

K. K. WAGH COLLEGE OF AGRICULTURAL BIOTECHNOLOGY, NASHIK - 3.

(Affiliated to Mahatma Phule Krishi Vidyapeeth, Rahuri)

-: Syllabus :-

PB-357 : Biotechnology for Biotic and Abiotic Stress Tolerance 2+1

Theory:

Prospects & Perspective of Biotic & abiotic stress resistant plants, Genetics of host-pathogen interactions, Mechanism of plant resistance. Role of jasmonates and salicylic acid in systematic resistance induction on wounding. Insect pest resistance – Structural/morphological changes ; Protease and amylase inhibitors; polyphenol oxidases; peroxidases; lectins; chitinase; seed proteins; their limitations and significance in multi-gene pyramiding. Vertical and Horizontal resistance to pathogens. Hypersensitive host response (HRGP) and apoptosis in relation to plant defense. Virulence- Avirulence in host–pathogens interaction. Race specific Resistance Gene Analogues (RGAs). Pathogenesis related proteins – groups with examples (Glucanases; chitinases; osmotin, chitin binding proteins; thaumatin like proteins; micropeptidal defensins; phytoalexins) and their role. Role of Phenylalanine ammonia lyase, callose synthases, detoxification for pathogen resistance. RIP. Field testing of transgenic for fungal, bacterial, viral and pest resistance. Prospects and perspectives. Biopesticides- Developing antagonistic strains suitable for varying stress conditions. Biochemical basis of abiotic stresses namely osmotic (drought, salinity), temperature, heavy metals, air and water pollutants, synthesis and functions of proline and glycine betaine in stress tolerance interaction between biotic and abiotic stresses; stress adaptation. Reactive oxygen species and biotic and abiotic stress, antioxidants, enzymes defense system. Role of calcium, nitric oxide and salicylic acid in plant development. Molecular strategies for imparting tolerance against biotic and abiotic stress.

Practicals:

Meristem culture for virus elimination. Screening toxins and culture filtrate of fungi and bacteria for in vitro screening of host resistance, toxin bioassay, selection of toxin resistant calli/ cell suspension culture and its regeneration. Agro-bacterium-mediated transformation protocol and selection of transformed regenerated plants. Biocontrol: Testing antagonism of Trichoderma and Pseudomonas fluorescens- mass production agents. Measurement of water status of plants, determination of osmotic potential by vapour pressure and freezing point depression, Stress imposition and quantification,

Stress–stomatal conductance. Root- shoot signals-ABA and cytokinin effect on stomatal behavior, Heat tolerance and membrane integrity. chilling tolerance Galactolipase and free fatty acid levels as biochemical markers for chilling damage,

Reference Books :

1. Basra A. S. 1997. Stress Induced Gene Expression in Plants.Harwood Academic Publ.
2. Chessin M., De Borde, D. & Zipf, A. 1995. Antiviral Proteins in Higher Plants.CRC Press.
3. Crute, I. R., Burdon, J. J. & Holub, E. B. (Eds.). 1997. Gene-for-Gene Relationship in Host- Parasite Interactions. CABI.

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-: Syllabus :-

PB-358:

Biodiversity Conservation

2+1

Theory

Definition, Historical and geographical causes of diversity. Types of diversity- Genetic, Species and population diversity. Distribution of diversity in life forms. Ecological diversity and stability. Biodiversity and centers of origins of plant. Hot spots in India. Principles of conservation biology. Biosphere concept, Genetical and evolutionary principles of conservation. Collection Maintenance and conservation of biodiversity. Assessing and documenting of vulnerability and extinction of biodiversity; red list categories as per IUCN (International Union for the Conservation of Nature and Natural resources): Extinct, Extinct in the wild, Critically Endangered, Endangered, Vulnerable, Lower risk, Data deficient and Non Evaluated. Bio-village concept: *in situ* and *ex situ* conservation. Community level Gene banks, Utilization of biodiversity. Global biodiversity system. Intellectual Property Rights and legal concerns of Bio-resources. Biodiversity and human welfare.

Practicals:

Collection of ITK (Flora) Study of species composition in surrounding areas. Morphological description of plant parts. Collection of seeds of rare species of forest and medicinal plants. Study the microclimate and forest soils. List of important medicinal plants used in healthcare.

