BHARATHIDASAN UNIVERSITY
TIRUCHIRAPPALLI – 620 024

M. Phil. Botany (FT / PT) PROGRAMME
(For the candidates admitted from the academic year 2007-2008 onwards)

Semester I

	Title of the Course	Marks			Credits
		IA	UE	Total	
Course -I	Research Methodology	25	75	100	4
Course - II	Plant Molecular Biology	25	75	100	4
Course- III	Biochemistry and Plant Physiology	25	75	100	4

Semester II

Course – IV	Elective (Any one) 1. Advances in Plant Development 2. Plant Pathology and Protection 3. Microbial Biotechnology 4. Plant Biotechnology	25	75	100	4
	Dissertation and Viva-Voce Viva Voce 50 marks Dissertation 150 marks	200 (150+50)			8

QUESTION PAPER PATTERN (Course I – IV)

Part - A: Two questions from each unit (without choice). Each question carries 2 marks. (10 x 2 = 20)

Part – B: One "EITHER OR" questions from each unit Each question carries 5 marks (5 x5 = 25).

Part – C: One question from each unit. Each question carries 10 marks. The candidate has to answer three questions out of the five questions (3 x 10 = 30)

M.PHIL. BOTANY SYLLABUS

(For the candidates to be admitted from the academic year 2007-2008 onwards)

SEMESTER 1 - COURSE 1 - RESEARCH METHODOLOGY

Unit 1. Centrifugation and microscopy

Centrifugation: Principle and Types of centrifuges. Ultracentrifugation, density gradient centrifugation and continuous centrifugation.

Microscopy - Light microscope, differential interference contrast (DIC), polarization, fluorescent Microscopy, dark field and phase contrast microscopy. Electron microscope- SEM and TEM. Atomic Force Microscopy.

Unit 2. Spectrometry, Electrophoresis and Separation techniques

Spectrometry- Principle – Beer Lambert's Law. UV, IR, FTIR, Atomic Absorption Spectroscopy, CD, Stop Flow, Mass, MALDI-TOF and NMR.

Electrophoresis: Principle of Gel electrophoresis, Polyacrylamide gel electrophoresis (PAGE & SDS PAGE) and Agarose gel electrophoresis, comet assay and capillary electrophoresis. Two dimensional electrophoresis and isoelectrofocussing.

Radioisotopes and their applications in biology - GM and Scintillation counter.

Chromatography: Principle and procedures- Functions and application of TLC, PC, Gel Filtration and Ion exchange, Affinity Chromatography, GC, GLC, HPLC/FPLC and HPTLC.

Unit 4. Molecular biological techniques

Molecular biological techniques: Isolation and amplification of nucleic acid-Genome DNA (*E.coli*), Plasmid DNA, total RNA, Polymerase chain reaction.

Gene cloning techniques: Phosphatase treatment of cloning vectors, use of adapters and linkers in cloning-screening of recombinants-labelling of

nucleic acids by radioactive methods-colony hybridization-southern hybridization-western blot-Northern blot-DNA finger printing and Microarray..

Gene transfer mechanisms: Agrobacterium mediated, Shot gun technique, electroporation and micro injection.

Unit 4. Biostatistics

Biostatistics: Collection and Presentation of Experimental data – Measures of Central Tendency and Location: Arithmetic Mean, Median, Mode, Position of averages, Geometric Mean, Harmonic mean and percentile – Measures of Dispersion: Range, Inter quartile range, variance and standard deviation.
Correlation and Regression: Correlation coefficient – Types of correlation - Regression equation – Principles of least squares – Linear regression – Biological significance of correlation and regression – Tests of significance: Basis of statistical inference – Student's 't' test for mean, difference of means and test for correlation and regression coefficients – Chi-square test – Analysis of variance and DMRT.

Unit 5. Data collection, analysis and Research publications

Data collection and analysis-Web browsing and searching- Electronic biological data bases – NCBI, PubMed, Sequence and Structure data bases.

Research publications and bioinformatics: Preparation of manuscripts-full paper, short communications, Review paper, Thesis writing, Bibliography, Index card.

References:

1. Batschelet, E. 1991. Introduction to Mathematics for Life Scientists. Springer International Student Edn., Narosa Publishing House, New Delhi.
2. Becker, J.M., Caldwell, G.A. and Zachgo, E.A. 1996. Biotechnology: A Laboratory Course, 2nd Edn. Academic Press, Inc., San Diego, California.
3. Cannel, J.P. 1998. Natural Products Isolation. Humana Press, New Jersey, USA.

