Horticultural Genetics and Biotechnology

Organized by

Mediterranean Agronomic Institute of Chania

Horticultural Genetics and Biotechnology

Scientific Coordinator: **Dr. Panagiotis KALAITZIS**
MAI Coordinator: **Mr. Alkinoos NIKOLAIDIS**

Objectives

The long course on Horticultural Genetics & Biotechnology provides a two-year Master of Science degree programme for scientists holding a university Bachelors degree in agronomy, biology, horticulture, agricultural engineering, chemistry, plant science or any related field.

The major goals of the programme of Horticultural Genetics and Biotechnology are to provide the students with a thorough grounding in the mechanisms, capabilities, uses and limitations of plant biotechnological methods so that they will be able to apply them to problems related to horticultural production and quality.

The first year students receive a solid theoretical background and practical training, leading to the attainment of a DSPU diploma, attending classes and extensive laboratory courses in the following fields: (i)Applied plant molecular genetics and biotechnology including the hormonal and developmental regulation of gene expression, *in vitro* and tissue culture techniques, and transformation strategies; (ii) Applied plant genetics including marker-assisted breeding, risk assessment for Genetically Modified Organisms, Genetically Modified Organism certification protocols and *Arabidopsis* genetics. Emphasis throughout the course is placed on horticultural crops and their products.

In the second year, students who have successfully completed the first year will develop advanced molecular biology technical skills and independent thinking by working on research projects in modern, well-equipped laboratories leading to a Master's of Science degree.

Part 1
Post graduate specialization programme

The programme is organized in 4 sections

Tools **ENGLISH, COMPUTERS**
The cycle includes introductory courses on Scientific English and Use of Computer.

Section 1 **INTRODUCTION TO ADVANCED BIOLOGY**
The section is dedicated to bioscience core courses. The scope of this module is to strengthen the students' training in the fundamental and time-independent aspects of sciences upon which all subsequent and ever-evolving specialization will rest.

Section 2 **IN-VITRO PRODUCTION TECHNIQUES**
The section deals with advanced propagation techniques. It focuses on *in vitro* techniques for plant propagation and also includes a week of hands-on laboratory training.

Section 3 **APPLIED GENETICS**
The section is devoted to applied genetics. The first week teaches the basic principles of genetics followed by the concepts of molecular breeding and how they may be applied to improve crop production and quality. One week's practical training on molecular breeding techniques will improve understanding of these advanced methods. The second part of this module is dedicated to *Arabidopsis thaliana*, the model system for plant genetics, and will cover mutant analysis and transformation protocols.

Section 4 **POST-HARVEST BIOTECHNOLOGY**
This section provides an overview of the recent advances on post-harvest biotechnology. One week on the physiology and the biochemistry of plant senescence will up-date students on the basic principles of this area. This course inter-relates with one week of classes on the regulatory role of ethylene in ripening and senescence process, followed by recent developments in the molecular biology of fruit ripening. Finally, one week of practical training on advanced techniques in biotechnology will provide students with the tools to better understand and exploit this field of research. The remaining courses deal with the issue of Genetically-Modified agricultural products and the ecological risk assessment of producing such crops followed by the laboratory on how to certify GMO agro-products.

TRAINING SEQUENCE

Tools	**ENGLISH, COMPUTER**
October	**Unit 1 –** Scientific english
	Unit 2 – Introductory computing
Section 1	**INTRODUCTION TO ADVANCED BIOLOGY**
(18 ECTS)	**Unit 1 –** Cell biology
October -	**Unit 2 –** Biochemistry
December	**Unit 3 –** Stress physiology
	Unit 4 – Genetics
	Unit 5 – Molecular biology
	Unit 6 – Molecular biology techniques [Lab]
Section 2	**IN-VITRO PRODUCTION TECHNIQUES**
(6 ECTS)	**Unit 1 –** In-vitro techniques for applied biotechnology
December	**Unit 2 –** Tissue culture and in-vitro techniques laboratory [Lab]
Section 3	**APPLIED GENETICS**
(18 ECTS)	**Unit 1 –** Molecular breeding of horticultural crops
January to	**Unit 2** – Genetic improvement and seed production
February	**Unit 3 –** DNA fingerprinting technology
	Unit 4 – Arabidopsis genetics
	Unit 5 – Mutant analysis of arabidopsis
	Unit 6 – Arabidopsis transformation and analysis of transgenic plants
Section 4	**POST-HARVEST BIOTECHNOLOGY**
(18 ECTS)	**Unit 1 –** Molecular biology and biochemistry of fruit ripening
March to May	**Unit 2 –** Biotechnology of the plant hormone ethylene
	Unit 3 – Nutritional genomics
	Unit 4 – CA storage and molecular basis of hypoxia
	Unit 5 – Advanced GMO detection technologies [Lab]
	Unit 6 – Environmental risk assessment of GMOs

