

**Syllabus of**

**PLANT GENETIC TRANSFORMATION, GENOME, SEED AND  
MARKER ANALYSIS**

**Botany of crops**

Importance of crops in food production and raw materials for industry. Field crops-Rice, Wheat, Sorghum, Maize and Sugarcane, Fibre crops-Cotton and Jute, 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*, 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, Spices and Condiments-Pepper, Cardomom, Turmeric, Zinger, Clove, Cinnamon and Nutmeg. Medicinal and Aromatic plants-*Withania somnifera*, *Raulfia serpentine*, *Andrographis*, *Coleus forskohli*, *Camptotheca acuminata*, *Acorus*, *Bacopa*, *Commiphora wightii*, *Artemisia anuua*, Citrus and Lemon grass.

**Genetics and Breeding Methods:** 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.

**Plant Tissue Culture:** Introduction to cell and tissue culture, Tissue culture media (composition, preparation), Initiation and maintenance of callus and cell suspension culture, organogenesis, Protoplast isolation-Culture 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 Publsihers, New Delhi.**

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Bhowjwani, 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

### **BTFS 102: Plant Molecular Biology**

**40 h**

**Cloning Vectors:** General characteristics of vectors, types of vectors, plasmids, phage vectors, cosmids, phagemids, gateway vectors and artificial chromosomes.

**Plant Genes:** Plant nuclear genes, plastid genes and mitochondrial genes,

**Gene Libraries:** Preparation of plant cDNA and genomic libraries in vector systems.

**Molecular Techniques:** Gel electrophoresis techniques for DNA, RNA and protein. DNA sequencing, Southern, Northern and Western blot.

**PCR Techniques:** Introduction and Basics of PCR, Polymerase Chain Reaction (PCR), Reverse transcriptase-PCR and Real Time PCR.

**Plant Transformation Techniques:** Types of plant vectors and their uses. 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.

**Gene silencing:** RNA silencing, micro RNA, siRNA, RNA silencing for plant functional genomics, in *silico* analysis and assignment of gene function.

### **References:**

Brown, TA, 1991. Molecular Biology, Bios Scientific Publishers Ltd.,Oxford,

Bernard R. Glick and John E. Thompson. 1993. Methods in Plant Molecular Biology and Biotechnology, CRC Press.

Clark, M.S. 1997. Plant molecular biology: a laboratory Manual. Springer-Verlag, Berlin, Heidelberg.

- Charles Neal Stewart, Alisher Touraev, Vitaly Citovsky and Tzvi Tzfira. 2011. Plant Transformation Technologies. Wiley-Blackwell Publishers.
- 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.
- Anthony Bretscher, Arnold Berk, Chris A. Kaiser, Harvey Lodish, Hidde Ploegh, Matthew P. Scott, Monty Krieger, Paul Matsudaira. 2011. Molecular Cell Biology (6<sup>th</sup> Ed). W.H. Freeman Publishers.
- Gregory J. H. 2003. RNAi: A Guide to Gene Silencing, Cold Spring Harbour Laboratory Press, New York.
- John M. S. Bartlett and David Stirling. 2003. PCR Protocols. Humana Press.
- Liang, GH and Skinner, DZ. 2004. Genetically modified crops: their development, uses, and risks. Routledge publishers.
- Maliga, P. 1995. Methods in plant molecular biology: a laboratory course manual. Cold Spring Harbor Laboratory Press, New York.
- 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.
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- 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.

**Transgenic plants tolerant to biotic and abiotic stresses:** Introduction to biotic and abiotic stresses. Transgenics to drought tolerance, salt tolerance and freeze tolerance, Pathogens-Bacteria, fungal and viruses, Insect resistance with cry proteins and non Bt proteins- Proteinase inhibitors and alpha amylase inhibitors. Pyramiding of genes.

**Engineering plants for Molecular farming:** Farming systems and importance of therapeutics- monoclonal antibodies, edible vaccines in human and animal health care, Avidin, B-glucuronidase etc.

**Engineering plants for raw materials for industry:** Biodegradable plastics, polyhydroxybutyrates, industrial enzymes- glycosidases, proteases and laccase, production of fatty acids, industrial oils and silk proteins of spiders and insects.