4. Chirikjian, J.G.1995. Biotechnology: Theory and Techniques Vol. I.Plant Biotechnology, Animal Cell Culture, Immunobiotechnology. Jones and Bartlett Publishers, London, England.
5. Cynthia Gibas and Per Jambek.2001. Developing Bioinformatics computer skills, Shroff Pub., Mumbai.
6. Forthofer, L. 1995. Introduction to Biostatistics, Academic Press, New York.
7. Gupta, S.C. and Kapoor, V.K. 2002. Fundamentals of Mathematical Statistics, (11th Edn.). Sultan Chand & Sons, New Delhi.
8. Harborne, J.B. 1998. Phytochemical Methods. Chapman & Hall, London.
9. Jordan, D.W. and Smith, P. 2002. Mathematical Techniques. Oxford University Press, New Delhi.
10. Primrose, et al.2005. Principles of gene manipulation. Black Well Science, London.
11. Sambrok and Russel. 2001. Molecular cloning-A laboratory manual. Cold Spring Laboratory Press, New York.
12. Sharma, B.K 1996. Instrumental Methods of Chemical Analysis. Goel Publishing House, Meerut.
13. Sokal, R. R. and Rohlf, F.J. 1987. Introduction to Biostatistics (Biology-Statistics Series). W.H. Freeman & Company, New York.
14. Snedecor,GW and Cochran,WG. 1967. Statistical methods.Oxford & IBH Pub.New Delhi.
15. Wilson, K. and Walker, J. 1997. Practical Biochemistry: Principles and Techniques. Cambridge University Press, Cambridge.
16. Zar, J. H. 2006. Biostatistical Analysis: Prentice-Hall.

COURSE II - PLANT MOLECULAR BIOLOGY

Unit 1. Plant genome organization

Plant genome organization : Structural features of a representative plant gene. Chromatin and gene families in plants. Organization of chloroplast genome. Nucleus-encoded and chloroplast-encoded genes for chloroplast proteins. Targeting of proteins to chloroplast. Organization of mitochondrial genome, nuclear and mitochondrial encoded genes for mitochondrial proteins. Targeting of proteins to mitochondria.

Unit 2. CMS, seed proteins, phytohormones and transposons

Mitochondrial genome, and origin and induction of cytoplasmic male sterility. Seed storage proteins. Regulation of gene expression in plant development. Transposons and their role in transgenic plants. Plant hormones and phytochrome.

Unit 3. Nitrogen fixation

Molecular basis of symbiotic nitrogen fixation in legumes by Rhizobia. Agrobacterium and crown gall tumours. Triparental mating. Mechanism of T-DNA transfer to plants. Types of Ti-plasmid vectors for plant transformation. Agrobacterium mediated infection.

Unit 4. Stress biology

Molecular biology of plant stress response-drought, salinity, dehydration, UV, osmotic stress and biotic stress**.** Direct transformation of plants by physical methods. Tagging, mapping and cloning of plant genes.

Unit 5. Genetic engineering

Genetic engineering in plants- selectable markers, reporter genes and promoters used in plant vectors. Genetic engineering of plants for virus resistance, pest resistance, herbicide tolerance, cytoplasmic male-sterility, delay of fruit ripening, resistance to fungi and bacteria. Production of antibodies, viral antigens and peptide hormones in plants. Gene silencing in transgenic plants.

References:

1. H.S.Chawla. 2001. Introduction to Plant Biotechnology. Oxford & IBH Publishing Co. Pvt. Ltd.
2. Peter J.Lea, Richard C.Leegood. 1999. Plant Biotechnology & Molecular Biology. John Wiley & Sons.
3. J. Hammond, P.McGarvey & V.Yusibov (Eds). 2000. Plant Biotechnology-New Products & Applications. Springer-Verlog.
4. Maarten J.Chrispeels and David E.Sadava. 2000. Plants, Genes and Agriculture. Jones and Barlett Publishers.

COURSE III - BIOCHEMISTRY AND PLANT PHYSIOLOGY

Unit 1. Amino acids, Peptides and Proteins

Amino acids - structure and classification. Peptides - naturally occurring peptides, Dipeptides, Glutathione synthesis and function, Neurotransmitter peptides, peptide hormones and growth hormones. Proteins - Peptide bond, primary structure and protein sequencing. Secondary structure and backbone folding. Tertiary structure and stabilizing forces in collagen. Quaternary structure of hemoglobin and its regulatory features.