Comprehensive oral or written examination (Modalities and dates)

During Introduction, participants attend classes on English TOEFL and Computers. They are also obliged to attend a 45-hour course in Scientific English, equally distributed during sections, and take a written examination.

Participants take written examinations or submit projects for every unit within one section in the exam week which is scheduled at the end of every section, each unit being independently graded. Written exams consist of a set of questions that require a concise answer. Some of the questions are multiple choices. Lengthy questions are avoided.

Participants have the option of retaking course examinations of their preference equivalent of 9 ECTS credits during a weeklong retake period. There is no retake examination period for the last section.

A comprehensive oral examination conducted by an Examination Board takes place at the end of the academic year, representing 15% of the total grade.

ANALYTICAL SYLLABUS

Tools **ENGLISH, COMPUTER**

Unit 1 – Scientific english
A review of the fundamentals of English grammar. Oral communication, public speaking and note-taking. Development of academic writing skills, study of scientific text and papers.

Unit 2 – Introductory computing

Section 1 **INTRODUCTION TO ADVANCED BIOLOGY**

Unit 1 – Cell biology
Eukaryotic versus prokaryotic cells. Cell structure and compartmentation. Cell membranes and their principles. Cell organelles and their principles. Cell organelles and their physiology. Intracellular and intercellular communication. Genetic information storage – nucleus. Nuclear cytoplasmic interactions. Cell specialization – animal and plant cells. Cell responses to environmental stimuli.

Unit 2 – Biochemistry
Water, solvent of life; Properties of solutions; buffers. Biological macromolecules; (proteins, nucleic acids, polysaccharides, lipids)
Proteins: Levels of protein organization, Protein interactions Cell environment factors controlling protein assembly, Protein synthesis
Post-translation modifications, Protein compartmentation, Protein stability and turnover, Multiple functional stages of proteins, Multiplicity of protein forms and functions, Plant and insect hormones and their receptors
Polysacharides: Cell surface diversification, Cell adhesion, Natural products
Lipids: Simple and complex lipids, Lipoproteins, Lipid messengers
Nucleic acids:
(See molecular biology)

Unit 3 – Stress physiology

Selected topics on environmental physiology: Water relationships (the soil-plant atmosphere continuum, water transport processes, the anatomy of the pathway, stomatal control and transpiration). Photosynthesis, physiological and ecological. Considerations (Photosynthetic responses to light, to CO2 and to temperature). Biological nitrogen fixation.

Selected topics on stress physiology: An overview of resistance mechanisms of plant responses to stresses. Recognition and signal transduction. Stress-induced gene expression. Oxidative stress, a constituent of all stresses. Stresses imposed by water deficits (drought, low and high temperatures, salinity) and by oxygen deficiencies (flooding).

Unit 4 - Genetics

Historical perspective of Genetics. Early genetic experiments. Experimental derivation of the laws of inheritance/ Mendel.
Critical experiments in the development of Genetics.
Chromosome theory of inheritance. The unit of heredity.The nature of the gene; Cistrons. Position effects of DNA (Mc Clintock's work). Gene synthesis. Genetic engineering. Impact of Genetics on Society.

Unit 5 – Molecular biology

Stereochemistry of DNA and DNA topology. DNA replication, In vitro genetics. Restriction enzymes. Transcription factors. Transcriptional machineries. Pleiotropic effects of transcription. The substrate of transcription (chromatin). Role of introns-exons. Post transcriptional regulation. Post translational regulation. Differential gene expression and chromosomal domains. Extranuclear DNA. Environmental effects of gene expression

Unit 6 – Molecular biology techniques [Lab]

Introduction to basic molecular biology techniques such as ligation, transformation and plasmid DNA isolation, use of restriction endonucleases plant RNA and DNA extraction and RT-PCR.

