Course title: Molecular Genetics for Plant Functional Genomics: Principles and Practice			
Course code: BBP 148	**No. of credits:** 3	**L-T-P:** 22-23-0	**Learning hours:** 45
Pre-requisite course code and title (if any): None			
Department: Department of Biotechnology			
Course coordinator(s): Prof. Anandita Singh	**Course instructor(s):** Prof. Anandita Singh / Prof. Shashi Bhushan Tripathi		
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Course type: Core course for Plant Biotechnology specialization	**Course offered in:** Semester 3		

Course description:

Transformative technological solutions emerging from plant biotechnology can tackle sustainability challenges in varied sectors including agriculture, energy, and environment. Crop genomics offers exciting possibilities to enhance production of nutritious food to feed the future world. Bio-synthetic potential of plants is being exploited to harvest solar energy for bio-fuel production and achieving CO_2 sequestration. Nonetheless, true potential of plant sciences is required to be harnessed by way of systematic, large-scale functional studies of candidate genes and intergenic regions at genome-wide level. The multi-disciplinary approach of functional genomics aims to unravel the complex relationship between genotype and phenotype. Functional genomics also aims to describe constituents of biological systems and how these interact to manifest traits.

Molecular genetics lies at the heart of functional genomics. The phenotype centric view derived from experimental validation is in sharp contrast with hypothesis driven OMICS and bioinformatics approaches. Analysis of mutant phenotypes, combined with forward mapping strategies are cornerstones for molecular genetics research. Integration of contemporary NGS driven, genome-wide tools, precision phenotyping and genome editing have accelerated gene discovery and functional characterization of genes. Advance statistical models and ML methods have been deployed to fast-track production of superior crops.

This advance level course has been designed to impart an in-depth knowledge on concepts and methodological repertoire of molecular genetics for the purpose of gene discovery and characterization. A basic understanding on principles of molecular biology, genetics and biochemistry is required to fully comprehend the topics covered in this course. Students will be briefly oriented to technologies and various online resources for functional genomics research. However, insights on genomics, genotyping methods, epi-genomics, transcriptomics, proteomics and metabolomics, are to be integrated from other courses taught in the programme. Relevant topics implied in molecular genetics are assembled in five modules given below. Case studies will be used to illustrate power of new technologies in decoding genomes and pangenomes, dissecting genetic architecture of traits, discovering novel alleles and translation of basic knowledge for design of low-input, high-yielding, climate resilient crop varieties.

Course objectives:

1. Building perspectives on integrative approaches of “Functional Genomics”
2. Promoting an understanding on genesis and scope of “Molecular Genetics”
3. Creating an in-depth understanding on forward and reverse genetics-based approaches for dissecting genotype-phenotype relationship
4. Introducing methodological repertoire of forward and reverse genetics
5. Inculcating an appreciation for power of molecular genetics and genomics in unravelling biological function, processes, and phenomena for crop improvement

Course contents				
Module		**L**	**T**	**P**
1	**Molecular genetics and functional genomics: An overview**	1	5	
	Introduction to genomics, functional genomics and molecular genetics within "OMICS" space, concepts implicit in functional genomics (transcription profiling, genotyping, epigenetic profiling, DNA/RNA-protein interactions, meta-analysis), select *in-silico* resources for gene prediction and plant functional genomics (Gramine, PlantGDB, FGENESH, eFP Browser, ArrayExpress, PlantPAN), orientation to ENCODE encyclopaedia, molecular genetics and conundrum of "forward-reverse" for establishing genotype-phenotype relationship			
2	**Linkage mapping: Identification of causal loci using experimental populations**	4	4	
	Linkage mapping and dissection of genetic architecture of traits: Phenotypes and endophenotypes; natural variation and discovery of alleles; construction of linkage maps, bi-parental and multi-parent mapping populations for high resolution trait mapping: F2, RILs (Recombinant Inbred Lines), backcross lines, NILs (Near Isogenic Lines), HIFs (Heterogeneous Inbred Families), AILs (Advanced Inter-cross Lines), pseudo-test-cross mapping, NAM (Nested Association mapping), MAGIC (multi-parent advanced generation inter-cross); map-based cloning (traditional candidate gene sequencing based positional cloning strategies; modern deep sequencing based simultaneous mapping and identification of causal mutation: SHORE maps)			
3	**Association mapping: Identification of causal alleles using natural populations**	2	2	
	Pangenomics, genomes and super-pangenomes; Linkage Disequilibrium (LD) mapping, Haplotype maps, Genome-wide association studies (GWAS), Case studies on crops including rice, wheat and orphan crops viz. chickpea and pigeonpea			
4	**Designing breeding approaches**	4	4	
	Marker assisted foreground and background selection (MAS, MABC, MARS); BSA (Bulked Segregant Analysis); natural variation and exotic genetic libraries, introgression lines; classical and modern approaches for enhancing genetic gains; integration of NGS approaches, statistical models and machine learning tools for genomic selection, AI based tools for precision phenotyping, plant to sensor and sensor to plants, GEBVs (Genomics estimated breeding values) in field crop breeding, case studies covering shuttle breeding, speed breeding and integrated framework on fast-forward breeding			
5	**Mutant Analysis for Functional Genomics**	10	8	
	Approaches for mutagenesis and mutant analyses: Chemical, physical and biological mutagenesis; genetic screens (enhancer, suppressor, modifiers); random and targeted mutagenesis; conceptual understanding of loss-, and gain-of-function mutants; integrated forward and reverse genetics for functional genomics using mutants; Insertional mutagenesis (T-DNA and transposon-tagging); systematic insertional mutagenesis for high-throughput functional genomics; genome-wide mutant libraries; TILLING (Targeted Induced Local Lesions in Genomes), Fast-neutron			

