

MEDITERRANEAN AGRONOMIC INSTITUTE OF CHANIA
(MAICH)

Horticultural Genetics and Biotechnology

POSTGRADUATE ACADEMIC GUIDE

&

ECTS GUIDE

2006 - 2007

Horticultural Genetics and Biotechnology

Postgraduate Specialisation and M.Sc. Programme

SCIENTIFIC COORDINATOR: **Dr. Panagiotis Kalaitzis**

EDUCATIONAL AND PROFESSIONAL GOALS

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 Department 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 PGD certificate, 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.

ACCESS TO FURTHER STUDIES

Statistics show that 28% of M.Sc. graduate students are accepted to Ph.D. programmes with full scholarship in European and North American Universities.

POST GRADUATE SPECIALISATION PROGRAMME

The programme is organized in 5 sections

Section I - Introductory Discipline

The Cycle includes introductory courses on Scientific English and use of Computer.

Section II - Introduction to Advanced Biology

The Cycle 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 III - In-Vitro Production Techniques

The Cycle 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 IV - Applied Genetics

The Cycle 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 V - Post-Harvest Biotechnology

This Cycle 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

Section I - Introductory Discipline [7 ECTS]

October to November

HOB.501 - Scientific English (3 ECTS)

HOB.502 - Introductory Computing (1 ECTS)

Section II - Introduction to Advanced Biology [18 ECTS]

from November to December

HOB.511 - Cell Biology (3 ECTS)

NPB.512 - Biochemistry (3 ECTS)

HOB.513 - Stress Physiology (3 ECTS)

HOB.514 - Genetics (3 ECTS)

HOB.515 - Molecular Biology (3 ECTS)

HOB.516 - Molecular Biology Technology (Lab) (3 ECTS)

Section III - In-Vitro Production Techniques [6 ECTS]

from January to February

HOB.521 - In-Vitro Techniques for Applied Biotechnology (3 ECTS)

HOB.522 - Tissue Culture and In-Vitro Techniques (Lab) (3 ECTS)

Section IV - Applied Genetics [18 ECTS]

from February to March

HOB.531 - Molecular Breeding of Horticultural Crops (3 ECTS)

HOB.532 - Genetic Improvement and Seed Production (3 ECTS)

HOB.533 - DNA Fingerprinting Technology (3 ECTS)

HOB.534 - *Arabidopsis* Genetics (3 ECTS)

HOB.535 - Mutant Analysis of *Arabidopsis* (3 ECTS)

HOB.536 - *Arabidopsis* Transformation & Analysis of Transgenic Plants (3 ECTS)

Section V - Post-Harvest Biotechnology [21 ECTS]

from March to June

HOB.541 - Molecular Biology and Biochemistry of Fruit Ripening (3 ECTS)

HOB.542 - Biotechnology of the Plant Hormone Ethylene (3 ECTS)

HOB.543 - Nutritional Genomics (3 ECTS)

HOB.544 - CA Storage and Molecular Basis of Hypoxia (3 ECTS)

HOB.545 - Advanced GMO Detection Methodologies (Lab) (3 ECTS)

HOB.546 - Environmental Risk Assessment of GMOs (3 ECTS)

ANALYTICAL SYLLABUS

HOB.501 - Scientific English (3 ECTS) _____

LECTURER: M. Verivaki

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: The development of scientific writing and communication skills.

PREREQUISITES: Good knowledge of English language.

COURSE CONTENTS

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.

RECOMMENDED READING

Papers provided by the instructor.

ASSESSMENT METHODS: Written exam

HOB.502 - Introductory Computing (1 ECTS) _____

LECTURER: N. Boretos

TYPE OF COURSE AND TEACHING METHODS: Computer-assisted teaching

OBJECTIVE OF THE COURSE: Introduce scientists to the computer-based resources available.

COURSE CONTENTS

Introduction to computer utilization

RECOMMENDED READING

European Computing Driving Licence.

ASSESSMENT METHODS: Written exam

HOB.511 - Cell Biology (3 ECTS) _____

LECTURER: Prof. K. Vlachonasis

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To introduce students to the fundamentals of plant cell biology. To strengthen their background on biological mechanisms that takes place in the eukaryotic and prokaryotic cell. Students understand the complexity of cells and the environment where fundamental processes preserve cell homeostasis.

COURSE CONTENTS

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.

RECOMMENDED READING

Biochemistry & Molecular Biology of Plants, B. Buchanan, W. Gruissem, R. Jones, eds. 2000, American Society of Plant Physiologist.

