Department of Plant Pathology
Faculty of Agricultural Sciences
University of the Punjab, Lahore

Course Outline

Programme	B.Sc. (Hons.) Agriculture (Plant Pathology) 4 Year program	**Course Code**	**PP-405**	**Credit Hours**	**2 + 1**
Course Title	**Introductory Molecular Plant Pathology**				

Course Introduction

This course provides an in-depth exploration of molecular techniques and their applications in plant pathology. It covers the molecular mechanisms of plant disease pathogenesis, host-pathogen interactions, and the genetics of virulence and resistance. Students will learn molecular approaches to control pathogens, including resistance gene engineering and the use of transgenic plants. Practical skills in DNA/RNA isolation, PCR, sequence analysis, and plant transformation will be developed. By combining theoretical knowledge with hands-on practice, the course prepares students to investigate and manage plant diseases using advanced molecular tools and techniques.

Learning Outcomes

On the completion of the course, the students will:

1. **Understanding Molecular Mechanisms in Plant Pathology**: Students will gain a comprehensive understanding of the molecular mechanisms underlying pathogenesis in plant diseases, including host-parasite interactions, biochemical mechanisms of pathogenesis, and signaling in plant disease development.
2. **Application of Molecular Techniques**: Students will be proficient in applying molecular techniques to investigate plant diseases, including DNA/RNA isolation, PCR reactions, hybridization, sequence analysis, and plant transformation. They will be able to design primers, conduct BLAST searches, align sequences, and perform sequence editing.
3. **Genetics of Pathogen Virulence and Host Resistance**: Students will understand the genetics of virulence in pathogens and resistance in host plants, including gene variability, co-evolution of hosts and pathogens, and the role of resistance genes. They will explore the molecular mechanisms of fungicide resistance and resistance gene engineering.
4. **Development of Disease Control Strategies**: Students will learn molecular approaches to control plant pathogens, including the use of cloned resistance genes, transgenic plants, RNA silencing, and quorum sensing. They will also study pre-existing structural and chemical defenses, as well as induced structural and biochemical defenses.
5. **Practical Skills in Molecular Plant Pathology**: Students will develop practical skills in molecular plant pathology, including methods for investigating plant diseases, library construction and screening, protein isolation, and the use of degenerate PCR for detecting plant disease resistance in crops. They will be familiar

with common molecular techniques and tools used in the field.

Course Content		Assignments/Readings
Week 1	**THEORY** **Unit-I** **Introduction to molecular techniques and their application** **1.1 Fundamental Molecular Techniques** 1.1.1. DNA/RNA Isolation and Purification 1.1.2. Polymerase Chain Reaction (PCR) 1.1.3. Hybridization Techniques 1.1.4. Sequence Analysis	**Reading (Theory + Practical)** Koshariya, A. K., Mahant, M. M., Afsana, C., & Reddypriya, P. (2023). Introduction to plants pathology. AG Publishing House (AGPH Books). Capote, N., Pastrana, A. M., Aguado, A., & Sánchez-Torres, P. (2012). Molecular tools for detection of plant pathogenic fungi and fungicide resistance. Plant pathology, 12, 151-202. Venbrux, M., Crauwels, S., & Rediers, H. (2023). Current and emerging trends in techniques for plant pathogen detection. Frontiers in Plant Science, 14, 1120968.
	Unit-I **1.2. Gene Manipulation** 1.2.1. Gene Cloning and Expression 1.2.2. Genome Editing 1.2.3. Functional Genomics and Proteomics **PRACTICAL** **Introduction to Molecular Approaches in Plant Pathology** o Overview of molecular techniques used in plant pathology o Importance and applications of molecular methods in disease investigation	
Week 2	**THEORY** **Unit-I** **1.3. Plant Transformation and Pathogen Control** 1.3.1. Plant Transformation Techniques	
	1.3.Plant Transformation and Pathogen Control 1.3.2. Pathogen Control Strategies **PRACTICAL** **Primer Design and BLAST Search** o Principles and considerations in primer design o Hands-on session on using BLAST (Basic Local Alignment Search Tool)	