Section 2 IN-VITRO PRODUCTION TECHNIQUES

Unit 1 - In-vitro techniques for applied biotechnology

Plant tissue culture is the science of growing plant cells, tissues or organs in defined media under sterile and optimized environmental conditions. This course deals with techniques and methods such as callus culture in liquid and solid media, protoplast cultures, and the production of haploid and triploid plants. Particular attention will be given to regeneration strategies, somatic embryogenesis and shoot organogenesis in relation to gene transformation protocols. Finally, prospects for the use of somaclonal variation to develop germplasm with useful traits will be discussed.

Unit 2 - Tissue culture and in-vitro techniques laboratory [Lab]

This practical training course will concentrate on basic methods used in tissue culture laboratories. These methods include working under aseptic conditions, media preparation from stock chemicals and cultures of meristematic tissues. In addition, students will gain hands-on experience of the detection and diagnosis of plant viruses using ELISA assays and also the methodology required for the production of certified plant material.

Section 3 APPLIED GENETICS

Unit 1 - Molecular breeding of horticultural crops

The selection of superior genotypes is often hampered by the significant influence that environmental factors have on the expression of a trait and the variability of these environmental factors. This is especially true for traits related to crop yield. The efficiency of selection for useful traits can be improved when the genes responsible for the traits or genes closely linked to them are known. Many molecular techniques are now available for the identification of such genes. This course will focus on molecular techniques such as AFLP (Amplified Fragment Length Polymorphism), ISSR-PCR (Inter-Specific Simple Repeat PCR), RAPD (Randomly-Amplified Polymorphic DNA) and how they can be used to assess genetic variability in plant germplasm collections or to fingerprint on commercial scale cultivars of horticultural crops.

Unit 2 - Genetic improvement and seed production

Introduction. The purpose of plant breeding. Basic principles of recombination. Reproduction and gametophytic generation. The process of Pollination and Fertilization. Variation. Natural vs artificial variation. Sources of natural variation and the Centers of origin. Inbreeding depression and Heterosis. The impact of inbreeding. Quantitative inheritance. Heritability. Selection methods for self pollinated crops.

Selection methods for cross pollinated crops. Variation in chromosome number (Polyploidy). Breeding for disease resistance. Basics of seed production. Self vs. cross pollinated species. Hybrid seed production systems. Seed production case studies for Maize, Tomato, Cucumber).

Crop genetic resources and seed conservation.

Unit 3 - DNA fingerprinting technology

This practical course will mainly focus on the AFLP technique, for which plant DNA will be extracted, and subjected to pre-amplification and amplification PCR reactions. The products of these reactions will be resolved on polyacrylamide gels, and exposed to film in order to visualize the variable DNA bands produced by using the different sets of primers. Finally the students will learn how the data produced is interpreted and may be used to evaluate genetic variability among plant samples.

Unit 4 - Arabidopsis genetics

The adoption of *A. thaliana* by the plant genetics community as the model of choice has resulted in the generation of an enormous amount of information concerning the genetics and biology of this plant. As an introduction to this system, the general features of the plant will be discussed, and also the large number of *Arabidopsis* resources available to researchers on the Internet. In addition, the course will discuss how functional genomics in *Arabidopsis* are used to study biotic and abiotic stress.

Unit 5 - Mutant analysis of arabidopsis

Chemical mutagenesis is frequently used to generate plants displaying phenotypic alterations. The genetic lesions resulting in these characteristics may then be mapped and the genes involved in the modified phenotype can be identified by using reverse genetic techniques. In this unit mutation protocols, phenotypic analysis, and genome analysis will be described. In addition, the RNA interference technology will be presented extensively (advantages and disadvantages)

Unit 6 - Arabidopsis transformation and analysis of transgenic plants
A powerful technique to confirm a phenotypic trait assigned to a particular gene by reverse genetics is to demonstrate that the introduction of a modified gene or the disruption of its function gene will cause the same phenotype in normal plants. In this unit the processes by which particular genes are incorporated into recombinant vectors and introduced into *Agrobacterium* strains which are then used to infect and transform *Arabidopsis* plants mutants, and the isolation and analysis of the resultant recombinant progeny will be discussed.

