Universitat de Lleida

DEGREE CURRICULUM

PLANT BIOTECHNOLOGY

Coordination: BASSIE , LUDOVIC

Academic year 2022-23

PLANT BIOTECHNOLOGY 2022-23

Subject's general information

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<tr><th>Degree</th><th>Course</th><th>Character</th><th>Modality</th></tr>
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<tr><td>Course number of credits (ECTS)</td><td>6</td></tr>
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<tr><th>Activity type</th><td>PRALAB</td><td>TEORIA</td></tr>
<tr><th>Number of credits</th><td>2.5</td><td>3.5</td></tr>
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<tr><td>Coordination</td><td>BASSIE , LUDOVIC</td></tr>
<tr><td>Department</td><td>CROP AND FORESTRY SCIENCES</td></tr>
<tr><td>Teaching load distribution between lectures and independent student work</td><td>60 hores presencials
90 hores no presencials</td></tr>
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PLANT BIOTECHNOLOGY 2022-23

Teaching staff	E-mail addresses	Credits taught by teacher	Office and hour of attention
BASSIE , LUDOVIC	ludovic.bassie@udl.cat	9,5	
CAPELL CAPELL, MARIA TERESA	teresa.capell@udl.cat	3	
CHRISTOU , PAUL	paul.christou@udl.cat	1	

Subject's extra information

The Plant Biotechnology course is a core subject that is taught during the first semester of the third year of the Degree of Biotechnology. The teaching load for this course is 6 credits which are distributed between theoretical lectures and laboratory practices representing 3.5 and 2.5 credits respectively.

The course is divided into 35 theoretical sessions of 50 minutes (including a video session on the theme of 'molecular farming') and 25 hours for conducting a series of laboratory practices. The course introduces students to the concepts of plant biotechnology and it is based on two main lines: molecular biotechnology and applied biotechnology.

Molecular biotechnology is considered fundamental for its contribution of theoretical notions. Here the main goal is to know and to understand the basis of some aspects of molecular mechanisms and organization that are relevant for generating successfully genetically engineered plants.

Molecular biotechnology part focuses on:

- Gene structure and (molecular) mechanisms of gene expression at RNA level.

- Molecular tools and the main plant transformation methods.

To develop the concept of the subject, **applied plant biotechnology** refers to various plants and crop species genetically engineered which have essential roles at economical level in agriculture, food industry and pharmaceutical industry, and more recently, in the bio-industry fuels. This part focuses on plant Molecular Farming and metabolic pathway engineering.

This course requires a sufficient basis in biochemistry, cell biology, molecular genetics and genetic engineering, among others.

Learning objectives

The main objectives of the course are to provide to the students the technical basis of molecular biotechnology in plants and to provide the most relevant conceptual bases of plant biotechnology. Part of the program is devoted to plant genetic engineering. It covers all relevant aspects which are applied to the pharmaceutical industry and agribusiness. It is intended that, once completed the course, the student has assimilated the theoretical and methodological bases which enable himself to access and understand the new discoveries and developments.

Students who pass the course must achieve knowledge of:

1 - Some aspects of molecular biology in plant cells.

2 - The molecular tools used in plant biotechnology.

3 - The different plant transformation systems.

4 - The applications of plant biotechnology.

5 - The key mechanisms involved in gene expression at RNA level, and the methods of analysis to study RNA expression.

6 - The use of plants as bio-factories.

7 - The strategies for optimizing the production of recombinant proteins in plants.

8 - The alternative use of plants by modifying endogenous metabolic pathways and / or by introducing a new metabolic pathway.

Competences

GC1 Being able to selectively search for and use sources of information necessary to achieve the training objectives.

GC3 Working in a team, with a multidisciplinary vision and with the ability to make a rational and efficient distribution of tasks among team members.

GC4 Knowing and adequately using the scientific and technical vocabulary of the different areas of Biotechnology.

GC5 Working in the laboratory applying criteria of quality and good practice.

GC11 Acquiring criteria for choosing the most appropriate analytical techniques for each specific practical case.