<table>
<tr><td rowspan="3">Week 3</td><td><u>THEORY</u>
Unit-II
2.1.Molecular Mechanisms of Pathogenesis in Plant Diseases
2.1.1. Introduction to Plant Pathogenesis
○ Overview of Plant Diseases
○ Importance and impact on agriculture
○ Types of pathogens affecting plants
2.1.2. Molecular Basis of Pathogenesis
○ Introduction to molecular mechanisms involved
○ General process of colonization and infection</td><td rowspan="3">Reading (Theory)

Haq, I. U., Ijaz, S., & Khan, I. A. (Eds.). (2022). Phytomycology and molecular biology of plant pathogen interactions. CRC Press.

Hariharan, G., & Prasannath, K. (2021). Recent advances in molecular diagnostics of fungal plant pathogens: a mini review. Frontiers in Cellular and Infection Microbiology, 10, 600234.

Reading (Practical)

Groth-Helms, D., Rivera, Y., Martin, F. N., Arif, M., Sharma, P., & Castlebury, L. A. (2023). Terminology and guidelines for diagnostic assay development and validation: Best practices for molecular tests. PhytoFrontiers™, 3(1), 23-35.

Assignments (Theory)

Research Paper Review

Objective: Select a recent research paper focusing on a molecular mechanism in plant pathogenesis (e.g., virulence factors, host manipulation).

Task: Summarize the paper, emphasizing the identified molecular mechanism and its role in disease progression. Discuss how this mechanism contributes to understanding plant diseases and its potential applications in disease management.

Case Study Analysis

Objective: Analyze a case study of a prominent plant disease.

Task: Identify and explain the molecular interactions between the pathogen and host that contribute to disease development. Propose strategies for disease management based on the insights gained from the molecular mechanisms</td></tr>
<tr><td>Unit-II
2.2.2. Host-Pathogen Interactions
• Recognition and Evasion Mechanisms
○ Plant defense mechanisms
○ Strategies used by pathogens to evade detection
• Signaling Pathways in Disease Development
○ Role of signaling molecules in pathogenesis
○ Communication between pathogens and host cells
<u>PRACTICAL</u>
Alignment of Sequences and Sequence Editing
○ Techniques for sequence alignment and editing
○ Software tools for sequence analysis and manipulation</td></tr>
<tr><td></td></tr>
<tr><td rowspan="2">Week 4</td><td><u>THEORY</u>
2.2.3. Biochemical Mechanisms of Pathogenesis
• Virulence Factors
○ Types and functions of virulence factors
○ Mechanisms by which pathogens cause disease
• Host Manipulation and Disease Progression
○ How pathogens manipulate host physiology
○ Disease progression at the molecular level</td></tr>
<tr><td><u>THEORY</u>
2.2.4. Case Studies and Applications
• Case Studies in Molecular Pathogenesis
○ Examples of specific plant diseases and their molecular mechanisms</td></tr>
</table>