ASSESSMENT METHODS: Written exam

NPB.512 - **Biochemistry** (3 ECTS) _____

LECTURER: Prof. K. Seceris

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To familiarize with all the important components of the chemistry of life. To introduce students to the structure and function of proteins and nucleic acids.

COURSE CONTENTS

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

Polysaccharides: Cell surface diversification, Cell adhesion, Natural products

Lipids: Simple and complex lipids, Lipoproteins, Lipid messengers, Nucleic acids.

RECOMMENDED READING

Lehninger: Chapter 3 (Biomolecules), Ch. 4 (Water), Ch. 5 (Aminoacids and peptides), Ch. 6 (An introduction to proteins), Ch. 7 (The three dimensional structure of proteins), Ch. 9 (Lipids), Ch. 11 (Carbohydrates), Ch. 12 (Nucleotides and nucleic acids) and Ch. 26 (Protein Metabolism)

ASSESSMENT METHODS: Written exam

HOB.513 - **Stress Physiology** (3 ECTS) _____

LECTURER: Dr. T. Awada

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To provide an overview of plant stress and environmental physiology. To strengthen the background on basic physiological mechanisms that plants employ to overcome adverse conditions.

COURSE CONTENTS

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).

RECOMMENDED READING

- Jones, H.G. Plants and microclimate.1994. Cambridge University press.
Fitter, A.H. and Hay, R.K.M. 2002. Environmental physiology of plants. Academic press.
Taiz, L. and Zeiger E. 1998. Plant Physiology. Sinauer Associates Inc., Publ.
Larcher, W. 2003. Physiological Plant Ecology. Springer.
Lambers et al. 2000. Plant Physiological Ecology. Springer.
Ajay Arora, R. K. Sairam and G. C. Srivastava. 2002. Oxidative stress and antioxidative system in plants. Current Sci. 82: 1227-38.

ASSESSMENT METHODS: Written exam

HOB.514 - **Genetics** (3 ECTS)

LECTURER: Prof. A. Tsaftaris

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: Provide an introduction into the science of Genetics, a historical perspective and understanding of the current issues.

COURSE CONTENTS

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.

RECOMMENDED READING

- The double Helix-50 years, Nature Publishing Group Supplement (2003) Vol. 421, p 395-453.
RNA-directed DNA methylation, (2000) M. Wassenegger, Plant Molecular Biology, 43: 203-220.
Status of genome projects for nonpathogenic bacteria and archaea, K. E. Nelson, Ian T. Paulsen, J. F. Heidelberg and C. M. Fraser (2000) Nature Biotechnology Vol. 18, 1049-1054.
Functional genomics, Nature insight, Reprinted from Vol. 405, no. 6788, 2000, p 819-822.
The Evolution of Epigenetics (2001), Science, Vol. 293, p 1063-1105.
Epigenetic Mechanisms of Gene Regulation, Edited by Vincenzo E.; A. Russo, Robert A. Martienssen and Arthur D. Riggs, p. 5-24.
Bioinformatics: A Biologist's Guide to Biocomputing and the Internet, Stuart M. Brown, Ch. 2 and Ch. 3.
DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*, J. F. Heidelberg, J. A. Elsen, W. C. Nelson, R. A. Clayton, M. L. Gwinn, R. J. Dodson, D. H. Haft, E. K. Hickey, J. D. Peterson, L. Umayam, S. R. Gill, K. E. Nelson, T. D. Read, H. Tettelin, D. Richardson, M. D. Ermolaeva, J. Vamathevan, S. Bass, H. Qin, I. Dragoi, P. Sellers, L. McDonald, T. Utterback, R. D. Fleishmann, W. G. Nierman, O. White, S. L. Salzberg, H. O. Smith, R. R. Colwell, J. J. Mekalanos, J. Graig Venter and C. M. Fraser (2000), Nature Vol. 406, p. 477-483.
Molecular Cloning, A Laboratory Manual, Joseph Sambrook and David W. Russell.
The Unseen Genome: Gems among the Junk, Scientific American (2003).
The Asteroid Tugboat, R. L. Shweickart, E. T. Lu, P. Hut and C. R. Chapman (2003), Scientific American.

ASSESSMENT METHODS: Written exam

HOB.515 - Molecular Biology (3 ECTS) _____

LECTURER: Dr. D. Kafetzopoulos

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To familiarise with DNA science and provide an understanding of the fundamental molecular processes taking place in a cell. To introduce students to the basic steps in the flow of genetic information.

PREREQUISITES: Basics knowledge of Genetics and Biochemistry.

