Universidade do Minho

universidade de aveiro

U.PORTO

**Programa Inter-Universitário de Doutoramento**

**em Biologia de Plantas - BioPLant**

***Joint Doctoral Programme in Plant Biology***

MAP JOINT DOCTORAL PROGRAMMES

# 1ª Workshop Anual Bioplant

## 29 - 30 de Março de 2010 / *29 - 30 March 2010*

**PROGRAMA** / ***PROGRAMME***

**LIVRO DE RESUMOS** / ***BOOK OF ABSTRACTS***

FUNDAÇÃO CALOUSTE GULBENKIAN

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Caixa Geral de Depositos

Natural Concepts

***Bem vindos*** *à 1ª Workshop Anual BioPlant organizada pela 1ª edição do Programa Inter-Universitário de Doutoramento em Biologia de Plantas – BioPlant, das Universidades do Minho, Aveiro e Porto (programa MAP).*

*Esta* ***1ª Workshop Anual BioPlant*** *funcionará como um* ***fórum de encontro*** *entre os alunos BioPlant, potenciais orientadores, investigadores e empresas / agentes sócio-económicos, que potenciará a implementação de interacções essenciais para o desenvolvimento de investigação de excelência, de cariz fundamental ou aplicado, pelos alunos BioPlant.*

*Estes são os primeiros passos de um Programa de Doutoramento que pretende potenciar ao máximo o conhecimento académico existente no nosso país em Biologia de Plantas para a formação avançada de jovens, ao mesmo tempo que promove a abertura da Universidade e a aplicação do seu saber à realidade sócio-económica de Portugal. Contamos com o interesse e o envolvimento de todos os presentes para fazer crescer o BioPlant!*

***Welcome*** *to the 1st Annual BioPlant Workshop organized by the 1st edition of the Joint Doctoral Programme in Plant Biology – BioPlant, from the Universities of Minho, Aveiro and Porto (MAP programme).*

*This* ***1st Annual BioPlant Workshop*** *should work as a* ***meeting forum*** *between the BioPlant students, potential supervisors, researchers and companies / socio-economical agents, which should potentiate the implementation of interactions essential to the development of investigation of excellence, with a fundamental or applied character, by the BioPlant students.*

*These are the first steps of a PhD Programme that intends to potentiate to its maximum the academic knowledge existing in our country in Biology of Plants for the advanced education of youngsters, at the same time it promotes the opening of the University and the application of its know-how to the socio-economical reality of Portugal. We count with the interest and involvement of all participants to make BioPlant grow!*

# Conteúdos / *Contents*



## Apoios / *Support*

O BioPlant tem o privilégio de usufruir de um financiamento da **Fundação Calouste Gulbenkian** ao abrigo do seu Programa de Reforço da Capacidade Científica para Projectos Inter-Universitários de Doutoramento, obtido em concurso nacional em que apenas dois programas foram subsidiados.
A organização desta Workshop contou também com o apoio da Unicer, da Caixa Geral de Depósitos e da Natural Concepts.

The BioPlant has the privilege of benefiting from the financing of the **Calouste Gulbenkian Foundation** under its Programme of Reinforcement of the Scientific Capacity for Inter-Universitary Doctoral Programmes, obtained through a national call in which only two Programmes were financed.
The organization of this Workshop has also counted with the support of Unicer, Caixa Geral de Depósitos and Natural Concepts.

## Comissão de Organização / *Organization Committee*

Mariana Sottomayor – Departamento de Biologia, FCUP
Paula Melo - Departamento de Biologia, FCUP
Carmencita Lino - EPALMO

## Comissão Directiva BioPlant / *Executive Committee BioPlant*

Mariana Sottomayor – UP (Directora do BioPLant 2009/2010)
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## Comissão Científica BioPlant / *Scientific Committee BioPlant*

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Alberto Dias - UM
Conceição Santos – UA
João Serôdio - UA
Manuel Ferreira - UM
Paula Melo - UP