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-: Syllabus :-

PB-369: Introduction to Bioinformatics 1+2

Theory :

1. Introduction, Scope and application of Bioinformatics
2. Biological database, NCBI, ENSEMBL, EBI, EXPASY, Genbank ,AgriCola
3. Computer tutorial, introductory programming,
4. Computers and biology, online resources for bioinformatics,
5. Simple pair wise alignment, Induction to Blast , FASTA, BLAST searching, BLAST and PHI/PSI-BLAST,
6. Introduction to Gene expression and microarrays
7. Introduction Micro array data analysis
8. Introduction to protein structure and structural databases
9. Molecular visualization, Basics of Proteomics, Bioinformatics methods for studying proteins,
10. sequence alignment, Pairwise alignment Multiple sequence alignment tools for alignments (CLUSTALX, CLUSTAL W),
11. Concepts in Protein structure prediction,
12. Phylogeny, cladistics, and evolution, Phylogenetic trees

Practicals:

1. Find Agribiotechnology related research paper fromAgriCola
2. Find Research paper from NCBI-pubmed
3. Study of NCBI database with different parameters
4. Primary work on Entrez Home Search engine
5. Find out 5 Plant genome related Genes from different Plant Genome database with specific characters (3)
6. Study on Plant genome database
7. Data analysis of Rice genome with Nutritional valuable genes
8. Find out the present stress tolerance gene

9. Study on Introduction of BLAST

10. Analysis of Basic sequence analysis with BLAST

11. A biochemist suspects that P450 protein sequences are characterized by the following motif: FMFEGHDTTA

a. Use the ScanProsite -- select the Search Swiss-Prot with a PROSITE entry part of the form! -- or FPAT (*server down?*) programs to search for matches to this motif in SwissProt.

b. Take these hits and use PRATT to construct *regular expressions* characterising these sequences. *A brief explanation of what PRATT does can be found here.*

c. Search back into SwissProt with FPAT or ScanProsite using (some of) these patterns and see what hits are obtained.

i. Do these searches return all of the original sequences?

ii. What other sequences (if any) are identified by these patterns? Are these also P450 sequences?

d. You can also try IBM's TEIRESIAS program to generate patterns.

12. Take the set of P450 sequences that you have identified with the FMFEGHDTTA motif

a. Randomly divide it into a training set and a test set. (Set the ratio of training to test sequences as somewhere between 1:1 and 1:3.)

b. Use PRATT to learn a significant pattern for the training set.

c. Search into the test set (or the entire database if you can't do this) with that pattern.

d. Use your results to compute the sensitivity and specificity for your pattern.

13. Nucleotide Analysis check for vector contamination, BLAST similarity search, FASTA similarity search, whole genome search, pairwise alignment, ClustalW2 multiple sequence alignment and translation of DNA/RNA into protein.

14. Protein Structure

Topics include; introduction to nucleotide structures, introduction to protein structures and bioinformatics for protein structure prediction.

15. Genome Browsing Topics include; information on the two main genome browsers available Ensembl in the UK and UCSC Genome Bioinformatics in the USA.

16. Database Browsing Topics include; summary of the interfaces available for searching multiple databases and also a detailed explanation of the Sequence Retrieval System (SRS) provided by the EBI.

17. EBI Web Services Topics include: What are SOAP Web Services, where to get these from; how to use them and how to develop applications with these.

Reference books:

1. Elementry Bioinformatics by I. Khan, 2007 Pharma Book Syndicate, HYD.
2. Bioinformatics by A. Baxevanis, 2007, Panima ND.
3. Practical Bioinformatics by Bujnicki, 2008 Springer.
4. Bioinformatics By Polanski, 2008 Springer.
5. Structural Bioinformatics by Philips Bourne, Panima ND.
6. Bioinformatics by Dear P., 2008, Panima, ND.

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-: Syllabus :-

PB3610:

Bio Safety Issues

2+0

Theory

Definition, Biosafety concerns. Biosafety regulations in various countries. International agreements related to biosafety. Regulatory frame work of biosafety in India . Guidelines for recombinant DNA technology . Status, prospectus and concerns of GM crops, Biosafety of environment and human health. Guidelines for research in transgenic plants and drugs. Social and ethical issues; Biosafety issues related to genetically modified organisms (GMOs) by Biotechnology Consortium India Ltd. and Department of Biotechnology Govt. Of India.

Gene

contamination, Super weed and super pest.

Reference books:

1. Food Biotechnology by K.Shetty,2006, CRC publication.
2. Biotechnology expanding Horizon, B.D.Singh,2004, Kalyani Publishing, ND.
3. Plant Biotechnology by Chawla,2002, Oxford IBH, ND.
4. WEB sites
 - 1) [www. agbios.com](http://www.agbios.com),
 - 2) [www. dbt.nic.in](http://www.dbt.nic.in),
 - 3) [www. bcil.nic.in](http://www.bcil.nic.in),
 - 4) [www. moef.nic.in](http://www.moef.nic.in),
 - 5) [www. Igmoris.nic.in](http://www.Igmoris.nic.in).

