

**Plant Genetic Engineering**  
**(Syllabus of the theory papers)**

**17PGE101: Plant Genetics, Breeding and Tissue Culture**

**52 h**

**UNIT I: Food Crops - Taxonomy, Ecology, Cultivation and Economic Importance** **13 h**

Importance of crops in food production. Field crops: Rice, Wheat, Sorghum, Maize and Sugarcane; Oil Crops: Groundnut, Brassicas, Sunflower, Soybean and Castor; Vegetable crops: Tomato, Brinjal, Gourds, Capsicum, Cucumber; Fruit crops: Banana, Sapota, Guava, Grapes, Mango, pomegranate, *Citrus sps*; Spices and Condiments: Pepper, Cardomom, Turmeric, Zinger, Clove, Cinnamon and Nutmeg.

**UNIT II: Industrial Crops - Taxonomy, Ecology, Cultivation and Economic Importance** **13 h**

Importance of crops as raw materials for industry. Fibre crops: Cotton and Jute; Plantation crops: Rubber, Coffee, Tea, Coconut, Areca and oil palm; Flower and ornamental crops: Rose, Gladiolus, Chrysanthemum, Anthurium, Dieffenbachia, Petunia, Caladium, Philodendron, Gerbera and Orchids; Medicinal and Aromatic plants: *Withania somnifera*, *Raulfia serpentine*, *Andrographis*, *Coleus forskohli*, *Camptotheca acuminata*, *Acorus*, *Bacopa*, *Commiphora wightii*, *Artemisia anuua*. Citrus and Lemon grass.

**UNIT III: Breeding Methods and Seed Technology**

**13 h**

Breeding methods of self and cross pollinated crops. Pure lines, inbred lines and hybrids. Sexual incompatibility, male sterility and their importance in hybrid seed production. Production of hybrid seeds in self and cross pollinated crops. Seed production and certification. Plant genetic resources and conservation. Barstar and Barnase system for production of hybrid seeds.

**UNIT IV: Plant Tissue Culture**

**13 h**

Introduction to cell and tissue culture, Tissue culture media (composition, preparation), Initiation and maintenance of callus and cell suspension culture, organogenesis. Protoplast Culture: isolation and fusion, cybrids. Production of haploids, Somaclonal variations, Embryo culture and embryo rescue. Production and processes for enhancing secondary metabolites from cell suspension cultures and hairy root cultures. Mass multiplication of commercially important crops. Virus indexing and genetic fidelity of micropropagated crops.

## References:

- Agarwal, P.K. and M. Dadlani. 1990. Techniques in Seed Science and Technology, South Asian Publishers, New Delhi.
- Allard, RW. 1999. Principles of Plant Breeding. John Wiley and Sons.
- Bhojwani, S.S. 1990. Plant Tissue Culture: Application and Limitations. Amsterdam, Elsevier.
- Bhojwani, S.S. and Rajdan. 2004. Plant Tissue Culture: Theory and Practice.
- Crispeels, M.J. and Sadava, D.E. 2003. Plants, Genes and Crop Biotechnology. (2<sup>nd</sup> Ed). Jones and Bartlett Publishers.
- Hartl, DL and Jones, EW. 1998. Genetics: Principles and Analysis (4<sup>th</sup> Ed). Jones and Bartlett publishers, Inc.
- Kochhar, S.L. 2000. Economic Botany In the Tropics, 3<sup>rd</sup> ed, Macmillan Publishers, India.
- Roberta Smith. 2000. Plant Tissue Culture: Techniques and Experiments. (2<sup>nd</sup> Ed), Academic Press.
- Sambamurthy, AVSS and Subrahmanyam, NS, 2000. Economic Botany of Crop Plants. Asiatech publishers, India.
- Singh, BD, 2009. Plant Breeding: Principles and Methods (8<sup>th</sup> Ed), Kalyani publishers, India.
- Vijendra Das, LD. 2005. Genetics and Plant Breeding, New Age International publishers.

**UNIT I: Plant Genes and Gene silencing****13 h**

Plant nuclear genes, plastid genes and mitochondrial genes, preparation of plant cDNA and genomic libraries in vector systems.

