot formation

Organogenesis via callus formation (Nicotiana)

Organogenesis via callus formation (Cereals: wheat, barley,

maize, rice, etc.)

Embryogenesis (Carrot)

7. CELL SUSPENSION AND SECONDARY METABOLITES

57

Types of suspension cultures

Batch culture

Continuous culture

Open continuous culture

Closed continuous culture

Semi continuous culture

Growth measurements

Synchronization of suspension culture cells

Technique for single cell culture - Bergmann cell plating technique

Applications

Production of secondary metabolites

Morphological and chemical differentiation

Medium composition for secondary product formation

Growth production patterns

Environmental factors

Selection of cell lines producing high amounts of a useful metabolite

Product analysis

Application

Problems associated with secondary metabolite production

Immobilized cell systems

Polymers for immobilization

Product release

Biotransformation

Protocols

Protocol for cell suspension culture (Nicotiana tabacum)

Protocol for cell suspension culture (Cereals - wheat, rice,

maize, barley, etc.)

8. IN VITRO PRODUCTION OF HAPLOIDS

74

Androgenic methods

Anther culture

Microspore culture

The various factors governing the success of androgenesis

Genotype

Physiological status of the donor plants

Stage of pollen

Pretreatment of anthers

Culture media

Process of androgenesis

The ploidy level and chromosome doubling

Diploidization

Significance and uses of haploids

Problems

Gynogenic haploids

Factors affecting gynogenesis

Chromosome elimination technique for production of haploids

in cereals (Barley and wheat)

Protocol

Protocol for anther culture of cereals (Rice, barley, wheat, etc.)

9. PROTOPLAST ISOLATION AND FUSION

Protoplast isolation

Mechanical method

Enzymatic method

Physiological state of tissue and cell material

Enzymes

Osmoticum

Protoplast purification

Protoplast viability and density

Culture techniques

Culture medium

Environmental factors

Protoplast development

Cell wall formation

Growth, division and plant regeneration

Somatic hybridization

Protoplast fusion

Spontaneous fusion

Induced fusion methods

Treatment with sodium nitrate

Calcium ions at high pH

Polyethylene glycol method

Electrofusion

Mechanism of fusion

Identification and selection of hybrid cells

Chlorophyll deficiency complementation

Auxotroph complementation

Complementation of resistance markers

Use of metabolic inhibitors

Use of visual characteristics

Compound selection system

Verification & characterization of somatic hybrids Morphology Isoenzyme analysis Chromosomal constitution Molecular techniques Genetic characterization Chromosome number in somatic hybrids Cybrids Potential of somatic hybridization Problems and limitations of somatic hybridization Protocol Protocol for protoplast isolation and fusion 110 10. SOMACLONAL VARIATION Nomenclature Schemes for obtaining somaclonal variation Without in vitro selection With in vitro selection Factors influencing somaclonal variation Factors important during in vitro selection of somaclonal variants Application of somaclonal variation Basis of somaclonal variation Disadvantages Gametoclonal variation 123 GERMPLASM STORAGE AND CRYOPRESERVATION 11. Cryopreservation Raising sterile tissue cultures Addition of cryoprotectants and pretreatment Freezing Storage Thawing Determination of survival/viability TTC method of staining Evan's Blue staining Plant growth and regeneration. Slow growth method **Applications** PART II: GENETIC MATERIAL AND ITS ORGANIZATION 133 12. GENETIC MATERIAL Sugars Amino acids **Nucleotides** Structural formulae of nucleotides Nomenclature of nucleoside and nucleotide compounds

Polynucleotides

Significance of differences between DNA and RNA Shorthand notation for polynucleotide structure Genetic material Discovery of DNA Double helix is a stable structure **DNA** replication ORGANIZATION OF DNA AND GENE EXPRESSION Different forms of DNA Supercoiling - Tertiary structure of DNA Linking number Cruciform - Tertiary structure of DNA Eukaryotic DNA organization into nucleosomes **DNA** content Denaturation **DNA** renaturation Renaturation rate and DNA sequence complexity-Cot curves Flow of genetic information: central dogma Organization of genes in DNA molecules Operon Multigene family Plant gene structure as a discontinuous gene Control sequences TATA box AGGA box Other regulatory elements Types of RNA molecules Messenger RNA and processing Ribosomal RNA Transfer RNA Small nuclear RNA Transcription **Nucleotide sequences** Transcription in prokaryotes

