University of Zagreb Faculty of Agriculture
Svetošimunska cesta 25, 10000 Zagreb
Phone: +385 (0)1 2393 777
E-mail: dekanat@agr.hr
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Molecular methods in plant pathology (146071)

Course coordinator

Prof. Edyta Đermić, PhD

Course description

Module is presenting facts on basic molecular techniques that are used in plant pathology intended for identification of plant pathogens (e.g. isolation and electrophoretic analysis of biological macromolecules, hybridization, diverse PCR techniques as well as serological techniques based on immunofluorescence). Students will participate in implementation of selected diagnostic protocols in molecular laboratory (PCR protocol for molecular detection of phytopathogenic bacterium Erwinia amylovora causing fire blight in apple and pear; identification of phytopathogenic virus by means of isolation of RNA and its electrophoretic analysis in agarose gel through "submarine" electrophoresis). Within this module students will gain basic knowledge on universal molecular diagnostic techniques as well as on equipment (accessories, utensils, appliances), reagents necessary for specified molecular protocols and required safety precautions essential for working in molecular laboratories in general.

ECTS: **3.00**

Teaching hours: 30
Lectures: 13
Auditory exercises: 2
Laboratory exercises: 5
Practicum: 4
Seminar: 6

Lecturer

- Prof. Edyta Đermić, PhD

Associate teacher for exercises

- Prof. Edyta Đermić, PhD
- Assoc. Prof. Snježana Topolovec-Pintarić, PhD
- Prof. Darko Vončina, PhD

Grading

Sufficient (2): 60 %
Good (3): 70 %
Very good (4): 80 %
Excellent (5): 90 %

Conditions for obtaining signature

Regular attendance and seminar presentation.

Description

Oral exam.

Type of course

- Graduate studies / Environment, agriculture and resource management (Elective course, 4 semester, 2 year)

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General competencies

Students gain knowledge about molecular techniques indispensable in detection of important groups of plant pathogens and in analysing their diversity. They acquire knowledge about the functioning of phytopathological laboratories and skills necessary for organizing the work in molecular laboratory. Competence in working in accordance with the diagnostic laboratory protocols as well as in obtaining, preparation and using of reagents, accessories and laboratory equipment is gained through the work in molecular phytopathological laboratory.

Types of instruction

- Lectures
  (both oral and e-learning)
- Auditory Exercises
  theoretical preparations for practical work in diagnostics
- Laboratory practice/exercises
  practical work in laboratory for molecular diagnostics.
- Practicum
  practical team work in order to prepare reagents for diagnostics
- Seminars
  reading of contemporary scientific articles from the field of molecular methods (based on the expressed student interest), critical thinking, commenting and writing on the topic together with presentation of gained knowledge.

Learning outcomes

Learning outcome	Evaluation methods
Acceptance of basic knowledge on most important universal molecular techniques used in plant pathology (A1)	Seminar, final exam
Achieving of skills important for decision making on electing of techniques needed to be performed in order to diagnose important plant pathogens (A1, B3, C3)	Seminar, final exam
Capability development in problem analysis in plant pathogen molecular diagnostics and synthesis of solutions (B3, C3)	Seminar, final exam
Experience acquisition in work on molecular diagnostics laboratory protocols based on technical, personal and interpersonal (solo and team work) as well as on systemic competencies applicable to work in diagnostic laboratory after graduation (A1, B3, C3)	Seminar, final exam
Development of capability for independent reading of original scientific articles, writing and presenting of seminar and presentation of gained knowledge (B3, C3)	Seminar, final exam

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Working methods

Teachers' obligations

All teaching materials are prepared and organized by the teachers. All lectures are available in Moodle system as well as tools for bidirectional communication students-teachers. On the same platform there are all information related to the course as well as calendar of major events in the module.

Students' obligations

Attending lectures, exercises and seminars is mandatory but students participate in learning through the system for e-learning, too. Students with more abscences from classes than allowed by official regulations lose possibility for exam accession. The same is for the attendance at exercises and seminar sessions.

Methods of grading

Evaluation elements	Maximum points or Share in evaluation	Grade rating scale	Grade	Direct teaching hours	Total number of average student workload	ECTS
Class attendance (P13+V(LAB)11)				24	24	
Seminar (S6; preparation+presentation)				6	18	0,6
Oral exam	100 %	0-59 % 60-70 % 71-80 % 81-90 % 91-100 %	Insufficient (1) Sufficient (2) Good (3) Very good (4) Excellent (5)		48	2,4
Total	100 %	0-100 %	1-5	30	90	3

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Evaluation elements	Description	Deadline	Recoupment
Attendance at seminars	on every session students are signed	before exam accession	student with more absences than allowed by official regulations lose possibility for exam accession
Activity in class (seminars, lectures, exercises, e-discussions)	Students can participate in discussions at seminars, actively participate in the work of teams and to be active in the exercises and practical work. Special activity in the discussions at the lecture, seminars and e-discussions could be scored, too.	before exam accession	
Attendance at exercises	on every session students are signed	before exam accession	students with more absences than allowed by official regulations lose the ability to apply for the exam
Oral exam	students answer questions	an exam accession	student's knowledge and understanding of the subjects covered in lectures, exercises and seminars are examined