# Programa / *Programme*

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## 29 Março / March 29th

09:00 - **Abertura** / ***Opening***
Maria de Lurdes Correia Fernandes, Vice-Reitora da Universidade do Porto

09.10 **- Apresentação do Bioplant** / ***Presentation of Bioplant***
Mariana Sottomayor, FCUP / IBMC, Directora do Bioplant

09:30 - **Conferencia convidada** / ***Invited lecture***
**Do negative results exist?** CC1
Robert Verpoorte, IBL **-** University of Leiden, The Netherlands

10:30 - **Coffee Break e/*and* Posters**

**Sessão 1 - Investigação Fundamental em Biologia de Plantas** / ***Basic Research in Biology of Plants***

11:00 – **Nitrogen Assimilation in the model legume *Medicago truncatula*** O1
Helena Carvalho, IBMC-UP

11:15 – **Flow Cytometry – an overview and potentialities** O2
Conceiçao Santos, DB-UA / CESAM

11:30 – **Health Improving Effects of Plant Metabolites through Induction of Stress Responses** O3
Cristóvão Lima, DB-UM / CITAB

11:45 – **Bacterial -plant symbiosis and the oxygen paradox: from a bad first date to a happily-ever-after finale** O4
Catarina Santos, IBMC-UP

12:00 – **Morphology, behaviour, ultrastructure and phylogeny of Dinophyta** O5 António José Brito Calado, DB-UA / CESAM

12:15 – **HP6 function during development of root lateral organs in *Arabidopsis thaliana* and *Medicago truncatula*** O6
Ana Campilho, IBMC-UP

12:30 - **Almoço e posters** / ***Lunch and Posters***

**Sessão 1 - Investigação Fundamental em Biologia de Plantas / *Basic Research in Biology of Plants***

14:30 **- Stress fotooxidativo em diatomáceas bênticas** O7
João Ezequiel, DB-UA / CESAM

14:45 - **New insights on metabolomics and bioactivities of *Catharanthus roseus*** O8
David Pereira, FFUP / REQUIMTE

15:00 - **Effects of microcystins in plants** O9
Ana Luisa Frazão Pereira, CIIMAR-UP

15:15 - **Genetic diversity in *Olea europaea* L. cultivars assessed by microsatellites markers and pollen allergens** O10
Isabel Amorim, FCUP / CGUP

15:30 - **Proteomics for analysis of protein expression in plants** O11
Alexandre Campos, CIIMAR-UP

15:45 - **The laboratory of Plant Peroxidases and Secondary Metabolism** O12
Mariana Sottomayor, FCUP / IBMC

16:00 - **Coffee Break e/*and* Posters**

**Sessão 1 - Investigação Fundamental em Biologia de Plantas / Basic Research in Biology of Plants**

16:30 - **Heavy metal environmental remediation by *Solanum nigrum* L.: the (metal-specific?) role of metallothioneins in heavy metal homeostasis** O13
Jorge Teixeira, FCUP / BioFIG

16:45 **- Understanding the molecular basis of plant recalcitrance against Agrobacterium mediated transformation** O14
Franklin Gregory, DB-UM / CITAB

17:00 - **Cardosins as tools to dissect vacuolar trafficking and sorting** O15
Cláudia Pereira, FCUP / BioFIG

17:15 - **Functional genomics in the model plant *Arabidopsis thaliana*** O16
Herlânder Azevedo, DB-UM / BioFIG

17:30 - 18:30 - **Posters**

# 30 Março / March 30th

**Sessão 2 - Investigação Aplicada em Biologia de Plantas / *Applied Research in Biology of Plants***

09:30 - **Ecological research applied to biodiversity conservation and Monitoring** O17
Helena Hespanhol, FCUP / CIBIO

09:45 - **Allergenic potential nature of airborne tree pollen in Porto assayed by aerobiological, immunochemical and hospital admissions data** O18 Helena Ribeiro, FCUP / CGUP

10:00 - **Agricultural research for Development: a contribution of Eco-Bio/IICT** O19
Ana Ribeiro, IICT, Lisboa