Unit 2. Carbohydrates, Lipids and Metabolism

Carbohydrates - Monosaccharides, disaccharides and polysaccharides, structure and their biological significance. Lipids -Triglycerides, phosphoglycerols, derived lipids – steroids, prostaglandins and leukotrienes. Lipid alignment and membrane proteins, isolation of membrane proteins.
Metabolism - basic concepts and design – glycolysis – gluconeogenesis – pentose phosphate pathway – TCA cycle. Fatty acid metabolism – amino acid biosynthesis – de novo synthesis and salvage pathways in nucleotide metabolism.

Unit 3. Enzymology

Enzyme nomenclature and classification. Catalytic power and specificity of enzymes – Enzyme kinetics- Michaelis - Menten constant- General properties of enzymes, effect of pH, temperature – Extraction, assay and purification of enzymes – Clinical and industrial applications of enzymes – enzyme engineering.

Unit 4. Water relations, Nitrogen metabolism and Photosynthesis

Water relations - Absorption and translocation of water – stomatal physiology – transpiration flux – anti transpirants – transport of organic food materials – Inorganic nutrition: mineral ion uptake – diffusion – passive and active uptake – cotransport (symport) – counter transport (antiport) - Nernst equation – Donnan's equilibrium – role of ATPase as a carrier –. Nitrogen metabolism including nitrogen fixation (Symbiotic and asymbiotic). Photosynthesis- C_3, C_4, and CAM pathways – C_2 cycle.

Unit 5. Growth and Stress physiology

Growth – definition – phases – internal and external factors affecting vegetative growth – hormones: discovery, synthesis, bioassay, mode of action and physiological effects of auxins, gibberellins, cytokinins, abscisic acid, ethylene and Brassino steroids. Stress - Definition of stress – strain – injury and resistance stress injury – kinds, resistance, avoidance and tolerance – water stress – drought stress – injury – Drought resistance – mechanism, temperature stress – mechanism, cold and heat resistance mechanism, radiation stress – UV injury and resistance – ionizing radiation – mechanism – salt stress – types, injury, biotic stress and resistance mechanism.

References:

1. Voet D. and Voet JG. 2006. Fundamentals of Biochemistry, John Wiley and Sons, New York.
2. Lehninger AL. Nelson DL. And Cox MM. 2002. Principles of biochemistry, Macmillan Worth publishers.
3. Stryer L. 2002. Biochemistry, WH Freeman and Company, New York.
4. Noggle GR and Fritz GJ. 1999. Introductory Plant Physiology, Prentice Hall, London.
5. Bray CM. 1983. Nitrogen metabolism in plants, Longman.
6. Kramer PJ. 1969. Plant and soil water relationship – A modern synthesis.
7. Trevor Palmer 2004. Enzymes Biochemistry, Biotechnology and Clinical Chemistry, East – West Press Pvt. Ltd. India.
8. Hopkins WG. 1995. Introduction to Plant Physiology. John Wiley and Sons Inc., New York.
9. Moore TC.1989. Biochemistry and physiology of plant hormones. Springer-Verlag, New York.
10. Nobel, PS. 1999. Physiochemical and Environmental Plant Physiology, Academic press, San Diego, USA.
11. Taiz L. and Zeiger E. 2006. Plant Physiology (6^{th} edition). Wordsworth Publishing Co.
12. Thomas B. and Vince-Prue D. 1997. Photoperiodism in plants Academic press, San Diego, USA.
13. Westhoff, P. 1998. Molecular plant development from gene to plant. Oxford University Press, Oxford, UK.

14. Battacharya D. 1989. Experiments in Plant Physiology: A laboratory manual. Narosa Publishing house, New Delhi.
15. Cooper TG. 1977. Tools in Biochemistry. John Wiley and Sons, New York, USA.
16. Dryer RL and Lata GF. 1989. Experimental Biochemistry, Oxford University press, New York.
17. Moore TC. 1974. Research experiences in Plant Physiology: A laboratory manual, Springer-Verlag, Berlin.
18. Plummer, DT. 1988. An introduction to practical Biochemistry. Tata McGraw Hill Pub. Co. Ltd., New Delhi.

SEMESTER 2 (OPTIONAL)
COURSE IV (Elective) - ADVANCES IN PLANT DEVELOPMENT

Unit 1. General introduction to molecular biological strategies in plant development

Pattern formation in development, important differences between plant and animal development, germ line development, role of the gametophyte, post embryonic development, cell movement and the planes of cell division, regeneration and totipotency. Variety of plant organs and cell types, intercellular communication, model plant systems, cloning genes relevant to plant development.