RNA silencing, micro RNA, SiRNA, RNA silencing for plant functional genomics, *insilico* analysis and assignment of gene function.

**UNIT II: Molecular and PCR Techniques****13 h**

Gel electrophoresis techniques for DNA, RNA and protein. DNA sequencing, Southern, Northern and Western blot. Introduction and Basics of PCR, Polymerase Chain Reaction (PCR), Reverse transcriptase- PCR and Real Time PCR.

**UNIT III: Cloning Vectors and Plant Transformation Techniques****13 h**

General characteristics of vectors, plasmids, phage vectors, cosmids, phagemids, gateway vectors and artificial chromosomes. Concepts of Genome editing.

Binary and co integration vectors, viral vectors and their applications. Methods of plant transformation: Particle bombardment, electroporation, microinjection and *Agrobacterium* mediated transformation. Transformation of chloroplasts. Screening and selection of transformants. Transgene stability. Generation and maintenance of transgenic plants.

**UNIT IV: Trait Improvement of plants****13 h**

Transgenics to drought tolerance, salt tolerance and freeze tolerance. Insect resistance with cry proteins and non Bt proteins: proteinase inhibitors and  $\alpha$  amylase inhibitors.

Pyramiding of genes. Improving the nutritional quality and functional properties of seed proteins, carotenoids and flavonoids. Improvement of shelf life of fruits and flowers through the use of ACC synthase, polygalacturonase and ACC oxidase. Herbicide resistance in plants: phosphinothricin, glyphosate and atrazine. Improving plant photosynthesis and growth. Nitrogen fixing genes and nod genes- structure, function and role in nodulation. Hydrogen metabolism and genetic engineering of hydrogenase genes. Development of transgenics for phytoremediation and phytomining.

## References:

- Bernard R. Glick and John E. Thompson. 1993. *Methods in Plant Molecular Biology and Biotechnology*, CRC Press.
- Brown, TA, 1991. *Molecular Biology*, Bios Scientific Publishers Ltd., Oxford.
- Charles Neal Stewart, Alisher Touraev, Vitaly Citovsky and Tzvi Tzfira. 2011. *Plant Transformation Technologies*. Wiley-Blackwell Publishers.
- Clark, M.S. 1997. *Plant molecular biology: a laboratory Manual*. Springer-Verlag, Berlin, Heidelberg.
- Dabre, P.D. 1988. *Introduction to Practical Molecular Biology*, John Wiley & Sons Ltd.
- Darnell, J, Lodish H and Baltimore D. 1994. *Molecular Cell Biology* (2<sup>nd</sup> Ed), Scientific American Books, Inc. USA.
- Gregory J. H. 2003 *RNAi: A Guide to Gene Silencing*, Cold Spring Harbour Laboratory Press, New York.
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- Matzke, MA and Matzke, AJM. 2000. *Plant Gene Silencing*. Springer Publishers.
- Mouldy Sioud. 2009. *siRNA and miRNA gene silencing: from bench to bedside*. Humana press.
- Potrykus, I and Spangenberg, G. 1997. *Gene Transfer to Plants (Springer Lab Manual)*, Springer Verlag.
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- Sambrook, J, Frisch, E and Maniatis, T. 2000. *Molecular Cloning: A Laboratory manual*, Cold Spring Harbor Laboratory Press, New York.
- Sohail, M. 2005. *Gene Silencing by RNA Interference: Technology and Application*. CRC Press.
- Stanton B. Gelvin and Robert A. Schilperoort. 1988. *Plant molecular biology manual*. Volumes 1-2. Kluwer Academic Publishers.
- Watson, JD. Baker, TA, Bell SP, Gann, A, Levine, M and Losick, R. 2004. *Molecular Biology of the Gene* (5<sup>th</sup> Ed). The Benjamin/Cummings Publ, Co. Inc, California.
- Charles Cunningham and Andrew J.R. Porter. 1997. *Recombinant Proteins from Plants: Production and Isolation of Clinically Useful Compounds (Methods in Biotechnology)*. Humana Press.
- Galun, E and Galun, E. 2001. *The manufacture of medical and health products by transgenic plants*. Imperial College Press.