	○ Application of molecular understanding in disease management • **Future Directions and Research Trends** ○ Emerging technologies in studying plant pathogenesis ○ Challenges and opportunities in developing novel disease control strategies **PRACTICAL** **Open Reading Frames (ORFs) and Gene Prediction** ○ Understanding open reading frames in genomic sequences ○ Methods for gene prediction and annotation	discussed in the case study.
Week 5	**THEORY + PRACTICAL** **Group Discussion**	**Assignment (Practical):** **Primer Design and PCR Optimization** **Objective:** To design specific primers for the amplification of a target gene associated with a plant pathogen and optimize PCR conditions.
	THEORY + PRACTICAL **Quiz** **PRACTICAL** **DNA and RNA Isolation** ○ Techniques for isolating DNA and RNA from plant tissues ○ Optimization and troubleshooting in isolation method	
Week 6	**THEORY** **Unit III** **3.1. Molecular Biology of Host-Parasite Interaction** • **Introduction, importance and overview** to Host-Parasite Interactions • **Molecular Mechanisms in Host Recognition** ○ Recognition receptors in plants ○ Pathogen-associated molecular patterns (PAMPs) and their recognition • **Signal Transduction in Plant Defense** ○ Activation of defense pathways (e.g., MAPK cascades) ○ Production of defense-related hormones (e.g.,	**Reading (Theory + Practical)**: Ashapkin, V. V., Kutueva, L. I., Aleksandrushkina, N. I., Vanyushin, B. F., Teofanova, D. R., & Zagorchev, L. I. (2023). Genomic and epigenomic mechanisms of the interaction between parasitic and host plants. International Journal of Molecular Sciences, 24(3), 2647. **Assignment (Theory)**: Summarize the paper, focusing on the key findings related to recognition receptors and PAMPs. Discuss the implications of these findings for understanding plant-pathogen interactions and

	salicylic acid, jasmonic acid)	developing disease-resistant crops.
	THEORY **Unit III** **3.2. Biochemical Mechanisms of Pathogenesis** • **Virulence Factors and Effector Proteins** ○ **Virulence Factors**: Types and functions ○ **Effector Proteins**: Role in manipulating host physiology • **Host Manipulation and Disease Progression** ○ **Suppression of Host Defenses**: Mechanisms employed by pathogens ○ **Biochemical Progression**: Disease development at the molecular level **PRACTICAL** **Hybridization Techniques** ○ Introduction to nucleic acid hybridization methods ○ Practical demonstration of hybridization assays	***Reading:*** *Case Study: Dong, X. (1998). SA, JA, ethylene, and disease resistance in plants. Current opinion in plant biology, 1(4), 316-323.* ***Assignment:*** *Analyze the molecular mechanisms discussed in the case study, focusing on the types and functions of virulence factors.* **Objective**: Propose strategies to mitigate the effects of these virulence factors, considering the biochemical mechanisms involved in pathogenesis and host defense responses.
Week 7	**THEORY** **Unit IV** **4.1. Molecular Approaches to Control Pathogens** • **Introduction to Pathogen Control Strategies** • **Genetic Basis of Pathogen Resistance** • **RNA Interference (RNAi) as a Control Strategy** **4.2. Advanced Molecular Approaches** • **Genome Editing Techniques** • **Use of Plant Defense Genes** **PRACTICAL** **PCR Techniques: Basic PCR** ○ Fundamentals of polymerase chain reaction (PCR) ○ Performing basic PCR reactions in the lab	**Reading (Theory + Practical)**: Taliansky, M., Samarskaya, V., Zavriev, S. K., Fesenko, I., Kalinina, N. O., & Love, A. J. (2021). RNA-based technologies for engineering plant virus resistance. *Plants*, *10*(1), 82. Read about RNA interference (RNAi) and its applications in targeting pathogen genes for crop protection.
Week 8	**THEORY** **Unit V** **Genes and Diseases** • **Introduction to Genes and Diseases**	**Reading**: **Book (Theory)** Lugtenberg, B. (2016). Principles of plant-microbe interactions. Springer International Pu.