Unit 2. Seed to seedling

Seed germination-nucleic acid metabolism, protein metabolism, mobilization of stored food reserves; Seedling growth-tropisms, photomorphogenesis of seedling, hormonal control of seedling growth, gene expression during seedling growth.

Seedling to adult plant: Organization of the shoot apex. Tissue differentiation in the shoot. Leaf determination and development, evolution of leaf form, evolution of leaf cells, differentiation of leaf cells and venation, senescence and abscission of leaves; Root growth and development-root apical meristem, differentiation of the root, regulation of root growth, root-microbe interactions.

Unit 3. Reproduction

Floral evocation and development of the floral meristem- acquisition of the competence to flower, physiology of floral evocation; Formation of floral organs-molecular basis of determination and differentiation of floral organs, homoetic mutants; Microsporogenesis and formation of the male gametophyte- anther differentiation, pollen development and maturation, male gametogenesis; Megasporogenesis and formation of the embryo sac-ovule determination and development, megasporogenesis, organization of the embryo sac; Pollen-pistil interactions and fertilization-pollen-stigma interactions and pollen tube guidance, self-incompatibility, double fertilization, *in vitro* fertilization.

Unit 4. Seed and fruit formation

Endosperm development and embryogenesis-gene action during endosperm development and embryogenesis, storage proteins of the endosperm and the embryo; Fruit growth and ripening, role of ethylene in fruit ripening; Dormancy of seeds and buds-induction of dormancy in seeds, overcoming seed dormancy and bud dormancy.

Unit 5. Vascular development

Cells of vasculature, development of the stem and leaf vasculature, regeneration of vascular strands, lineage of vascular cells in the leaf, differentiation of xylem, phloem. Programmed cell death, gene expression during vascular differentiation. Wound callus morphogenesis, insect galls, crown gall tumors, hairy root disease.

References:

1. S.H.Howell. 1998. Molecular Genetics of Plant Development. Cambridge University Press, Cambridge.
2. P.Wesrhoff.1998. Molecular Plant Development. From Gene to Plant. Oxford University Press, Oxford.
3. V.Raghavan.2000. Developmental Biology of Flowering Plants. Springer-Verlag, New York.
4. P.M.Gilmartin and C.Bowler.2002. Molecular Plant Biology. Vol. I & II. Oxford, University Press, Oxford.

COURSE IV (Elective) - PLANT PATHOLOGY AND PROTECTION

Unit 1. Introduction

History of plant pathology. Discovery of Bordeaux mixture. Nature and concept of plant diseases. Causal factors and Causal agents (Fungi, Bacteria, Virus, Protozoa, Phytoplasma, Spiro plasma etc.,), Plant Diseases (Powdery mildew, Smuts, Bunts, Rust, Wilt, Root rot, Leaf spot, blight, Anthracnose, Damping off, Downy mildew). Direct and indirect transmission of plant pathogens. Significance of Plant diseases.

Unit 2. Penetration and Pathogenesis

Penetration and entry by pathogens. Prepenetration. Direct penetration. Entry through natural openings, wounds, root hairs, buds. Permeability changes in diseased plant. Overcoming host barriers (detoxification, cell wall lytic enzymes, enzymes for waxes and cutins). Toxins, Mycotoxins. Virulence factors. Avirulence genes.

Unit 3. Defense mechanism in plants:

Natural and induced defense mechanism. Structural defense mechanism. Programmed cell death. Biochemical defense mechanism – PR proteins, Phytoalexins, hyper-sensitive reactions. Systemic acquired resistance. Post-transcriptional gene silencing. Gene for gene resistance mechanism. Induced systemic resistance. Signalling in plant disease resistance mechanism (MAP kinases, Ion fluxes, Calcium homeostasis, Oxidative burst, Nitric Oxide, Low molecular weight signalling molecules)

Unit 4. Molecular biology and diagnostics

Arabidopsis thaliana – as a model host in molecular plant pathology. Transformation techniques. Forward and reverse genetics. Signalling pathways. Gene expression profiling.
Molecular diagnostics: Polyclonal, monoclonal and recombinant antibodies. ELISA. Immunoblot. Nucleic acid based technology. Identification of pathogen specific markers. PCR based techniques. Southern, northern and tissue-print hybridization. Gene array based techniques. Quantitative PCR. Uses of bioinformatics in plant pathology.