- John Hammond, Peter McGarvey and Vidadi Yusibov. 1999. Plant Biotechnology: New Products and Applications, Springer Verlag.
- Meran R. L. Owen and Jan Pen. 1996. Transgenic plants: a production system for industrial and pharmaceutical proteins. John Wiley and Sons.
- Oksman-Caldentey, KM and Barz, WH. 2002. Plant Biotechnology and Transgenic Plants. Marcel Dekker, Inc. New York.
- Pena , L 2004. Transgenic Plants: Methods and Protocols. Humana Press.

## **17PGE103: Plant Genomics and Proteomics**

**52 h**

### **UNIT I: Genome structure**

**13 h**

Genetic architecture of plant genomes in nucleus, mitochondria and chloroplast. C-Values of genomes. Early sequencing efforts. Methods of preparing genomic DNA for sequencing, Whole genome sequencing- methods and perspectives. Arabidopsis and rice as a model genome, Mechanism of genome evolution.

### **UNIT II: Genome Analysis**

**13 h**

Importance of mapping-genetical and physical maps. Breeding requirements for maps. Molecular markers- Isozymes, RFLP, RAPD, SSR, ISSR, AFLP, SNP and SCAR. Marker assisted breeding for crop improvement. Map based cloning, T-DNA and transposon tagging, TILLING, Differential display, Microarray in functional genomics. ESTs, transcriptional profiling and metabolic profiling. Serial Analysis of gene expression (SAGE).

### **UNIT III: Proteomics**

**13 h**

Introduction to proteins, Methods of protein isolation, purification, quantification. Proteomic data bases, proteins as drugs, Mass-spec based analysis of protein expression and post translational modifications. Protein chips-interactions and detection techniques. Two dimensional PAGE for proteosome analysis. Automation in proteomics, Proteomics as a tool in plant improvement.

### **UNIT IV: Plant Bioinformatics**

**13 h**

Introduction to Data Mining, Sequence Comparison and Alignment Techniques, Primer design. Databases for functional information and Biological pathway resources and Plant Promoter Database. Application of molecular phylogenetics- phyogenetic data analysis in plant biotechnology, Comparative and subtractive genomics-relevant resources, Studies on SNP and EST-scope and computational biology tools. Need for molecular modeling -homology modeling and model refinement, Molecular docking studies-need and utility in agricultural biotechnology, Computational biology tools and resources for molecular modeling and docking.

## References:

- Brown, TA. 2006. Genomes 2, Wiley-Liss.
- Durbin, R, Eddy, SR, Krogh, A and Mitchison, G. 2000. Biological Sequence Analysis, Probabilistic Models of Proteins and Nucleic Acids. Cambridge University Press.
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- Lankenau, D-H and Volff, J-N. 2009. Transposons and the Dynamic Genome. Springer, Dordrecht.
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- Weising, K, Nybom, H, Wolff, K and Kahl, G. 2005. DNA fingerprinting in Plants: Principles, Methods and Applications. 2<sup>nd</sup> Ed. CRC Press.

**17PGE104: Syllabus of the Elective papers**  
**(choose any one from the following)**

**17PGE1041: Product Development – Biologist**

**52h**

**Unit 1 – Essentials of product development**

**12h**

Company protocols for research, privacy policies, institutional and professional code of ethics and standards of practice, IPR guidelines, Knowledge of basic laboratory procedures, GLP and GMP, relevant EOPs, SOPs, competitor products. Biosafety assessment procedures for transgenic food crops, case studies of relevance. Use of transgenics and their release in environment, legal implications. Stability studies – generate stability data & prepare stability reports for innovation products.

**Unit 2 - Reporting and documentation**

**10h**

Reporting – product development, adverse reactions, process details, statistical analysis of test data- Correlation and Regression. Chi-square test- Analysis of variance and Covariance. Documentation – methods and procedures of writing and maintaining lab, research records, research performance reports, schemes and guidelines, power point presentations, tables, charts, word documents, development of research objectives and proposal writing for funding and contractual purposes, publications and technical writing, Regulatory compliance of the final documents.