	• **Gene Variability in Hosts and Pathogens**	
	<u>THEORY</u> **UNIT VI** **Genetics of Virulence in Pathogens** • **Mechanisms of Pathogen Virulence** • **Resistance in Host Plants** **<u>PRACTICAL</u>** **PCR Techniques: Nested PCR** o Applications and setup of nested PCR for enhanced specificity o Hands-on practice with nested PCR protocols	
Week 9	**MID-TERM**	
Week 10	**<u>THEORY</u>** **UNIT VII** **Co-evolution of Hosts and Pathogens** • **Co-evolutionary Dynamics** • **Molecular Insights into Co-evolution**	**Assignment (Theory):** Exploring Co-evolution: Signaling and Transcription in Pathogen-Plant Interactions" **Assignment Description:** Propose a research project investigating how co-evolution shapes pathogen virulence, focusing on signaling pathways and MYB transcription factors in *Gibberella zeae*.
	<u>THEORY</u> **UNIT VIII** **Signaling in plant disease development** • **Signal Perception and Transmission** • **Signaling Networks in Disease Response** • **Manipulation of Signaling by Pathogens** **<u>PRACTICAL</u>** **PCR Techniques: Real-Time PCR (qPCR)** o Principles and advantages of real-time PCR (qPCR) Performing and analyzing qPCR assays	
Week 11	**<u>THEORY</u>** **UNIT IX** **Functional analysis of MYB transcription factors in *Gibberella zeae*** • **Overview of MYB Transcription Factors** • **Functional Roles in *Gibberella zeae*** • **Applications and Future Directions**	
	<u>THEORY</u> **UNIT X** **Molecular Mechanisms of Fungicide Resistance in Plant Pathogenic Fungi** • **Mechanisms of Fungicide Action** • **Genetic Basis of Fungicide Resistance** • **Molecular Adaptations in Fungal Populations**	**Reading:** **(Theory)** Baibakova, E. V., Nefedjeva, E. E., Suska-Malawska, M., Wilk, M., Sevriukova, G. A., & Zheltobriukhov, V. F. (2019). Modern fungicides: Mechanisms of action, fungal resistance and phytotoxic effects. Annual Research

	• **Management Strategies for Fungicide Resistance** **PRACTICAL** **Library Construction and Screening** ○ Methods for constructing and screening genomic and cDNA libraries ○ Hands-on experience in library construction technique	& Review in Biology, 32(3), 1-16.
Week 12	**THEORY + PRACTICAL** **Group Discussion**	**Assignment (Practical): Sequence Analysis and Phylogenetic Tree Construction** **Objective:** To analyze DNA sequences of a gene family involved in plant disease resistance and construct a phylogenetic tree to study evolutionary relationships.
	THEORY + PRACTICAL **Quiz**	
Week 13	**THEORY** **UNIT XI** **Pre-existing Structural and Chemical Defenses** • **Structural Defenses** • **Morphological Adaptations**	**Reading** **Book (Theory + Practical)**: Windham, M. T., Trigiano, R. N., & Windham, A. S. (2003). Plant pathology: concepts and laboratory exercises. CRC Press. **Research Paper**: Taliansky, M., Samarskaya, V., Zavriev, S. K., Fesenko, I., Kalinina, N. O., & Love, A. J. (2021). RNA-based technologies for engineering plant virus resistance. Plants, 10(1), 82.
	THEORY ***Chemical Defenses*** • **Secondary Metabolites** • **Role of phytochemicals in plant defense** • **Defense through lack of essential factors** • **Induced structural and biochemical defenses**	
Week 14	**THEORY** **UNIT XII** **Transgenic plants, Resistance gene engineering; vectors for gene engineering** • **Introduction to Resistance Gene Engineering Vectors for Gene Engineering** • **Applications and Strategies in Resistance**	**Assignment:** Designing the Future: Genetic Engineering for Plant Disease Resistance **Description:** Prepare a research proposal focusing on genetically