COURSE CONTENTS

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.

RECOMMENDED READING

Molecular Cell Biology, 5th Ed., Ch. 4, Ch. 9

ASSESSMENT METHODS: Written exam

HOB.516 - Molecular Biology Technology (Lab) (3 ECTS) _____

LECTURER: Dr. P. Kalaitzis

TYPE OF COURSE AND TEACHING METHODS: Laboratory

OBJECTIVE OF THE COURSE: To introduce students to the basic plant molecular biology techniques in the laboratory.

COURSE CONTENTS

Introduction to basic molecular biology techniques. Ligation, Transformation of E.coli, Plasmid DNA isolation, plant RNA and DNA extraction, RT-PCR.

RECOMMENDED READING

Molecular cloning - A Laboratory Manual, Third Edition, Sambrook and Russell, Volumes 1, 2, 3

ASSESSMENT METHODS: Written exam

HOB.521 - In-Vitro Techniques for Applied Biotechnology (3 ECTS) _____

LECTURER: Prof. E. Rugini

TYPE OF COURSE AND TEACHING METHODS:

OBJECTIVE OF THE COURSE: To provide the students with an overview of plant tissue culture techniques and their potential in the production of propagative material

COURSE CONTENTS

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.

RECOMMENDED READING

Plant tissue culture concepts and laboratory exercises, Second edition, Chapter 14, Chapter 43.

Tissue culture techniques for horticultural crops, K.C. Torres, Chapter 18.

ASSESSMENT METHODS: Written exam

HOB.522 - **Tissue Culture and In-Vitro Techniques (Lab)** (3 ECTS) _____

LECTURER: N. Leventakis, K. Grigoriadou, Ch. Dovas

TYPE OF COURSE AND TEACHING METHODS: Laboratory

OBJECTIVE OF THE COURSE: To provide hands on experience in tissue culture techniques and production of virus-free material

COURSE CONTENTS

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.

RECOMMENDED READING

Power Point Presentation, notes provided.

ASSESSMENT METHODS: Written exam

HOB.531 - **Molecular Breeding of Horticultural Crops** (3 ECTS) _____

LECTURER: Dr. J. Garcia / Dr. P. Verus

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To introduce students into molecular markers and how they are used in molecular breeding

COURSE CONTENTS

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.

RECOMMENDED READING

Introduction to quantitative genetics, Third edition, D. S. Falconer, Chapter 10 Heritability.

The genetic architecture of quantitative traits, Trudy F. C. Mackay, Annu. Rev. Genet. 2001.

From plant to genomics to breeding practice, M. Morgante and F. Salamini, Current Opinion in Biotechnology 2003.

Mapping polygenes, S.D. Tanksley, Annu. Rev. Genet. 1993.

QTL analysis in plants; where are we now?, M.J. Kearsey and A.G.L. Farquhar, Heredity (80), 1998.

ASSESSMENT METHODS: Written exam

HOB.532 - **Genetic Improvement and Seed Production** (3 ECTS) _____

LECTURER: Prof. N. Fanourakis

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To provide basic principles of plant breeding and seed production techniques

COURSE CONTENTS

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.

RECOMMENDED READING

Breeding Field Crops, by J. Poehlman Covers very well some general topics. Very good for specific crops of agronomic importance. AVI Publish. Co.

Principles of Plant Breeding, by A. Alard Good classic book (quite old but still in use!) John Willey.

Plant Breeding, by M. Hayward et. al. (CINEAM) Good book covers all main subjects. Also Biotechnology. Chapman & Hall.

Breeding for Disease Resistance by R. Johnson and G. Ellis. Good for specific topics. Kluwer Acad. Publishers.

Breeding Vegetable Crops, by M. Basset Very good for breeding the main vegetables AVI Publish. Co.

ASSESSMENT METHODS: Written exam

HOB.533 - **DNA Fingerprinting Technology** (3 ECTS) _____

LECTURER: Dr. G. Kotoulas, Dr. K. Tsingenopoulos

TYPE OF COURSE AND TEACHING METHODS: Laboratory

OBJECTIVE OF THE COURSE: To provide students hands on experience on molecular marker techniques such as RAPD, SSRs and AFLP

COURSE CONTENTS

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.

RECOMMENDED READING

Plant Genotyping: The DNA Fingerprinting of Plants / by Robert J. Henry (2001),Molecular Markers, Natural History, and Evolution / by John C. Avise (April 2004).

Principles of Genetics / by D. Peter Snustad, Michael J. Simmons (July, 2002).