CE30 To know the technological processes based on the use of living beings and their optimization strategies.

CE32 To know the use of animal, plant and microbial cells in biotechnological processes.

CE34 Be able to design the protocol of a specific biotechnological process with the necessary practical requirements to carry it out and its evaluation parameters.

CE35 To know how a biotechnology laboratory works and be able to work in it.

CE44 To know the main fields of application of biotechnology and acquire basic training in some of them.

Subject contents

The program is structured in two parts. The first part '**Molecular Plant Biotechnology- Methods of Plant transformation'** explains the concepts, techniques and terminology of the process leading to the generation of genetically engineered plants. This part essentially includes the objectives of knowledge 1, 2, 3 and 5 from the program. The second part **'Applied Plant Biotechnology'** describes how the use of transgenic plants may have an important role at economical level in agriculture, in medicine and in food industry. This part meets the objectives 4, 6, 7 and 8, although that the generalist objectives of knowledge 1 and 2 are covered.

Chapter I. Molecular Plant Biotechnology- Methods of Plant transformation

Theme 1. Introduction

Brief history of genetic engineering. Basic features of plant biotechnolog. The tools used in plant biotechnology.

Theme 2. Gene structure and features of gene transcription

Gene structure. Numbers of genes in plant genome. Expressed sequence tag (ESTs). Types of homologous sequences. Overview of basal transcription. Promoter structure (Concept of *Cis/ Trans* acting elements; Core promoter elements). RNA processing. Regulation of transcription. Untranslated regions (UTRs).

Theme 3. Main molecular tools used in plant biotechnology

Promoters used in plant biotechnology. Selectable markers: genes of selection. Reporter genes: screenable marker

Theme 4. Plant transformation-1

Generalities. Protoplast electroporation. *Agrobacterium*-mediated transformation: Characteristics of Agrobacterium tumefaciens. Characteristics of Ti-Plasmid. Process of natural transformation. Binary vector system: the modified Ti plasmid. T-DNA integration into the plant chromosome. Choice of plant tissues for infection. Infection and co-culture.

Thema5. Plant transformation-2

Plant transformation via particle bombardment. Principle: development of a method for transferring genes by using a gene gun. Physical and chemical principles of particle bombardment. Instrumentation. Biological principles of particle bombardment.

Theme 6. Methods for analyzing the presence and integration of the transgene

Genomic DNA isolation from plant tissues. Southern blot analysis: Genomic DNA digestion. Techniques of nucleic acid hybridization; Transfer method. Hybridization with specific probes: labeling systems. Pre-hybridization and hybridization. Sequential washes of stringency and Probe detection. Southern blot interpretation.

Theme 7. Targeted genome editing with the CRISPR-Cas technology

CRISPR History and background. CRISPR in the lab-a practical guide: Overview. The principle of CRISPR/Cas9-mediated gene disruption. Knock-out: To disrupt the gene of interest (via Insertions / Deletions). To edit / modify the endogenous genome via Homology Directed Repair. Off-target effects and Cas9 nickase. Expanded uses of the CRISPR system for genome manipulation.

Chapter II. Applied Plant Biotechnology: Applications of Plant Biotechnology in Industry and Agriculture

Theme 8. Plant molecular farming

Definition of plant molecular farming. Plants as bioreactors. Comparison of production systems. The beginning of molecular farming. Advantages of plant molecular farming. Types of recombinant proteins produced in plants: proteins as pharmaceuticals, recombinant antibodies, recombinants subunit-vaccines, other proteins of medical or industrial relevance. Type of plant-expression hosts: tobacco production systems (nuclear transgenic plants and transplastomic plants), cereals, legumes, fruits and vegetables. Edible vaccines.

Theme 9. Strategies in molecular farming

Strategies in gene construct: product targeting. Post-translational modifications: N-glycosylation in plants. The humanization of glycoproteins produced in plants. Seed protein storage organelles: protein bodies and protein storage vacuoles. Purification

strategies.