**Trait Improvement of plants:** 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, polygalactonase and ACC oxidase. Herbicide resistance 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.

**Safety of Transgenic plants:** Biosafety of transgenics and containment practices and ethical issues.

**Seed Technology:** Barstar and Barnase system for production of hybrid seeds. Terminator Technology. Production of normal and transgenic seeds. IPR and Plant breeders Rights.

### **References:**

Charles Cunningham and Andrew J.R. Porter. 1997. Recombinant Proteins from Plants: Production and Isolation of Clinically Useful Compounds (Methods in Biotechnology). Humana Press.

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Meran R. L. Owen and Jan Pen. 1996. Transgenic plants: a production system for industrial and pharmaceutical proteins. John Wiley and Sons.

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Pena , L 2004. Transgenic Plants: Methods and Protocols. Humana Press.

Tzosts, GT, Head, GP and Hull, R. 2010. Genetically Modified Plants: Assessing Safety and Managing Risk. Academic Press.

### **BTFS 104 Plant Genomics:**

**40 h**

**Genome structure:** Genetic architecture of plant genomes in nucleus, mitochondria and chloroplast. Whole genome sequencing- methods and perspectives. Arabidopsis and rice as a model genome, Mechanism of genome evolution.

**Genome Analysis:** 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. Methods of molecular mapping, 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).

**Proteomics:** 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. Proteomics as a tool in plant improvement.

### **Plant Bioinformatics:**

Introduction to Data Mining, Sequence Comparison and Alignment Techniques, Primer design. Databases for functional information and Biological pathway resources and Plant Promoter Database.

### **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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Meksem, K and Kahl, G. 2005. The Handbook of Plant Genome Mapping. Wiley-VCH, Weinheim.

Samaj, Z and Thelen, JJ. 2007. Plant Proteomics. Springer Publishers.

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Twyman, RM and Primrose, SB. 2003. Principle of Genome Analysis. Blackwell Publisher.

Varshney RK and Tuberosa R. 2007. Genomics-Assisted Crop Improvement. Springer, Dordrecht, vol. 1 and 2.

Veenstra, TW and Tate III, JR. 2006. Proteomics for Biological Discovery. Wiley-VCH.

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.

## **PRACTICALS**

- 1. Good Laboratory practices**
- 2. Estimation of protein content of leaves and seeds**
- 3. Estimation of oil content of seeds: Groundnut/Soybean/Sunflower**
- 4. Estimation of starch content of potato.**
- 5. Estimation of glucose content during seed germination-chick pea.**
- 6. Plant tissue culture: Instruments used in Tissue culture**
- 7. Tissue culture: Media composition, preparation and sterilization.**
- 8. Initiation and maintenance of callus cultures**
- 9. Initiation and maintenance of suspension cultures and secondary metabolite production.**
- 10. Use of fungal elicitors for enhancing secondary metabolites**
- 11. Extraction and analysis of secondary metabolites of medicinal plants by TLC/HPLC**
- 12. Meristem culture**

13. Anther/ovule culture
14. Chemical mediated induction of somaclones
15. *Agrobacterium* mediated transformation of plants.
16. Biolistic transformation of callus cultures and analysis-GUS/PCR
17. Cryopreservation techniques and germplasm conservation.
18. Genomic DNA isolation from plants and agarose gel analysis.
19. Polymerase Chain Reaction
20. Genetic fingerprinting of plants by RAPD/ISSR
21. Detection of genetically modified commercial crops by PCR-Cotton
22. Plasmid isolation and restriction digestion analysis.
23. Preparation of DNA insert by restriction digestion and gel purification
24. Ligation and transformation.
25. Selection of recombinants and their analysis for inserts.
26. SDS-PAGE analysis of seed proteins.
27. Dot blot and Western blot of seed proteins.
28. Southern Blotting
29. Genomic library preparation in plasmid vector.
30. Virus indexing of mother plants and micropropagated plants by Dot blot and PCR
31. Genetic fidelity testing of micropropagated crops-Banana

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Bhojwani, S.S. and Rajdan, 2004. Plant Tissue Culture: Theory and Practice.

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