Modern Genetic Analysis, Second Edition: Integrating Genes and Genomes / by Richard C. Lewontin, Anthony J.F. Griffiths, Jeffrey H. Miller, William M. Gelbart (February, 2002).

Genetics of Populations / by Philip W. Hedrick (2004).

Genetics: Analysis of Genes and Genomes / by Daniel L. Hartl, Elizabeth W. Jones (August 2004).

Ecological Genetics: Design, Analysis, and Application / by Andrew Lowe, Stephen Harris, Paul Ashton, Blackwell Pub (June, 2004).

A Primer of Ecological Genetics / by Jeffrey K. Conner, Daniel L. Hartl (February, 2004).

Applied Molecular Genetics / by Roger L. Miesfeld (1999).

Handbook of Plant Biotechnology (Life Sciences) / by Paul Christou, Harry Klee (May, 2004).

Molecular Plant Biology (The Practical Approach, 259) / by Philip M. Gilmartin, Chris Bowler (July, 2002).

ASSESSMENT METHODS: Written exam

HOB.534 - **Arabidopsis Genetics** (3 ECTS) _____

LECTURER: Prof. K. Vlachonasis

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To introduce students into the advantages and disadvantages of Arabidopsis as a model plant for genetic studies.

COURSE CONTENTS

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.

RECOMMENDED READING

Arabidopsis: A Laboratory Manual, Chapters 1, 2, 3.

Arabidopsis, the Rosetta Stone of Flowering time?, G. G. Simpson and Caroline Dean, Science, vol. 296, 2002.

Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway, Sarah Fowler and M. F. Thomashow, The Plant Cell, vol. 14, 2002.

ASSESSMENT METHODS: Written exam

HOB.535 - **Mutant Analysis of Arabidopsis** (3 ECTS) _____

LECTURER: Dr. K. Kalantidis

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To provide students with the available techniques for mutant analysis and how they are used to answer fundamental questions in plant biology

COURSE CONTENTS

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).

RECOMMENDED READING

Arabidopsis: A Laboratory Manual, Chapter 4.

Arabidopsis, Chapter 1: The Arabidopsis thaliana genome: towards a complete physical map.

Positional cloning in Arabidopsis. Why it feels good to have a genome initiative working for you, W. Lukowitz, C. Stewart Gillmor, W. Scheible, Plant Physiology, vol.123, 2000.

The art and design of genetic screens: *Arabidopsis thaliana*, D.R. Page and U. Grossiklaus, *Nature Reviews*, vol3, 2002.

Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*, The Arabidopsis Genome Initiative, *Nature*, vol. 408, 2000.

Plant functional genomics, H. Holtorf, M.C. Guitton, R. Reski, *Naturwissenschaften*, vol. 89, 2002.

RNA interference - 2001, P.A. Sharp, *Gene and development*, vol. 15, 2001.

ASSESSMENT METHODS: Written exam

HOB.536 - **Arabidopsis Transformation & Analysis of Transgenic Plants** (3 ECTS) ___

LECTURER: Prof. P. Chatzopoulos

TYPE OF COURSE AND TEACHING METHODS: Laboratory

OBJECTIVE OF THE COURSE: To introduce students into the nuts and bolts of agrobacterium-mediated transformation techniques and how it can be used in horticultural biotechnology

COURSE CONTENTS

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.

RECOMMENDED READING

Plant transformation technology, C.A. Newell, *Molecular biotechnology*, vol. 16, 2000.

Lessons in gene transfer to plants by a gifted microbe, G. Hansen and M.D. Chilton.

ASSESSMENT METHODS: Written exam

HOB.541 - **Molecular Biology and Biochemistry of Fruit Ripening** (3 ECTS) _____

LECTURER: Prof. G. Casadoro

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To introduce students into the mechanisms of fruit ripening and how it can be regulated in horticultural crops

COURSE CONTENTS

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 (*Lycopersicon 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..

RECOMMENDED READING

Regulation of Carotenoid Formation During Tomato Fruit Ripening and Development, Peter M. Bramley, *Journal of Experimental Botany*, vol 53, 2002.

Current Trends in the Embryology of Angiosperms, 2001, Chapter 17 - Parthenocarpy-State of the Art, Angelo Spena and Giuseppe Leonardo Rotino.

Molecular Aspects Of Cell Wall Modifications During Fruit Ripening, M. D. Brownleader et al., *Critical Reviews in Food Science and Nutrition*, Vol 39, 1999.