Theme 10. Metabolic pathways engineering in plants

Applications of metabolic pathway engineering. Strategies for metabolic pathway engineering. The antisense technology. Case of the Amflora potato. RNA interference pathways: transcriptional and post-transcriptional gene silencing. The use of siRNA strategies. Co-suppression: gene silencing induced by transgene overexpression. Case of the chalcone synthase co-suppression in petunia. Carotenoid pathway engineering.

Tema 11. Bt technology

Bt generalities. Mechanism of action (specificity; target insects). *Topical* use of Bt: development of Bt topical pesticides. The development of Bt transgenic crops.

Theme 12. Interesting examples of commercial uses of plant biotech. GMO database. Overview of GMO legislation

Finally, knowledge and ideas explained in the themes 2,3,4,5, 6 and the concepts discussed in the second chapter will be reinforced with a session of problems during the laboratory practices.

Program of practices

The program from laboratory practices is focusing on the **first steps** involved in the methodology of a Southern blot analysis (DNA blot analysis). During the process of production of transgenic plants it is required to monitor at molecular level if plants are transformed. Southern blot analysis is the best method for this purpose. As well this technique provides valuable information on transgene integration into the host genome. This information is essential for:

- The selection of the most suitable parental line for producing the next generation (presence of complete cDNA, low number of insertions, no segregation)

- The study of the structure and organization of transgenic loci

Students are organized by subgroups of two individuals among a group of 10 persons per session. Each session of laboratory practices requires 5 consecutive days of work.

Objectives of the practices

- To extract DNA from plant tissues.

- To learn to prepare and manage with electrophoresis agarose gel.

- To use specific restriction enzyme to digest the isolated DNA.

- To identify the digested DNA samples.

- To be able to interpret results from DNA and RNA blot analysis selected from research articles: identification of the transformed plants; analysis of transgene integration the pattern and gene expression analysis.

- To use and analyse DNA sequences obtained from database and sequencing results.

Program content:

-Day1. DNA extraction from rice plant

Using the NucleoSpin® Plant II kit (Macherey-Nagel)

-Day2. DNA quantification and digestion

DNA quantification by spectrophotometry using the NanoDrop device.

Digestion of plant genomic DNA by using a Master mix procedure.

-Day3. Amplification of the transgene by PCR

Calculations for dilutions and master mix preparation. Assembly of components.

Interpretations of several examples of Southern blot analysis.

-Day4. Electrophoresis and interpretations of molecular analyses

Preparation and casting of agarose gel in electrophoresis unit. Sample preparation and loading (gDNA; digested products; PCR products).

Interpretation of the results.

Brief evaluation of the cost of the lab sessions.

-Day 5. *In-silico* analysis of DNA sequences

Different types of DNA sequences (resulting from sequencing reactions/mRNA homologous/ plasmid vector) are analyzed with various software: Notepad++; FinchTV; ApE plasmid editor; MegaX.

Laboratory report

The members of each subgroup will write a lab report that reflects the work done during the 5 days of practices. The report will be organized in a daily format describing: the objective(s) of the day; the experiment(s); the process of the methodology indicating the important and critical steps/ points; the details of the calculations and the volumes used; the unexpected problems/mistakes and the interpretation of the results.

Methodology

Tipus Activitat	Descripció resumida de l'activitat (Títol de tema o activitat pràctica)	Dedicació (hores)
TEO	**Tema 1. Introducció**	2
TEO	**Tema 2. Estructura dels gens i característiques de la transcripció de gens**	3
TEO	**Tema 3. Les principals eines moleculars en biotecnologia vegetal**	3
TEO	**Tema 4. Transformació de les plantes-1**	3
TEO	**Tema 5. Transformació de les plantes-2**	4
TEO; *PRO*	**Tema 6. Mètodes d'anàlisis de la presencia i de la integració del transgen**	4
TEO	**Tema 7.Targeted genome editing with the CRISPR-Cas technology**	2
TEO	**Tema 8. El Molecular Farming**	4
TEO	**Tema 9. Les estratègies en el Molecular Farming**	2
TEO	**Tema 10. L'enginyeria de les vies metabòliques en plantes**	4