Section 4 POST-HARVEST BIOTECHNOLOGY

Unit 1 - Molecular biology and biochemistry of fruit ripening
Fruit ripening is a developmentally- and hormonally-regulated process that encompasses a wide range of cellular changes including fruit softening, chlorophyll degradation, anthocyanin production etc. The existence of a plethora of ripening mutants, detailed genetic maps and common transformation protocols have made the tomato (*Lycopersicum esculentum*) the model system for genetic, molecular and biochemical studies of fruit ripening. Emphasis will be given to molecular aspects of cell wall modifications during ripening. How genomics can be used to identify key components of ripening process. How biochemical events, such as carotenoid formation, are regulated during ripening..

Unit 2 - Biotechnology of the plant hormone ethylene
The plant hormone ethylene has profound effects on plant growth and development, including the induction of ripening in climacteric fruits, the promotion of seed germination, in the abscission of various organs and in senescence. The ethylene biosynthetic pathway has been determined and tremendous progress has been made towards elucidating the ethylene response signal transduction pathway, from signal perception to gene regulation. In addition to a general introduction to the topic, this class will focus on:
1. The manipulation of the ethylene biosynthetic genes in transgenic horticultural crops.
2. The genetic and molecular dissection of the ethylene response pathway.

Unit 3 - Nutritional genomics
In the near future we will notice an unprecedented change in the production and range of available agro-foods. Functional foods, defined as those that affect beneficially one or more target functions in the body, will be produced and utilized more and more in the coming years. The scope of this class is to define classes of metabolites, such as ascorbic acid, analyze the metabolic pathways of their production and how these pathways can be engineered to produce functional foods.

Unit 4 - CA storage and molecular basis of hypoxia
Controlled Atmosphere storage: Physiological significance of CA storage, Differential effects on ripening process.
Hypoxia and metabolic adaptation: Oxygen status of cells and tissues. Metabolic adaptation and energy balance. Role of ethylene in hypoxic triggering.
Carnation flowers and *Arabidopsis* plants as models to study hypoxia triggering; State of the art on cut carnation flowers. Expression profiles under hypoxia in *Arabidopsis*.
Oxygen sensors in prokaryotes.
Hypoxia sensing and signalling in mammalian systems.

Unit 5 – Advanced GMO detection technologies [Lab]

The latest techniques for the analysis of genetically modified common agricultural produce (soya, maize, cotton, tomato), and their products will be demonstrated.

The lab will include the sampling methodology necessary to establish the status of the produce with regard to its transgenic element content, and to examine for possible contamination of non-transgenic produce. DNA isolation will be carried out on various samples of seeds, fruits and their processed products. PCR analysis of these samples will then be performed to confirm the presence of species-specific marker genes, and to test for the presence of defined transgene sequences, and other genetic elements commonly found in transformed plant strains.

Unit 6 - Environmental risk assessment of GMOs

The potential environmental risks due to gene flow from genetically modified crops into wild plant populations are currently of great concern. Methodologies will be described for the detection of hybridization between genetically modified plants and their wild relatives, including PCR–based analyses such as ISSR and RAPD. In addition, the possible consequences of the accidental introduction of transgenes into other plants will be discussed and elaborated.

Part 2
The Master of Science Program

Research Project (9 months duration)

This part of the programme is carried out in cooperation with well-recognized institutions (universities, research centres or firms), generally throughout Greece or in the participant's country of origin, under the scientific supervision of a thesis director that must be a doctor of renowned prestige. Participants choose the topic according to their interest of training.

A wide range of research topics is available for Master of Science theses. Many of the theses focus on topics related with Laboratory Techniques, related to: Molecular signalling in plant senescence using as models cut carnation flowers and tomato fruit; Molecular basis of hypoxic and anoxic response using Arabidopsis as model plant; Development of quantitative GMO standards for crops such as cotton; DNA genotyping of horticultural crops such as olives and their food derivatives.

INDICATIVE MASTER THESIS REALIZED WITHIN THE AREA

Title: Supression of a Cotton Actin Transcript by Ethylene (2003)
Author: Amal Bijamane, Moroccan
Place of realization: MAICh, Greece
Thesis directors: Nikoforos Kapranos, Hospital Amalia Fleming, Greece, Panagiotis Kalaitzis, MAICh, Greece

Title: The Use of RNA Interference to Knockout Arabidopsis P4H Genes (2004)
Author: Ihab Lolas
Place of realization: MAICh, Greece
Thesis directors: Dr. Kriton Kalantidis, Greece, Dr. Panagiotis Kalaitzis, Greece