Unit 5. Control of Plant Diseases

Plant disease forecasting. Chemical methods. Fungicides (for treatment of soil,seeds,plants). Biological control. Mode of application. Integrated disease management. Breeding for disease resistance. Use of tissue culture in plant breeding. Marker-assisted breeding. Transgenic approaches for crop protection – pathogen-derived resistance (coat-protein-mediated resistance, replicase mediated resistance, RNA-mediated resistance) Plantibodies. Over-expressing defense genes.

References:

1. R.M. Twyman. 2004. Gene transfer to plants.. Bios Publishers, London.
2. M.Dickinson. 2003. Molecular Plant Pathology. Bios Publishers. London.
3. Gurr and Sarah Jane. 1997. Molecular Plant Pathology (vol.2). Oxford University Press,New York.
4. R.P. Singh and Uma S. Singh. 1995. Molecular methods in plant pathology. CRC press, London.
5. R.S.Mehrothra .1990.Plant Pathology. Tata-McGrow-Hill Publishers, New Delhi.
6. .J.A. Callow. 1984. Biochemical Plant Pathology. Ed John-Wiley & Sons,UK.

COURSE IV (Elective) - MICROBIAL BIOTECHNOLOGY

Unit 1. Introduction

Definition and scope of microbiology – A general account on microbial diversity: Bacteria including cyanobacteria, microfungi, microalgae, acellular "organisms" include viruses, molecular evolution and relationship between microbes classification: Whittaker's five kingdoms and Woese et al's three domains – A general account on microbial habitats and environments (including Extremophiles).

Unit 2. Microbial physiology

Nutrition (Photoautotrophs, Chemoautotrophs, Parasitism, Saprophytism, Mutualism and Symbiosis, Commensalism, endozoic microbes)—microbial pathogens of plants, animals and humans. Respiration and fermentation. Nitrogen metabolism including nitrogen fixation (Symbiotic and asymbiotic), Lipid metabolism, Primary and Secondary metabolism, Production of enzymes and antibiotics –Role of microbes in biogeochemical cycles.

Unit 3: Genetic improvement of Microbial strains

Basic principles of biochemical engineering, Screening methods for industrially important microbes –Strain selection and improvement –

Mutation, protoplast fusion and recombinant DNA technique for strain improvement.

Unit 4. Biofermentors and Bioprocessing

Selection of bioreactors – types of reactors submerged reactors – surface reactors mechanically agitated reactors – non-mechanically agitated reactors. Design of fermenters – Body construction – basic function of fermentor for microbial and animal cell cultures, aeration and agitation. Computerized fermentors. Downstream processing- Recovery and purification of fermented products.

Unit 5. Metabolites from microorganisms

Peptides, amino glycosides, alcohol, acids (citric acids and acetic acid), solvents (glycerol & butanol), antifungal agents, antibiotics (b lactams, tetracycline, penicllllin and streptomycin), antitumour antibiotics, amino acids (lysine and glutamic acid), methane and Biogas. Recombinant proteins and synthetic vaccines; microbial insecticides (*Bacillus thruingiensis*, *B. spaerinus*, *B. popilliae* and Baculo – viruses), Industrial applications of microbial enzymes - starch processing, textile desizing, detergents and cheese making.

References:

1. Edward A. Brige. 1992. Modern Microbiology – Principles and application, WMC Brown Publishers, USA.
2. Gerard J, Tortora, Berdell R, Funke, Christine, Case L. 2001. Microbiology – An introduction, Benjamin cummings, USA.
3. Stanbury PF, Whitaker. 1995. Principles of fermentation technology, Pergamon Press, Oxford.
4. Prescott JP, Harley, Klein DA. 2002. Microbiology, Mc Graw Hill, Boston.
5. Pelzer MJ, Chan ECS, Kreig NR. 1993. Microbiology, Mc Graw Hill Inc., New York.
6. Zhou J, Thomson DK, Xu Y, Tiedje JM. 2004. Microbial functional Genomics, J. Wiley & Sons publishers.
7. Alexander N.Glazer and Hiroshi Nikaido. 1998. Microbial biotechnology. W.H.Freeman & Co., USA.

COURSE IV (Elective) - PLANT BIOTECHNOLGY

Unit 1. Plant tissue culture - introduction

Introduction-history, scope, and concepts of basic techniques in plant tissue culture. Laboratory requirements and organization. Sterilization-filter, heat and chemical. Media preparation-inorganic nutrients, organic supplements, carbon source, gelling agents, growth regulators and composition of important culture media.