Weekly class schedule

1. Introduction to modul content. Laboratory equipment in molecular biology L, Lab - Introducton to modul content. Introductory presentation of molecular laboratory equipment and accessories with usage instructions and safety precautions. Details on equipment needed for polymerase chain reaction (PCR) protocols realization and visualisation of their results (thermocycler, electrophoretic appliances, UV-transiluminator, gel documentation systems). Preparation of equipment, accessories and reagents for molecular analysis (weighing, calculating of required concentrations, dilutions preparation, suspending of primers and other concentrates).
2. Introduction to modul content. Laboratory equipment in molecular biology L, Lab - Introducton to modul content. Introductory presentation of molecular laboratory equipment and accessories with usage instructions and safety precautions. Details on equipment needed for polymerase chain reaction (PCR) protocols realization and visualisation of their results (thermocycler, electrophoretic appliances, UV-transiluminator, gel documentation systems). Preparation of equipment, accessories and reagents for molecular analysis (weighing, calculating of required concentrations, dilutions preparation, suspending of primers and other concentrates).
3. Basic molecular techniques in plant pathology L, E, Pe-L - General molecular methods in plant pathology - intoduction. Basics of serological diagnostical IF protocol (immunofluorescence). PCR method. Specificity of different PCR protocols. Electrophoretic techniques used in analysis off different biological macromolecules (nucleic acids, etc.). Methods of molecular hybridization based on using of different types of molecules.
4. Basic molecular techniques in plant pathology L, E, Pe-L - General molecular methods in plant pathology - intoduction. Basics of serological diagnostical IF protocol (immunofluorescence). PCR method. Specificity of different PCR protocols. Electrophoretic techniques used in analysis off different biological macromolecules (nucleic acids, etc.). Methods of molecular hybridization based on using of different types of molecules.
5. Basic molecular techniques in plant pathology L, E, Pe-L - General molecular methods in plant pathology - intoduction. Basics of serological diagnostical IF protocol (immunofluorescence). PCR method. Specificity of different PCR protocols. Electrophoretic

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techniques used in analysis off different biological macromolecules (nucleic acids, etc.). Methods of molecular hybridization based on using of different types of molecules.
6. Methods for DNA and RNA isolation L - Protocols for nucleic acids isolation.
7. Methods for DNA and RNA isolation. PCR reaction. L+Lab - Protocols for nucleic acids isolation. Preparations for implementing procedure of polymerase chain reaction (PCR). Implementation of diagnostic protocol of polymerase chain reaction (PCR) in viral plant pathogen diagnostics.
8. PCR reaction Lab - Preparations for implementing procedure of polymerase chain reaction (PCR). Implementation of diagnostic protocol of polymerase chain reaction (PCR) in viral plant pathogen diagnostics.
9. Electrophoretic analysis of nucleic acids Lab - Implementation of „submarine“ horizontal electrophoresis of plant pathogen's nucleic acids in agarose gel.
10. Applications of molecular methods in diagnostics of most important plant pathogens L, E - Implementing diagnostic protocol of PCR in bacterial plant pathogen. Molecular diagnostics of bacterium Erwinia amylovora based on amplification of DNA fragments.
11. Advantages of molecular methods in plant pathogens diagnostics L - Comparation of general and advanced molecular diagnostic techniques vis-a-vis classical diagnostic techniques in plant pathology. Analysis of advantages and suitability of molecular diagnostic methods vis-à-vis classical techniques in terms of real obstacles that appear during implementation of molecular protocols (rapidity, precision, simultaneous analysing of large number of samples, storage/analysis of large set of results (data) obtained).
12. Advantages of molecular methods in plant pathogens diagnostics L - Comparation of general and advanced molecular diagnostic techniques vis-a-vis classical diagnostic techniques in plant pathology. Analysis of advantages and suitability of molecular diagnostic methods vis-à-vis classical techniques in terms of real obstacles that appear during implementation of molecular protocols (rapidity, precision, simultaneous analysing of large number of samples, storage/analysis of large set of results (data) obtained).
13. Seminar S, Pe-L - Selection of topics (current topics related to mol. methods in plant pathology) that will be elaborated by students and on which they will prepare presentation using the recent literature (topics elected will be mainly based on the expressed student interest). Presentations of seminar topics that have been revised by the lecturer. Group discussion.
14. Seminar S, Pe-L - Selection of topics (current topics related to mol. methods in plant pathology) that will be elaborated by students and on which they will prepare presentation using the recent literature (topics elected will be mainly based on the expressed student interest). Presentations of seminar topics that have been revised by the lecturer. Group discussion.
15. Seminar S, Pe-L - Selection of topics (current topics related to mol. methods in plant pathology) that will be elaborated by students and on which they will prepare presentation using the recent literature (topics elected will be mainly based on the expressed student interest). Presentations of seminar topics that have been revised by the lecturer. Group discussion.

Obligatory literature

1. Lectures in the form of multimedia presentations (available for students on Merlin e-platform)
2. Klement Z, Rudolph K, Sands DC (1990) Methods in Phytobacteriology. Akadémiai Kiadó, Nyomd Vàllalat, Budapest. (selected chapters)
3. Khan, A.J., Dijkstra, J. (2006). Handbook of Plant Virology. New York, London, Oxford: Food Products Press. (selected chapters)
4. Agrios, G.N., (2005). Plant Pathology . -5th ed., Elsevier Academic Press. (selected chapters)

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Recommended literature

1. Dimmock N, Easton A, Leppard K (2007) Introduction to modern virology, Wiley-Blackwell. (selected chapters)
2. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (2002) Short protocols in molecular biology: A Compendium of methods from current protocols in molecular biology. Wiley. (selected chapters)
3. Paterson RRM, Bridge PD (1994) Biochemical techniques for filamentous fungi, CAB International. (selected chapters)
4. Juretić N (2002): Osnove biljne virologije, Sveučilište u Zagrebu i Školska knjiga, Zagreb.

Similar course at related universities

- Toolbox molecular biology, University of Wageningen, Netherlands
- Applied Molecular Microbiology, University of Wageningen, Netherlands
- Phytopathological diagnosis and biotechnology, University of Bari, Italy