Transcription in euokaryotes

Genetic code and translation

Genetic code

Translation

Post-translational modifications

PART III: RECOMBINANT DNA TECHNOLOGY

14. BASIC TECHNIQUES

Agarose gel electrophoresis Pulsed field gel electrophoresis Polyacrylamide gel electrophoresis Isoelectric focusing

145

```
Southern blot analysis
               Northern blot analysis
          Protein blotting
               Western blot analysis
          Dot blot technique
          Autoradiography
          E. coli transformation
          Protocol
               Agarose gel electrophoresis
15. GENE CLONING: Cutting and Joining DNA Molecules
                                                                                            189
          Introduction
          Enzymes for cutting: Restriction endonucleases
                Type I, II, III endonucleases
                Other restriction enzymes
          Joining DNA molecules
                DNA ligase
          DNA modifying enzymes
                Kinase
                Alkaline phosphatase
                Terminal transferase
                DNA polymerase
                S 1- Nuclease
                λ-Exonuclease
                Exonuclease III
                Bal 31 nuclease
           Linkers and adaptors
           Protocol
                Restrictive digestion of plasmid DNA
                                                                                            201
16. GENE CLONING: Vectors
           Cloning vectors - features
           Biology of E. coli K-12
           Plasmids
                pBR322, pACYC184, pUC vectors, pUN121
                Yeast plasmid vectors
                      Integrating plasmids, episomal plasmids,
                      replicating plasmid, centromeric plasmid,
                      linear plasmid
                Ti plasmids
           Cosmids
           Bacteriophage vectors
                 Biology of bacteriophage \lambda
                 \lambda phage cloning vectors (\lambdagt10, \lambdagt11, EMBL3 and 4, Charon)
                 M13 phage
```

2-Dimensional gel electrophoresis

Nucleic acid blotting

Phagemids

Yeast artificial chromosome (YAC)

Bacterial artificial chromosome (BAC)

P1 phage vectors

P1 derived artificial chromosome (PAC)

Shuttle vectors

Expression vectors

Protocols

Isolation of plasmid DNA: mini preparation Isolation of genomic DNA by SDS-Proteinase K method

17. GENE CLONING: cDNA and Genomic Cloning and Analysis of Cloned DNA Sequences

Gene libraries

Complimentary DNA(cDNA) libraries and cloning

Isolation or extraction of mRNA of mRNA

Synthesis of first strand of cDNA

Synthesis of second strand of DNA

Cloning of cDNA

Introduction to host cells

Clone selection

Genomic cloning

Isolation of DNA

Partial digestion

Vectors for cloning

Ligation of fragment to a vector

Packaging

Identification and analysis of cloned genes

Probes

Approaches for identification of genes

Colony and plaque hybridization

Immunological detection

Southern blot analysis;

Detection of nucleic acid sequences

Radioactive labeling

Nick translation

Random primed radio labeling of probes

Probe preparation by PCR

Non-radioactive labeling

Horseradish peroxidase system

Digoxigenin (DIG) labeling system

Biotin-streptavidin labeling system

DNA sequencing

Sanger-Coulson method

Maxam-Gilbert method

High throughput DNA sequencing

		Contents XV
18.	POLYMERASE CHAIN REACTION	248
	Introduction	
	Procedure	
	Components in a polymerase chain reaction	
	Inverse PCR	
	Reverse transcriptase mediated PCR (RT-PCR)	
	RACE: Rapid amplification of cDNA ends	
	Quantitative RT-PCR	
	Amplification of differentially expressed genes	
	Differential display reverse transcriptase PCR	
	Suppression subtraction hybridization	
	Cloning of PCR products	
	Restrictive site addition	
	T/A cloning	
	Blunt end ligation	
	Genetic engineering by PCR Applications	
	Advantages	
	Problems	
	Protocol	
	PCR for detection of transgenes	
19.	IN VITRO MUTAGENESIS	269
	Site directed mutagenesis	
	Deletion mutagenesis	
	Unidirectional deletions	
	Gap sealing mutagenesis	
	Linker scanning mutagenesis	
	Cassette mutagenesis	
	Oligonucleotide directed mutagenesis Gapped duplex method	
	dUTP incorporation strategy	
	Phosphorothioate method	
	Chemical mutagenesis	
	PCR mediated in vitro mutagenesis	
	Advantages of site directed mutagenesis	
	Random mutagenesis	
	Insertional mutagenesis	
	Transposon mediated insertional mutagenesis	
	T-DNA mediated insertional mutagenesis	
20.		290
	Transposable elements in bacteria	
	IS elements	
	Composite transposons	
	Complex transposons	