	Gene Engineering **THEORY** **Delivering genes to the plant, the use of cloned resistance genes** • **Introduction to Gene Delivery Methods** • **Cloned Resistance Genes: Types and Functions Techniques for Gene Delivery to Plants Applications and Challenges in Cloned Resistance Gene Deployment** **PRACTICAL** **Protein Isolation and Analysis** o Techniques for protein extraction from plant tissues o Analysis methods such as SDS-PAGE and Western blotting	engineered plants with enhanced disease resistance, utilizing resistance gene engineering, vectors for gene delivery, and cloned resistance genes.
Week 15	**THEORY** **UNIT XIII** **Quorum sensing** • **Introduction to Quorum Sensing** • **Mechanisms of Quorum Sensing** • **Quorum Sensing in Microbial Communities** • **Applications and Implications of Quorum Sensing**	**Reading (Theory)** Miao, B., Han, Y., Gao, M., Yu, L., Ma, W., Chen, Z., ... & Liu, S. (2024). Global Trends in and Hotspots of Bacterial Quorum Sensing: A Bibliometric Analysis for the Period 2012–2022. Integrative Medicine in Nephrology and Andrology, 11(2), e23-00026.
	THEORY **UNIT XIV** **Programmed cell death** **PRACTICAL** **Plant Transformation Techniques** o Overview of plant transformation methods o Practical demonstration of plant transformation techniques	**Reading (Theory + Practical)** Ali, M. S., Hajam, A. H., Suhel, M., Prasad, S. M., & Bashri, G. (2023). The Dual Role of Reactive Oxygen Species as Signals that Influence Plant Stress Tolerance and Programmed Cell Death. In: *Reactive Oxygen Species: Prospects in Plant Metabolism* (pp. 161-177). Singapore: Springer Nature Singapore.
Week 16	**REVISION/TEST (THEORY + PRACTICAL)** **PRACTICAL** **Project Presentation and Review** o Presentation of projects or research conducted during practical sessions o Review and discussion of practical outcomes and findings	
	FINAL-TERM	
Textbooks and Reading Material		

Suggested Readings

BOOKS

- Koshariya, A. K., Mahant, M. M., Afsana, C., & Reddypriya, P. (2023). Introduction to plants pathology. AG Publishing House (AGPH Books).
- Haq, I. U., Ijaz, S., & Khan, I. A. (Eds.). (2022). Phytomycology and molecular biology of plant pathogen interactions. CRC Press.
- Ali, M. S., Hajam, A. H., Suhel, M., Prasad, S. M., & Bashri, G. (2023). The Dual Role of Reactive Oxygen Species as Signals that Influence Plant Stress Tolerance and Programmed Cell Death. In: Reactive Oxygen Species: Prospects in Plant Metabolism (pp. 161-177). Singapore: Springer Nature Singapore.
- Desi, L. 2007. Molecular Plant Pathology. Paragon International.
- Devi, P. 2005. Principles and Methods of Plant Molecular Biology, Biochemistry, Biotechnology and Genetics. Student Edition, India.
- Dickinson, M. 2003. Molecular Plant Pathology. NIOS Scientific Publishers. 273 pp.
- Gurr, S.J., M.J. McPherson and D.J. Bowles. 1992. Molecular plant Pathology: A Practical Approach. IRC Press at Oxford University Press. 328 pp.
- Hafeez, F., Y. Zafar and A. M. Khalid. 2005. Modern Techniques in Biotechnology. A Theoretical Manual. NIBGE, Faisalabad.
- Molecular Plant-Microbe Interaction by Kamal Bouarab, Normand Brisson and Fouad Daayf Molecular Plant Pathology by M. Dickinson, 2003. BIOS Scientific Publisher, Tylor and Francis Group