Cell, tissue and organ culture-Isolation of single cells, selection and types of cells, tissue explants and organs for culture – paper, raft nurse technique, plating method, microchamber technique, cell suspension cultures-batch, continuous, chemostat cultures. Cellular potency (morphogenesis)-cytodifferentiation, dedifferentiation and redifferentiation. Cytogenetics of differentiation in cell and tissue cultures, habituation; origin of nuclear variations, factors affecting variation, morphogenesis-nature of induction, direct and indirect organogenesis.

Unit 2. Micropropagation, Organogeneis and somatic embryogenesis

Micropropagation-clonal propagation of elite germplasm, factors affecting morphogenesis and proliferation rate, technical problems in micropropagation.

Organogenesis-formation of shoots and roots-role of growth regulators and other factors, somaclonal and gametoclonal variations.

Somatic embryogenesis-process of somatic embryogenesis, structure, stages of embryo development, factors affecting embryogenesis, synthetic seeds.

Unit 3. Haploid production, in vitro pollination, somatic hybridization, secondary metabolite production, cryopreservation

Haploid production-Androgenesis, gynogenesis-techniques of anther culture-segmentation pattern in microspore-isolated pollen culture-plantlets from haploids-diploidisation-factors influencing androgenesis, haploidy through gynogenesis, haploid mutants, utilization of haploids in plant breeding.

In vitro pollination-ovule and ovary culture, importance, techniques overcoming incompatibility barriers, embryo rescue.

Protoplast culture: Isolation of protoplasts-mechanical and enzymatic sources, culture of protoplasts, viability. Protoplast fusion-spontaneous, mechanical, induced-electrofusion, selection of somatic hybrids, cybrids, importance.

In vitro production of secondary metabolites- classification of secondary metabolites, biosynthetic pathways, cell suspension cultures, immobilized cell cultures, biotransformation and elicitors.

Cryopreservation and gene bank-Modes of preservation, preparation of materials for deep freezing, cryoprotectors, storage strategies, assessment of successful cryopreservation, application and limitations.

Unit 4. Plant transformation

Plant transformation technology: *Agrobacterium* mediated gene transfer,-basis of tumor and hairy root formation,features of Ti and Ri plasmids mechanisms of DNA transfer, role of virulence genes, use of Ti and Ri as vectors, binary vectors, use of 35S and other promoters, genetic markers, use of reporter genes.

Vector less or Direct DNA transfer- elctroporation, microprojectile bombardment, microinjection and transgenic plants. Transgene stability and gene silencing.

Application of plant transformation for productivity and performance-herbicide resistance, insect resistance (Bt genes), virus resistance, nematode resistance, abiotic stress, post harvest losses, long shelf life of fruits and flowers and male sterility.

Unit 5. Molecular marker-aided breeding

Molecular marker-aided breeding-RFLP maps, linkage analysis, RAPD markers, STS, micro satellites, SCAR (Sequence Characterized Amplified Regions), SSCP (Single strand Conformational Polymorphism), AFLP, QTL, map base cloning, molecular marker assisted selection.

References:

1. J. Hammond, P.Mc Garvey and V.Yusibov. 2000. Plant biotechnology. Springer Verlag.
2. E.F.George . 1993. Plant propagation Part II &, Exegetics, England.
3. Dixon et al. Plant cell culture-A practical approach. IRL Press, Oxford.
4. SS Bhojwani and MK Razdan. Plant tissue culture. Elseiver, Amsterdam.
5. M.K.Razdan. 1993. An introduction to plant tissue culture. Oxford & IBH Pub.Co. New Delhi.
6. Kalyan Kumar De. 1992. Plant tissue culture, , New Central Book Agency,
   Calcutta.
7. S.B.Primrose, R.M.Twyman and R.W.Old. 2001. Principles of gene manipulation, Blackwell Science.
8. J.Sambrook, E.F.Fritsch and T.Maiatis. 2000. Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press, New York. 2000.
9. S.B.Primrose. 1994. Molecular biotechnology, Blackwell Scientific Pub. Oxford.
10. J.Hammond, P.McGarvey and V.Yusibov. 2000. Plant biotechnology. Springer Verlag.
11. A. Slater, N.Scott and M.Fowler. 2003. Plant biotechnology. The genetic manipulation of plants. Oxford University Press.