**Unit 3 - Planning and communication**

**8 h**

Research planning – resource, time, timeline & budget considerations, technical feasibility analysis, plan day to day activities. Research communications - preparation of progress reports/ research outcomes for steering groups/ bodies, principal investigator, communication with laboratory and field trial teams.

**Unit 4 - Problem solving and decision making**

**6h**

Research initiatives – use new areas of research, techniques and methods, extend research/ product portfolio, creative analysis & interpretation of research data. Decision making – collaborative, appropriate, optimum & best possible solution, Trouble- shoot & Resolve problems to avoid delays.

**Unit 5 – Safety and Security at workplace**

**8h**

Different types of occupational health hazards, knowledge of chemical substances, characteristics & safety measures, use of safety gears, masks, gloves & accessories, evacuation procedures for workers & visitors. Health, safety & security issues – types (illness, fire accidents), company policies and procedures, When and how to report, summon medical assistance & emergency services

**Unit 6 – Interpersonal Skills**

**8h**

Understand work output requirements, company rules, guidelines & policies related to the process flow, identifying and reporting issues requiring intervention, delivery of quality work on time & report any anticipated reasons for the delay, effective interpersonal communication, conflict-resolution techniques, importance of collaborative working, multi-tasking, training the team members, knowledge of project management



## References:

- Kothari C.R, Research Methodology- Methods and Techniques, New Age International, New Delhi
- IPR in Agricultural Biotechnology by Erbisch F H and Maredia K M. Orient Longman Ltd.
- Safety Considerations for Biotechnology, Paris, OECD. Biosafety Management by P.L. Traynor, Virginia polytechnic Institute Publication.
- Bryman, Alan & Bell, Emma (2011). Business Research Methods (Third Edition), Oxford University Press.
- Tzosts, GT, Head, GP and Hull, R. 2010. Genetically Modified Plants: Assessing Safety and Managing Risk. Academic Press.

**17PGE1042: Quality Control/Quality Assurance Biologist****52 h****Unit 1 – Essentials of quality control****16 h**

Molecular and PCR techniques for cloning and expression of genes, Marker techniques for trait improvement, analytical techniques for determining the seed quality, Virus indexing and genetic fidelity tests for micropropagated crops. Data analysis with Statistical Packages – Correlation and Regression. Chi-square test- Analysis of variance and Covariance. The Cartagena protocol on biosafety. Guidelines for research in transgenic plants- APHIS, FDA, EPA, DBT, labeling of transgenic products. Safe handling of transgenics & microbial strains, procedures for handling infectious spillage control, GLP/GMP, working of instruments/apparatus/equipment, biosafety levels and biosafety hazards.

**Unit 2 - Quality Assurance****10 h**

Quality checks - quality assurance samples, master sample, internal controls, statistical analysis of test data, techniques and concepts of statistical quality control and statistical process control, non-conformities. Operational aspects – calibration, accuracy checks of quality control equipments like stability BOD incubators, HPLC, ELISA, Flow cytometry, etc.,

**Unit 3 – Safety and Security at workplace****6 h**

Different types of occupational health hazards, knowledge of chemical substances, characteristics & safety measures, use of safety gears, masks, gloves & accessories, evacuation procedures for workers & visitors. Health, safety & security issues – types (illness, fire accidents), company policies and procedures, When and how to report, summon medical assistance & emergency services.

**Unit 4 – Interpersonal Skills****6h**

Understand work output requirements, company rules, guidelines & policies related to the process flow, identifying and reporting issues requiring intervention, delivery of quality work on time & report any anticipated reasons for the delay, importance of team work, resolution of conflicts, multi-tasking, training the team members, knowledge of project management.