10:15 - **Diatoms as indicators of environmental change: from the cell to the community level** O20
Salomé Almeida, DB-UA / CESAM

10:30 - **Constructed Wetlands for domestic wastewater treatment – what do we know about its performances?** O21
Isabel Mina, DB-UM / CITAB

10:45 - **Plant physiological performance studies** O22
Celeste Dias, DB-UA / CESAM

11:00 - **Coffee Break e/*and* Posters**

**Sessão 3 - Actividades e Investigação em Empresas / *Activities and Research in Companies***

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Maria do Carmo Tavares da Silva, AmBioDiv – Valor Natural, Lisboa

11:45 - **From the lab to the market: exploring medicinal and aromatic plants** O24
Paulo Sérgio Carvalho Braga, Natural Concepts, Guimarães

12:00 - **Agricultura Biológica – Uma oportunidade com futuro** O25
Hélder Almeida, Mercatu – Alimentos Autênticos, Porto

12:15 - **Botânica Jardins** O26
Hernâni Madail e Cláudia Vaz - Botanica Jardins, Braga

12:30 - **Almoço e posters / *Lunch and Posters***

14:30 - **Conferencia Convidada / *Invited lecture***
**The Omic´s Era: - Impact on Plant Systems Biology and Plant Biotechnology** CC2
Maria Salomé Pais, BioFIG / FCUL

15:30 - **Coffee Break e/*and* Posters**

**Sessão 3 (continuação) - Actividades e Investigação em Empresas / *Activities and Research in Companies***

16:00 - **First line challenges for young entrepreneurs** O27
Luís Pontes, Peak Plants, Aveiro

**Sessão 1 (continuação) - Investigação Fundamental em Biologia de Plantas / *Basic Research in Biology of Plants***

16:15 - **Salt effects on growth, nutrient and secondary compound contents of *Diplotaxis tenuifolia*** O28
Cátia Guerra, DB-UA

16:30 - **Presence of atypical flowers in Aragonez (*Vitis vinifera* L.) cv.** O29
Ricardo Pinto, FCUP / CGUP

16:45 - **Identification and analysis of second site mutations in the *Arabidopsis thaliana dry2* mutant by Map-Based Cloning** O30
Vitor Amorim-Silva, DB-UM / BioFIG

17.00 - **Biopesticides made from MAP extracts: antinematode effects of *Hypericum* sp.** O31
Manuel Ferreira, DB-UM / CITAB

17: 15 - **Conferência de Encerramento / *Closing lecture***
**"GrapeBerryFactory" - Sugars, acids, phenolics and water on grape development and ripening** CE
Hernâni Gerós, DB-UM / CITAB

17:45 - **Sessão de encerramento / closing session**

18:00 - ***Chill Out***

# Conferencias Convidadas / *Invited Lectures*

## CC1

### Do negative results exist?

*R. Verpoorte; Y.H. Choi and H.K. Choi; Department of Pharmacognosy, Section Metabolomics, Institute of Biology Leiden, PO Box 9502, 2300RA Leiden, The Netherlands, Email: VERPOORT@LACDR.LeidenUniv.NL*

Metabolomics as the latest of the –omics has got quite some attention in the past years. It is a major tool, for example, in functional genomics, quality control of botanicals, studies on the activity of medicines and medicinal plants, and systems biology type of studies of the plant cell factory (Verpoorte et al. 2007, 2008). Metabolomics has the very ambitious objective to identify and quantify all metabolites in an organism. Numerous reviews have been written in the meantime pointing out the various advantages and limitations of the possible analytical methods. A major area for the application of metabolomics is systems biology. There are many definitions of systems biology. But basically systems biology is an unbiased measurement of as many different parameters as possible under different conditions (e.g. healthy plant versus infected plant) and uses various statistical/mathematical methods to determine possible correlations between certain compounds present and the effects observed. With other words there is no starting hypothesis, systems biology is fully based on observations, which are subsequently analyzed using various chemometric methods to find possible correlations between the different data, and based on that try to find (novel) The methods used in systems biology include metabolomics (determining as many as possible metabolites in an organism, or in an extract), proteomics (to determine possible changes in an organism on the level of proteins) and transcriptomics (which should detect up- and down regulated genes), as well as all kind of physiological measurements (e.g. plant growth, leave size). Such a systems biology approach is quite promising as, for example, for phytomedicines it offers new possibilities to relate activity to certain compounds, including the possibility to detect synergy and pro-drugs. So coming back to the title, when you understand what systems biology means you have the answer. This might also help to understand the problem noticed by Ioannidis (2005) who has shown that most research findings in life sciences are false, due to biased experiments. I very much look forward to further discuss this with you at the meeting!