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-: Syllabus :-

PB 4711

Advances in Plant Tissue Culture

0+4=4

Practical:

1. Sterilization and culturing of seeds, bulbs, leafs, stems, roots, suckers, and flower buds etc.
2. Seed germination, Embryo culture and embryo rescue after wide hybridization.
3. Meristem tip culture for virus elimination.
4. *In-vitro* pollination and Fertilization.
5. Cell suspension culture.
6. Organogenesis and embryogenesis.
7. Protoplast isolation and fusion (Somatic hybrid production).
8. Secondary metabolite production,
9. Somaclonal Variations,
10. Agrobacterium mediated gene transfer,
11. Gene transfer by biolistic method.

References:

1. An introduction to plant biotechnology by H. S. Chawala.
2. Biotechnology in crop improvement by H. S. Chawala, International book distributing co. Lukhnow.
3. Plant Biotechnology – Practical Mannual by C. C. Giri and Archana Giri, I. K. International Publishing house, Mumbai.
4. Biotechnology by B. D. Singh, Kalyani publishers.

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-: Syllabus :-

PB 4712 Techniques in Genetic Transformation 1+3=4

Theory:

Definition, introduction and history of genetic transformation, Need of genetic transformation, Agrobacterium mediated gene transfer Ti plasmid and Ri plasmid, Infection of wounded plants with Agrobacterium strains, Co cultivation, Leaf disc method, Advantages of Agrobacterium gene transfer. Direct gene transfer Particle gun/ microprojectile/biolistic method, Chemical method, Electroporation, Lipofection, Microinjection method, Macroinjection method, DNA delivery via growing pollen tubes, Direct DNA uptake by mature zygotic embryos, etc, Expression of induced genes by using selectable and scorable markers (Reporter genes). Application of genetic transformation in production of transgenic plants with various resistance characters and other improved qualities.

Practicals:

1. Induction of transformed Hairy roots using wild strains of *Agrobacterium rhizogenes* and production of secondary metabolites.
3. Induction of transformed shoots using wild specialized strains of *Agrobacterium tumifaciens* and production of secondary metabolites.
4. Introduction of foreign DNA into plants by using Particle bombardment/Microprojectile/ Biolistic method.
6. Transformation of Chloroplast genome in higher plants by using *Agrobacterium* and particle gun method.
7. Regeneration of transformed plants.
8. Study of transformed plants for regulated gene expression.

References:

1. An introduction to plant biotechnology by H. S. Chawala.
2. Biotechnology in crop improvement by H. S. Chawala, International book distributing co. Lukhnow.
3. Plant Biotechnology – Practical Mannual by C. C. Giri and Archana Giri, I. K. International Publishing house, Mumbai.
4. Genetic engineering and its applications by P. Joshi, Student Edition, Jodhpur.
5. Biotechnology by B. D. Singh, Kalyani publishers.

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-: Syllabus :-

PB 4713 PCR Techniques and its Application 1+3=4

Theory:

Introduction, History, Stages and components of the PCR, Working principle, Thermo stable DNA polymerases used in PCR, Primer design in PCR, Sensitivity of PCR, Variants of PCR: Inverse PCR, Anchored PCR, PCR for site directed mutagenesis, Asymmetric PCR for DNA Sequencing, RT-PCR, Overlap Extension PCR, Nested PCR, Touchdown PCR, Hot Start PCR, ARMS PCR, etc. Analysis of PCR products, Applications of PCR in biotechnology Study of DNA polymorphism using PCR, PCR & RAPD markers, PCR & VNTR loci, PCR & SSR loci, Molecular Mapping using PCR, Gene tagging using PCR, PCR for the conformity of the presence of transferred gene DNA fingerprinting using PCR, Gradient PCR.

Practicals:

1. Amplification of DNA.
2. Study of DNA polymorphism.
3. Cultivar identification using PCR,
4. Studying genetic diversity using PCR,
5. DNA fingerprinting by using PCR,
6. Screening of varieties for characterization,
7. Detection of transgene by using PCR,
8. RAPD, AFLP, SSR analysis.
9. Primer Designing

References:

1. PCR, second edition by C. R. Newton and A. Graham, Bios Scientific publishers.
2. Molecular Biology and biotechnology, Fourth edition by J. M. Walker and R. Rapley, Panima publishing house.
3. Biotechnology by B. D. Singh, kalyani publishers.
4. Elements of biotechnology by P. K. Gupta, Rastogi Publication.