TEO	**Tema 11. Bt technology**	3
TEO	**Tema 12- Exemples interessants d'usos comercials de la biotecnologia vegetal. Bases de dades d'OMGs. Visió general de la legislació dels OGMs.**	1
PLB	**Extracció d'ADN d'arròs**	5
PLB	**Continuació de protocol del dia1- Digestió**	5
PLB	**Dia3- PCR i preparació del gel d'agarosa**	5
PLB	**Dia4. Electroforesi i interpretació d' anàlisis moleculars**	5
PRO	**Dia5. Anàlisi *In-silico* de seqüencies** (sala d'informatica)	5

TEO: teoría; PRO: problemas; PLB: pràctiques de laboratori

Type of activity	**Description**	**Classroom Student work Activitat presencial Alumne**		**Student Work Outside of the classroom**		**Evaluation**	**Total Time**
		Objectives	**Hours**	**Student work**	**Hours**	**Hours *Weight%***	**Hours**
Lectures	Lecture (Class. Large group)	Explanation of the main concepts	35	Study: Learn, understand and synthesize knowledge	65	3.5 *70%*	105
Problemes i casos	Class participation (Small- sized group)	Problem solving	2	Learning how to solve problems	8-16	1.5 *20%*	10
Seminari	Class participation (Medium- sized group)	Activities of discussion or implementation		Problem solving and discussion			
Laboratory	Laboratory Practice (Small- sized group)	Implementation of the practice: to fully understand, measure…	25	Study and monography writing	10	Report *10%*	35
Computer room	Computer classroom practice (Medium- sized group)	Implementation of the practice: to fully understand, measure...		Study and monography writing			
Field Work	Practice Fieldwork (Medium- sized group)	Implementation of the practice: to fully understand, measure…		Study and monography writing			

Visits	Visit farms or industries	Making the Visit		Study and monography writing			
Guided Activities	Student work (individual or group)	Guiding Student study (in tutoring hours)	0	Bibliographic work, study, etc.	10	Report *4%*	10
Others							
Totals			60				160

Development plan

Theory classes will be held as **presential** sessions. In the event of confinement these sessions will be held as **non-presential**.

The practice sessions at the laboratory and at the computer room, in the event of confinement, will be substituted by **alternative activities.**

Evaluation

The evaluation of the subject is based on the following distribution:

-Theory (**57%**): partials 1 and 2 (Type test + short questions)

-Exercises and problems (**33%**): partials 1 and 2

-Report of laboratory practices (**10%**)

Bibliography

Bibliografia bàsica

- Hanbook of Plant Biotechnology volume1&2. 2004. P. Christou, H. Klee. John Wiley & Sons, Ltd. Chichester. *Disponible com llibre-e (www.bib.udl.cat).*
- Molecular Plant Biology Volume1, A practical approach. 2002. Philip M. Gilmartin and Chris Bowler. Oxford University Press.
- Principles of Gene manipulation. (sixth edition). 2001. SB. Primrose, RM. Twyman, RW. Old. Blackwell Sciences Ltd. Oxford
- Introduction to plant biotechnology (3rd edition). 2009. H.S. Chawla- Enfield, N.H. : Science Publishers, cop.
- Plant biotechnology and transgenic plants (2002). Kirsi-Marja Oksman-Caldentey, Wolfgang H. Barz. New York : Marcel Dekker, 2002
- GMO Compass: www.gmo-compass.org
- GMO safety: www.gmo-safety.eu
- International Service for the Acquisition of Agri-biotech Applications: www.isaaa.org

Bibliografia complementària

• Molecular Cloning- A Laboratory Manual. Vol 1,2,3. (Third Edition). 2001. J. Sambrook, DW. Russell. Cold Spring Harbor Laboratory Press, New York.

• Henry Stewart talks: www.hstalks.com

• National Center for Biotechnology Information: www.ncbi.nlm.nih.gov