	bombardment (DeleteAgene); RNAi based gene silencing (Intron-hairpin constructs, artificial miRNAs); Ectopic mis-expression, Activation mutagenesis (Enhancer activation tagging, promoter activation tagging) Discovery and functional analysis of cis-regulatory elements: Gene, enhancers, and promoter traps; expression domain analysis of promoters, two component systems for regulated gene expression Genome editing for gene-function analysis: TALENs and advanced CRISPR derived suites and programmable nucleases for altered gene expression; modification of promoters, coding, non-coding sequences; epigenome editing, insertion of reporter gene-drives (knock-ins) Cases studies from model plant *A. thaliana*: Unravelling molecular basis of developmental and adaptive traits using integrated molecular genetics and functional genomic approaches			
	Total	**22**	**23**	

Evaluation criteria:
1. Minor test 1 30%
2. Minor test 2 30%
3. Major test (end semester) 40%

Learning outcomes:
1. An integrated view on genetics, genomics and bioinformatics for deciphering molecular basis of phenotypes **(**Minor test 1, Minor test 2 and Major test**)**
2. An understanding on principles of forward mapping, classical reverse genetics and genomics powered high-throughput schemes for functional analyses of genomic sequences **(**Minor test 1, Minor test 2 and Major test**)**
3. Technical know-how on types of mutagenesis approaches, generation and application of gain- and loss-of-function mutants **(**Minor test 1, Minor test 2 and Major test**)**
4. Perspectives on contemporary genome editing methods for gene function analysis **(**Minor test 1, Minor test 2 and Major test**)**

Pedagogical Approach:
Lectures, tutorials supported by critical appraisal of original research articles, reviews, books and book chapters, hands-on-training and demonstration of online resources

Skill Set:
1. Rationalizing deployment of suitable strategies for gene discovery and gene functional analysis
2. Proficiency in use of relevant *in-silico* tools and online resources for functional characterization of genes
3. Generating mapping populations for trait mapping
4. Constructing of linkage maps using contemporary genotyping methods
5. Using natural populations for association mapping of traits
6. Using NGS data creatively for trait mapping
7. Applying MAS, MABC and MARS in breeding programmes
8. Designing and implementing mutagenesis screens
9. Generation of knock-out, knock-down, over-expression and genome edited mutant lines for activation or interference of target genomic sequences
10. Analysing mutant genotypes, phenotypes and endo-phenotypes
11. Innovating novel strategies for gene function analysis and characterization of genomic sequences

Employability:
1. Genotyping and sequencing companies
2. Agri-biotechnology, agri-genomics and seed companies
3. Law firms and knowledge processing organizations, IP management consultancy
4. Regulatory bodies and funding agencies

Materials:
Suggested readings (Representative)
Books

1. Sharples, F. (2020) Next Steps for Functional Genomics (Proceedings of a Workshop, National Academies of Sciences, Engineering, and Medicine; Division on Earth and Life Studies; Board on Life Sciences) National Academies Press (US); Washington (DC) ISBN-13: 978-0-309-67673-1
2. Varshney, R., Pandey, M., Chitikineni A. (2018) Plant Genetics and Molecular Biology: Advances in Biochemical Engineering / Biotechnology series number 164, Springer Nature, Switzerland, ISBN: 978-3-319-91312-4
3. Varshney, R., Roorkiwal, M., Sorells M. (2017) Genomic Selection for Crop improvement: New Molecular Breeding strategies for crop improvement. Springer Nature, Switzerland, ISBN-10: 3319631683, ISBN-13: 978-3319631684
4. Alonso J.,M., Stepanova, A.,N. (2015) Plant Functional Genomics: Methods and Protocols, Humana Press, ISBN 10: 1493924435, ISBN 13: 9781493924431
5. Grotewold, E. (2010) Plant Functional Genomics. Methods and Protocols, Humana Press, ISBN 13: 9781617373862
6. Meksem, K., Kahl, G. (2005) The Handbook of Plant Genome Mapping: Genetic and Physical Mapping, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, https://doi.org/10.1002/3527603514

Required texts:
Research articles, reviews on relevant topics, websites and relevant links as provided by the instructor in lectures and tutorials

Student responsibilities:
1. Class attendance
2. Study of course materials as specified by the instructor
3. Self-study

Course Reviewers:

1. **Professor Rajeev K Varshney**
   ML, FTWAS, FAAAS, FAAS, FCSSA, FASA, FNA, FASc, FNASc, FNAAS, Director, State Agricultural Biotechnology Centre, Director, Centre for Crop & Food Innovation, International Chair in Agriculture & Food Security, Food Futures Institute, Murdoch University, Australia

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