**Unit 5 – Clean work station****6 h**

Cleaning the work area and equipments, materials and equipments required for cleaning, adequate ventilation for the work area, personal protective equipments, dealing with accidental damage, procuring and storing housekeeping equipment and supplies, disposal of wastes, maintain schedules and records for housekeeping

**Unit 6 - Reporting and documentation in quality****8 h**

Reporting – company procedures, escalation matrix for reporting identified issues - defects, problem, incidents, quality issues and test results, feedback to field manager and R&D staff. Documentation – procedures and good documentation practices, accuracy, details, controlled document files and test records, regulatory and compliance requirements, inspection - procedures, protocols and checklists, inspection reports

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- Kothari C.R, Research Methodology- Methods and Techniques, New Age International, New Delhi
- IPR in Agricultural Biotechnology by Erbisch F H and Maredia K M. Orient Longman Ltd.
- Safety Considerations for Biotechnology, Paris, OECD. Biosafety Management by P.L. Traynor, Virginia polytechnic Institute Publication.
- Bryman, Alan & Bell, Emma (2011). Business Research Methods (Third Edition), Oxford University Press.
- John M. S. Bartlett and David Stirling. 2003 PCR Protocols. Humana Press.
- Tzosts, GT, Head, GP and Hull, R. 2010. Genetically Modified Plants: Assessing Safety and Managing Risk. Academic Press.

## PRACTICALS

### 17PGE105: Plant Biochemistry, Breeding and Plant Tissue Culture Lab

1. Good Laboratory practices.
2. Extraction and Estimation of protein content of leaves and seeds – Cauliflower leaves and Pea seeds.
3. Estimation of oil content of seeds: Groundnut/Soybean/Sunflower.
4. Extraction and Estimation of starch content of potato.
5. Estimation of glucose content during seed germination-chick pea.
6. Thin Layer Chromatography.
7. Plant tissue culture: General Introduction and Instruments used in Tissue culture.
8. Tissue culture: Media composition, preparation and sterilization.
9. Seed Culture.
10. Initiation and maintenance of callus cultures.
11. Initiation and maintenance of suspension cultures.
12. Meristem culture.
13. Anther/ovule culture.
14. Embryo Culture.
15. Micropropagation.
16. *Agrobacterium* mediated transformation of plants-GUS/PCR.
17. Protoplast Isolation and Fusion.
18. Microscopy- Light and electron Microscope
19. Determination of size of cell or spore by Micrometry
20. Study of divisional stages in Mitosis using onion root tips.
21. Study of divisional stages in Meiosis using onion flower buds.
22. Plant Breeder's Kit
23. Classification of plants, Botanical description and floral biology of Rice, Maize, Wheat, Bajra, Ragi, Groundnut, Castor, Sunflower, Sesamum, Brassica, Greengram, chilli, Tomato.
24. Emasculation Methods- Hand Emasculation, Suction method, Hot water Treatment, Alcohol treatment, Cold treatment, Genetic Emasculation, Use of Gametocides
25. Selfing Techniques- Bagging, Tagging, Ringing, Lantern method, Mud smearing

## **17PGE106: Plant Molecular Biology and Genetic Engineering Lab**

1. Genomic DNA isolation from plants- Leaves and Cauliflower.
2. Quantification of DNA by Spectrophotometric method.
3. Analysis of DNA by Agarose gel Electrophoresis.
4. Preparation of Competent Cells- *E. coli* and *Agrobacterium*.
5. Genomic DNA isolation from Bacteria- *E. coli*.
6. Isolation Plasmid DNA- *E. coli*.
7. Restriction Digestion analysis.
8. Ligation.
9. Transformation- *E. coli*.
10. Selection of Recombinants and their analysis for inserts.
11. Polymerase Chain Reaction.
12. Reverse Transcriptase PCR.

13. Dot blot.
14. Southern Blotting.
15. SDS-PAGE analysis of seed proteins- Green Pea.
16. Western blotting.
17. Detection of genetically modified commercial crops by PCR-Cotton.
18. Gene Cloning.
19. Genetic fingerprinting of plants by RAPD marker Analysis.
20. RFLP marker analysis.

### **References:**

- Bhojwani, S.S. and Rajdan, 2004. Plant Tissue Culture: Theory and Practice.
- Charles Neal Stewart, Alisher Touraev, Vitaly Citovsky and Tzvi Tzfira. 2011. Plant Transformation Technologies. Wiley-Blackwell Publishers.
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- Sambrook, J, MacCallum, P and Russell, D. 2001. Molecular Cloning: A Laboratory Manual (Third Edition). Cold Spring Harbor Laboratory Press.
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