- Cell Wall Metabolism in Fruit Softening and Quality and its Manipulation in Transgenic Plants, D. A. Brummell and M. H. Harpster, *Plant Molecular Biology*, Vol 47, 2001.
- Molecular Biology of Fruit Maturation and Ripening, J. Giovannoni, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, Vol 52, 2001.
- Single Transduction Systems Regulating Fruit Ripening, L. Adams-Phillips et al., *TRENDS in Plant Science*, Vol 9, 2004.
- Role of Cell Wall Hydrolases in Fruit Ripening, R. L. Fisher and A. B. Bennett, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, Vol 42, 1991.
- Biochemistry of Fruit Ripening. Edited by G. Seymour, J. Taylor and G. Tucker, 1993, Chapter 4 and Chapter 12.
- Regulation of Fruit Dehiscence in Arabidopsis, Cristina Ferrandiz, *Journal of Experimental Botany*, Vol 53, Fruit Development and Ripening Special Issue, 2002.
- Use of Genomics Tools to Isolate Key Ripening Genes and Analyse Fruit Maturation in Tomato, S. Moore et al., *Journal of Experimental Botany*, Vol 53, Fruit Development and Ripening Special Issue, 2002.
- Increasing Antioxidant Levels in Tomatoes Through Modification of The Flavonoid Biosynthetic Pathway, M. E. Verhoeyen et al., *Journal of Experimental Botany*, Vol 53, Fruit Development and Ripening Special Issue, 2002.

ASSESSMENT METHODS: Written exam

HOB.542 - **Biotechnology of the Plant Hormone Ethylene** (3 ECTS) _____

LECTURER: Dr J. Pech

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To introduce students into the biology and biotechnology of the plant hormone ethylene and how affects the post-harvest behaviour of horticultural crops

COURSE CONTENTS

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.

RECOMMENDED READING

- Fruit and Vegetable Biotechnology: V. Valpuesta Ed. Woodhead Pub Limited, Cambridge UK, 2002, ISBN 1 85573 467 2 Ed.
- Ethylene, Agricultural Sources and Applications: M. Arshad and W.T. Frankenberger Ed. Kluwer Acad publisher, New York, 2002, ISBN 0 306 46666 X.
- Ethylene in Plant Biology (second edition): F. Abeles and M.E. Salveit Eds, Academic Press, San Diego USA, 1992, ISBN 0 12 041451 1.
- Plant Hormones, Biosynthesis, Signal Transduction, Action: P. Davies Ed., Kluwer Acad Pub. Dordrecht, NL, 2004, ISBN 1 4020 2684 6 (HB) ISBN 1 4020 2686 2 (e-book).

ASSESSMENT METHODS: Written exam

HOB.543 - **Nutritional Genomics** (3 ECTS) _____

LECTURER: Prof. A. Kanellis

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To provide students an oversight of secondary metabolites with nutritional value, and how genetic engineering can alter the concentration of important metabolites from plants

COURSE CONTENTS

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.