Title: Assessing Olive Biodiversity by Genomic AFLP Methodology (2003)
Author: Elena Craita Bita
Place of realization: MAICh, Greece
Thesis director: Prof. Polydevkis Chatzopoulos, Greece, Dr. Panagiotis Kalaitzis, MAICh, Greece

Title: Expression Profile of the Arabidopsis Prolyl 4-Hydroxylase Gene Family in Response to Hypoxia (2003)
Author: Daniela Vlad, Agronomist, Romanian
Place of realization: MAICH, Greece
Thesis director: Dr. Panagiotis Kalaitzis, MAICh, Greece

Title: A Study on the Expression of the P4H7 and HSAP90 Genes in Response to Hypoxia and Anoxia (2004)
Author: Rachid Ben Hamm, Moroccan
Place of realization: MAICh, Greece
Thesis directors: Prof. Polydevkis Chatzopoulos, Greece, Dr. Panagiotis Kalaitzis, MAICh, Greece

Title: Supression of a Cotton Actin Transcript by Ethylene (2003)
Author: Amal Bijamane, Moroccan
Place of realization: MAICh, Greece
Thesis directors: Dr. Nikiforos Kapranos, Greece, Dr. Panagiotis Kalaitzis, MAICh, Greece

Title: Development of Scar Markers Linked to Resistance to *Fusarium Oxysporum* F.Sp. *Cucumerium* in Cucumber *(Cucumis sativus*) (2004)
Author: Emad Jaber, Jordan
Place of realization: Heraklion, Greece
Thesis directors: Dr. Andreas Doulis, Dr. Demetrios Vakalounakis, Greece

Title: Alternative Methods to Control Botrytis cinerea and Preserve the Quality of Hayward Kiwifruit (2004)
Author: Kamer Cetiz, Turkey
Place of realization: MAICh
Thesis director: Prof. Anastasia Lagopodi, Greece, Prof. E. Sfakiotakis, Greece

Title: High CO2 Storage of Truffles in Air-Tight and Temperature Insulated Shipping Box and its Effects on Polyphenoles, Polyamides, Antioxidant Activity,k ADH and LDH Activity (2001)
Author: Shady Hajjar, Lebanon
Place of realization: MAICh, Greece and University of Viterbo, Italy
Thesis director: Prof. F. Mencarelli, Italy

REFERENCES OF THE MAIN ACADEMIC STAFF TEACHING WITHIN THE M.SC.

More than 25 invited lecturers from about 6 countries participate in each edition of the M.Sc. programme of which, 45% came from Research Centres, 45% from Higher Education Institutions, 10% from Private Companies and 5% from International Centres.

- Tala AWADA, Researcher, University of Nebraska, USA
- Giorgos CASADORO, Professor, Universita di Padova, Italy
- Polydevkis CHATZOPOULOS, Professor, Agricultural University of Athens, Greece
- Chrisostomos DOVAS Scientist, Aristotle University of Thessaloniki, Greece
- Nikos FANOURAKIS, Professor, Technical Educational Institute of Crete, Greece
- Katerina GRIGORIADOU, Scientist, Vitro Hellas, Greece
- Dimitrios KAFETZOPOULOS, Researcher, Institute of Molecular Biology, Heraklion, Greece
- Kriton KALANTIDIS, Researcher, Plant molecular Biology Unit, The Forth Institute, Heraklion, Greece
- Angelos KANELLIS, Professor, School of Pharmaceutical Sciences, Aristotle University of Thessaloniki, Greece
- Olga KOUTITA, Scientist, Hellenic Sugar Industry, Thessaloniki, Greece
- Nikos LEVENTAKIS, Scientist, Vitro Hellas, Greece
- Jordi Garcia MAS, Scientist, IRTA, Spain
- Jean PECH, Researcher, ENSAT, France
- Eddo RUGINI, Professor, Universita degli Studi della Tuscia, Italy
- John SCANDALIOS, Professor, North Carolina State University, USA
- Konstantinos SEKERIS, Researcher, National Research Institute, Athens, Greece
- Athanasios TSAFTARIS, Professor, Department of Agriculture, Aristotle University of Thessaloniki, Greece
- Konstantinos TSINGENOPOULOS, Institute of Sea Biology, Heraklion, Greece
- Konstantinos VLACHONASIOS, Associate Professor, Department of Agriculture, Aristotle University of Thessaloniki, Greece
- Mike WILKINSON, Professor, University of Reading, U.K.