Journal Articles/ Reports

- **Li, J., Ai, M., Hou, J., Zhu, P., Cui, X., & Yang, Q. (2024). Plant–pathogen interaction with root rot of Panax notoginseng as a model: Insight into pathogen pathogenesis, plant defence response and biological control. Molecular Plant Pathology, 25(2), e13427.**
- **Venbrux, M., Crauwels, S., & Rediers, H. (2023). Current and emerging trends in techniques for plant pathogen detection. Frontiers in Plant Science, 14, 1120968.**
- **Hariharan, G., & Prasannath, K. (2021). Recent advances in molecular diagnostics of fungal plant pathogens: a mini review. Frontiers in Cellular and Infection Microbiology, 10, 600234.**
- **Ashapkin, V. V., Kutueva, L. I., Aleksandrushkina, N. I., Vanyushin, B. F., Teofanova, D. R., & Zagorchev, L. I. (2023). Genomic and epigenomic mechanisms of the interaction between parasitic and host plants. International Journal of Molecular Sciences, 24(3), 2647.**
- **Taliansky, M., Samarskaya, V., Zavriev, S. K., Fesenko, I., Kalinina, N. O., & Love, A. J. (2021). RNA-based technologies for engineering plant virus resistance. Plants, 10(1), 82.**
- **Baibakova, E. V., Nefedjeva, E. E., Suska-Malawska, M., Wilk, M., Sevriukova, G. A., & Zheltobriukhov, V. F. (2019). Modern fungicides: Mechanisms of action, fungal resistance and phytotoxic effects. Annual Research & Review in Biology, 32(3), 1-16.**

- **Taliansky, M., Samarskaya, V., Zavriev, S. K., Fesenko, I., Kalinina, N. O., & Love, A. J. (2021). RNA-based technologies for engineering plant virus resistance. Plants, 10(1), 82.**
- **Dong, X. (1998). SA, JA, ethylene, and disease resistance in plants. Current opinion in plant biology, 1(4), 316-323 (CASE STUDY).**
- **Capote, N., Pastrana, A. M., Aguado, A., & Sánchez-Torres, P. (2012). Molecular tools for detection of plant pathogenic fungi and fungicide resistance. Plant pathology, 12, 151-202.**

Note:

- It is preferable to use latest available editions of books. Mention the publisher & year of publication.
- The References/ bibliography may be in accordance with the typing manual of the concerned faculty/subject. Preferably follow APA 7th Edition publication manual.

Teaching Learning Strategies

1. **Active Engagement:** Encourage active participation and interaction among students through discussions, group activities, and problem-solving tasks.

2. **Differentiated Instruction:** Tailor teaching methods and materials to accommodate diverse learning styles, abilities, and interests of students.

3. **Assessment for Learning**: Use formative assessment techniques to monitor student progress, provide timely feedback, and adjust instructional approaches accordingly.

4. **Integration of Technology**: Incorporate educational technologies to enhance learning experiences, facilitate collaboration, and provide access to resources beyond the classroom.

Assignments: Types and Number with Calendar

Assignments (Theory)

1. Summarize the paper, focusing on the key findings related to recognition receptors and PAMPs. Discuss the implications of these findings for understanding plant-pathogen interactions and developing disease-resistant crops.
   Type: Research Paper Review
   Number: First assignment
2. Understanding and Countering Virulence Factors: Analyzing Molecular Mechanisms and Proposing Defense Strategies
   Type: Case Study Analysis
   Number: Second assignment
3. Exploring Co-evolution: Signaling and Transcription in Pathogen-Plant Interactions
   Type: Research Proposal
   Number: Third assignment
4. designing the Future: Genetic Engineering for Plant Disease Resistance
   Type: Project Proposal

Number: Final assignment

Assignments (Practical)

1. Assignment 1: Primer Design and PCR Optimization (Type: Laboratory Practical)
2. Assignment 2: Sequence Analysis and Phylogenetic Tree Construction (Type: Bioinformatics and Data Analysis)

Sr. No.	Elements	Weightage	Details
1.	Midterm Assessment	35%	Written Assessment at the mid-point of the semester.
2.	Formative Assessment	25%	Continuous assessment includes: Classroom participation, assignments, presentations, viva voce, attitude and behavior, hands-on-activities, short tests, projects, practical, reflections, readings, quizzes etc.
3.	Final Assessment	40%	Written Examination at the end of the semester. It is mostly in the form of a test, but owing to the nature of the course the teacher may assess their students based on term paper, research proposal development, field work and report writing etc.