RECOMMENDED READING

- Plants as “Chemical Factories” for the production of polyunsaturated fatty acids, Research review paper, D. L. Alonso and F. G. Maroto, *Biotechnology Advances* 18, 200.
- The biosynthesis and nutritional uses of crotonoids, Review, P. D. Fraser and P. M. Bramely, *Progress in Lipids Research* 43, 2004.
- Plant L-ascorbic acid: Chemistry, Function, Metabolism, Bioavailability and Effects of Processing, Review, M. W. Davey et al., *J Sci Food Agric* 80, 2000.
- Folic acid and folates: The Feasibility for nutritional enhancement in plant foods, Review, J. Scott et al., *J Sci Food Agric* 80, 2000.
- Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition, Review, E. Frossard et al., *J Sci Food Agric* 80, 2000.
- The potential for the improvement of carotenoid levels in food and the likely systematic effects, Review, H. Van Den Berg et al., *J Sci Food Agric* 80, 2000.
- Vitamin E, Review, PM Bramely et al., *J Sci Food Agric* 80, 2000.
- Plant sterols: Biosynthesis, Biological Function and their Importance to Human Nutrition, Review, V. Piironen et al., *J Sci Food Agric* 80, 2000.
- The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods, Review, R. F. Mithen et al., *J Sci Food Agric* 80, 2000.
- Immuno-nutrition: Designer diets in cancer, Review Article, R. Imoberdorf, *Support Care Cancer* 5, 1997.
- Vaccine antigen production in transgenic plants: Strategies, Gene Constructs and Perspectives, F. Sala et al., *Vaccine*, Vol.21, 2003.
- Engineering vitamin E content: From arabidopsis mutant to Soy oil, A. L. Van Eenennaam et al., *The Plant Cell*, Vol. 15, 2003.
- Improving iron, zinc and vitamin A nutrition through plant biotechnology, M. B. Zimmermann and R. F. Hurrell, *Current Opinion in Biotechnology*, Vol. 13, 2002.
- Synthesis and turnover of folates in plants, A. D. Hanson and J. F. Gregory III, *Current Opinion in Biotechnology*, Vol. 5, 2002.
- Increasing antioxidant levels in tomatoes through modification of the flavonoid biosynthetic pathway, M. E. Verhoeven et al., *Journal of Experiment Botany*, Vol. 53, No. 377, 2002.
- Antisense suppression of L-galactose dehydrogenase in *Arabidopsis thaliana* provides evidence for its Role in ascorbate synthesis and reveals light modulates L-galactose synthesis, S. Gatzek et al., *The Plant Journal*, Vol. 30(4), 2002.
- Metabolic engineering of Xanthophyll content in tomato fruits, S. Dharmapuri et al., *Federation of European Biochemical Societies (FEBS) Letters*, Vol. 519, 2002.
- Vitamin production in transgenic plants, K. Herbers, *J. Plant Physiol.* Vol. 160, 2003.
- What is beneficial for health? The Concept of Functional Food, M. B. Roberfroid, *Food and Chemical Toxicology*, Vol. 37, 1999.
- General introduction to the importance of genomics in food biotechnology and nutrition, C. T. Verrips et al., *Current Opinion in Biotechnology*, Vol. 12, 2001.
- Metabolic engineering of an alternative pathway for ascorbic acid biosynthesis in plants, A. K. Jain and C. L. Nessler, *Molecular Breeding*, Vol. 6, 2000.
- Over-expression of ascorbate oxidase in the apoplast of transgenic Tobacco results in ascorbate and glutathione redox states and increased sensitivity to ozone, Original article, M. Sanmartin et al., *Planta*, 2002.

Immunonutrition – supplementary amino acids and fatty acids ameliorate immune deficiency in critically ill patients, H. Grimm and A. Kraus, *New Surgical Horizons*, Langenbeck's Arch Surg 386, 2001.

Flavonoid biosynthesis. A Colorful Model for Genetics, Biochemistry, Cell Biology, and Biotechnology, B. Winkel-Shirley, *Plant Physiology*, Vol. 126, 2001.

Engineered polyamine accumulation in tomato enhances phytonutrient content, Juice Quality, and Vine Life, R. A. Mehta et al., *Nature Biotechnology*, Vol. 20, 2002.

ASSESSMENT METHODS: Written exam

HOB.544 - CA Storage and Molecular Basis of Hypoxia (3 ECTS) _____

LECTURER: Dr. P. Kalaitzis

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To provide students with the basic principles of CA storage and the molecular basis of hypoxia

COURSE CONTENTS

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.

RECOMMENDED READING

Power Point Presentation, notes provided.

Andrews DL, Drew MC, Johnson JR, Cobb BG, 1994. The response of maize seedling of different ages to hypoxic and anoxic stress. *Plant Physiology* 105, 53-60.

Chang WW, Huang L, Shen M, Webster , Burlingame AL, Roberts JK. 2000. Patterns of protein synthesis and tolerance of anoxia in root tips of maize seedlings acclimated to a low-oxygen environment, and identification of proteins by mass spectrometry. *Plant Physiology* 122, 295-318.

Chang C, Meyerowitz E. 1986. Molecular cloning and DNA sequence of the Arabidopsis thaliana alcohol dehydrogenase gene. *Proceedings of the National Academy of Sciences of the United States of America* 83, 1408-1412.

Chen X, Solomos T, 1996. Effects of hypoxia on cut carnation flowers (*Dianthus caryophyllus* L.): longevity, ability to survive under anoxia and activities of alcohol dehydrogenase and pyruvate kinase. *Postharvest Biology and Technology* 7,317-329.

Dolferus R, Jacobs M, Peacock WJ, Dennis ES. 1994. Differential interactions of promoter elements in stress responses of the Arabidopsis Adh gene. *Plant Physiology* 105, 1075-1087.

Dolferus R, Marbaix G, Jacobs M. 1985. Alcohol dehydrogenase EC 1.1.1.1 in Arabidopsis analysis of the induction phenomenon in plantlets and tissue cultures. *Molecular and General Genetics* 199, 256-264.

Drew, M.C. 1997. Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. *Annual Review of Plant Physiology and Plant Molecular Biology* 48, 223-250.

Droillard MJ, Bureau D, Paulin A, Daussant J. 1989. Identification of different classes of superoxide dismutase in carnation petals. *Electrophoresis* 10(1), 46-8.

Ellis MH, Dennis ES, Peacock WJ, 1999. Arabidopsis roots and shoots have different mechanisms for hypoxic stress tolerance. *Plant Physiology* 119, 57-64.

Freeling M, Bennett DC .1985. Maize Adh1. *Annual Review of Genetics* 19, 297-323.

Geigenberger P, Fernie AR, Gibon Y, Christ M, Stitt M. 2000. Metabolic activity decreases as an adaptive response to low internal oxygen in growing potato tubers. *Biol. Chem.* 381, 723-740.

- Hagermann RH, Flesher D. 1960. The effect of anaerobic environments on the activity of alcohol dehydrogenase and other enzymes in corn seeds. *Archives of Biochemistry and Biophysics* 87, 203-209.
- Hoeren F, Dolferus R, Wu Y, Peacock WJ, Dennis ES. 1998. Evidence for a role for AtMYB2 in the induction of the Arabidopsis alcohol dehydrogenase (ADH1) gene by low oxygen. *Genetics* 149, 479-490.
- Ismond KP, Dolferus R, De Pauw M, Dennis ES, Good AG. 2003. Enhanced low oxygen survival in Arabidopsis through increased metabolic flux in the fermentative pathway. *Plant Physiology* 132, 1292-1302.
- Jacobs M, Dolferus R, Van Den Bosshe VB. 1988. Isolation and biochemical analysis of ethyl methyl sulfonate induced alcohol dehydrogenase null mutants of Arabidopsis thaliana (L.) Heynh. *Biochemical Genetics* 26, 102-112.
- Jones ML, Woodson WR. 1999. Differential expression of three members of the 1-aminocyclopropane-1-carboxylate synthase gene family in carnation. *Plant Physiology* 119(2), 755-64.
- Kader AA. 1986. Biochemical and physiological basis for effects of controlled and modified atmospheres on fruit and vegetables. *Food technology* 40(5), 94-104.
- Kanellis AK, Solomos T, Mattoo A. 1989. Hydrolytic enzyme activities and protein pattern of avocado fruit stored in low oxygen atmosphere. *Plant Physiology* 90, 257-266
- Ke DL, Goldstein M, O'Mahony, Kader A. 1991. Effects of short term exposures to low O₂, or high CO₂ atmospheres on quality attributes of strawberries. *J. Food Sci.* 56, 50-54.
- Kelley PM, 1989, Maize pyruvate decarboxylase mRNA is induced anaerobically. *Plant Molecular Biology* 13, 213-222.

ASSESSMENT METHODS: Written exam

HOB.545 – **Advanced GMO Detection Methodologies (Lab)** (3 ECTS) _____

LECTURER: Dr. O. Koutita

TYPE OF COURSE AND TEACHING METHODS: Laboratory

OBJECTIVE OF THE COURSE: To provide hand on experience on GMO detection using PCR technology

COURSE CONTENTS

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.

RECOMMENDED READING

Detection of genetically modified organisms (GMOs) by PCR: a Brief review of methodologies available, Review, E. Gachet et al., *Trends in food science and technology* 9, 1999.

PCR detection of genetically modified Soya and Maize in foodstuffs, C. D. Hurst et al., *Molecular Breeding* 5, 1999.

Detection of genetically modified organisms in foods, Review, Farid E. Ahmed, *Trends in Biotechnology*, Vol.20 No.5, 2002.

ASSESSMENT METHODS: Written exam

HOB.546 - **Environmental Risk Assessment of GMOs** (3 ECTS) _____

LECTURER: Prof. M. Wilkinson

TYPE OF COURSE AND TEACHING METHODS: Lectures

OBJECTIVE OF THE COURSE: To introduce students into the concepts of the potential risk of GMO release into the environment

COURSE CONTENTS

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.

RECOMMENDED READING

Risk assessment of GM plants: Avoiding gridlock?, M. J. Wilkinson et al., *Opinion, Trends in Plant Science*, Vol.8 No.5, 2003.

Hybridisation between *Brassica napus* and *B. rapa* on a national scale in the United Kingdom, M. J. Wilkinson and al., *Science*, vol.302, 2003.

Gene flow and introgression from domesticated plants into their wild relatives, N. C. Ellstrand et al., *Annu. Rev. Ecol. Syst.* 30, 1999.

ASSESSMENT METHODS: Written exam

ACADEMIC SUPPORT FACILITIES

Laboratory of Plant Biotechnology and GMO Tesing

The laboratory of Plant Biotechnology & GMO Testing serves two purposes: a) to provide the infrastructure for training of students and support the research interests of the Horticultural Genetics & Biotechnology Department. b) To offer services to food industries on qualitative and quantitative analysis for the presence of GMOs in agricultural products.

The research interests of this laboratory focus on plant physiology, post-harvest biotechnology and technology, Arabidopsis genetics and development of GMO testing technology.

The molecular basis of hypoxic and anoxic response

Molecular signalling in plant senescence

The molecular characterization of the abscission process and its manipulation to improve crop characteristics using cotton as model plant

DNA genotyping of horticultural crops such as olives

Development of quantitative GMO standards for cotton

The facility includes an extended line-up of up-to-date computer-assisted apparatus, including: Automated (Capillary) DNA Sequencing Apparatus, Real Time / Quantitative PCR, 2 PCR Thermocyclers, In-situ PCR, 2 Centrifuges (up to 24,000 rpm), Bench-top Centrifuges (up to 6,000 rpm), microcentrifuges, DNA and protein Electrophoresis Apparatae, Shaking Incubators, Microscopes linked to digital camera, Microtome, Laminar Flow, Gel Documentation System, Autoclave, a Photosynthesis meter, Controlled Environment Chambers, Deep Freeze Chambers (-70°C), Cold Rooms (4°C), Tissue Culture Room, Growth Room, Radioactive Work Room, Photo dark room.

This laboratory supports the following research sector:

Laboratory of Plant Molecular Histology

This facility is part of the Plant Biotechnology & GMO Testing laboratory and comprises infrastructure necessary for histochemical staining of plant tissues, in-situ hybridization and in-situ PCR experiments. The equipment includes a state of the art LEICA RM2155 microtome, an Applied Biosystems in-situ PCR, a LEICA EG1140H Parafin embedding station, a LEICA EG1140C cooling plate and a LEICA TP1020 automatic tissue processor.

Laboratory of Horticultural Products Quality

This facility is part of the plant biotechnology laboratory and comprises infrastructure necessary for post-harvest quality assessment of fruits and vegetables. The equipment includes a gas-chromatograph, facility for controlled atmosphere experiments, an oxygen/CO₂ analyzer and necessary equipment for assessing the physicochemical properties of hort products.

MASTERS OF SCIENCE PROGRAM

Research Project (9 month duration)

Requirement

60 ECTS credits. Laboratory techniques (Related to the subject of the Master Thesis)

Research Subject Areas (topics generally available for Master of Science thesis):

- Molecular signalling in plant senescence using as models cut carnation flowers and tomato fruits in order to extend the storage life of horticultural products
- The 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: The Use of RNA Interference to Knockout Arabidopsis P4H Genes (2004)

AUTHOR: Ihab Lolos

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 Bită

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: Suppression 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, 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 30 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. Considering their implication in the programme, the following academic staff is taken as reference (Annex I includes further details of these lecturers):

GREECE

Higher Education Institutions

- E. Tsouvelekas, University of Rethymnon
- N. Fanourakis, Technical Educational Institution, Heraklion
- K. Vlachonassios, A. Tsaftaris, A. Kanellis, E. Sfakiotakis, D. Gerasopoulos, Aristotle University of Thessaloniki
- G. Nanos, University of Thessaly
- P. Chatzopoulos, Agricultural University of Athens

Research Centres

- K. Sekeris, National Research Institute
- D. Kafetzopoulos, K. Kalantidis, Institute of Molecular Biology
- Dr. G. Kotoulas, Dr. K. Tsingenopoulos, Institute of Sea Biology
- O. Koutita, Hellenic Sugar Industry

Private Companies

- Mrs. K. Grogoriadou, Mr. N. Leventakis, Mr. Chr. Dovas, Vitro Hellas

FRANCE

- J.C. Pech, ENSAT

ITALY

Higher Education

- E. Rugini, University of Tuscia
- G. Casadoro, University of Padova

SPAIN

Research Centres

- Jordi Garcia Mas, Pere Arus, Laboratori de Genètica Molecular Vegetal CSIC-IRTA, Cabriels-Barcelona

UNITED KINGDOM

Higher Education Institutions

- M. Wilkinson, Reading University

UNITED STATES

Higher Education Institutions

- E. Moudrianakis, John-Hopkins University
- J. Scandalios, North Carolina State University

The Institute reserves the right to replace its visiting faculty according to its evaluation process.