## CC2

### The Omic's Era: - Impact on Plant Systems Biology and Plant Biotechnology

*Maria Salomé Pais - Laboratory of Plant Systems Biology – Unit of Molecular Biology & Plant Biotechnology (BioFIG) – Sciences Academy of Lisbon*
*Ed. ICAT, Campo Grande, 1749-016 – Lisboa, Portugal*
*Email address :- mspais@fc.ul.pt or msalomepais@gmail.com*

Only understanding Man efforts to survive, along the history, making use of the surrounding environment we can have an idea of the present human crisis when millions of people are confronted with extreme hunger or are fighting for arable land for water and for better quality of life. Since Neolithic period Man inhabiting regions as far as the Near East and terraces and valleys of Andean mountains was engaged on an increasing domestication of plants and animals as well as on adaptation of culture practices as a way to face different environmental conditions and personal needs. These efforts constituted the known Neolithic revolution. The conditions determining the type of culture in a region are: Climate, water availability, type of soil and social conditionings. After the last glaciation (11000BC) an important climatic change was responsible for very dry seasons that favoured the appearance of annual or perennial plants able to survive either by seeds production or formation of bulbs, stolons or tubers. These organs

enabled the capacity of food storage thus the change of dispersed social structures to organized villages. It is thus evident that the global changes at that time were responsible for plant adaptations / vegetation changes responsible for the appearance of agricultural practices. As far as and like in Neolithic period, the 21th century Man is again challenged with climatic changes with known problems in water availability and quality, soil quality and availability of arable land, appearance of more and more pests and diseases among others. Would it be responsible for a new Agriculture revolution, being said the Molecular Agriculture Revolution? Despite of the knowledge accumulated since year 1000AD that enabled a tremendous improvement of agriculture and of new hybrids more adequate to needs, and the available knowledge in the last decades of complete genome of model plants and animals including man, an integrated knowledge on plant systems biology is still far from being a reality. Living organisms are extremely well organised, this organization depending on an harmonized functional complexity. Although genes in the organisms seem to have a limited range of options, the functions they govern are almost unlimited. Humans and other mammals as well as potatoes, tomatoes or other plants like Arabidopsis share many genes. Major differences in appearance (phenotype) often disguise surprising similarities at the genetic level (genotype).

It is a common knowledge that phenotypic variation is not much based on drastic alterations on genome structure, the accountability of this variation being found in the level of expression of those genes in the genome and how they interplay. These differences in expression levels are responsible for significant changes in the level and ratio of encoded proteins and metabolites that ultimately are responsible for the phenotype as well as for the development and life capabilities of whatever is the living organism.

If and when humans, plants or microorganisms are unbalanced they can develop towards a disease state. An imbalance in biological pathways commonly leads to differences in dynamics and rhythms of the systems, thus eventually leading to strong changes accounting for undesirable symptoms that may end in stopping development, emerging diseases or death. Tracing those differences requires the analysis of millions of biological elements gathered by using different approaches such as transcriptomics, proteomics, metabolomics, metalomics and celomics (the omics), all together being networked by the bioinformatics, enabling its integration in a meaningful fingerprint of a particular state of systems – *The systems biology* -.

According to Hiroaki Kitano, (Science, Vol. 295, No. 5560. pp. 1662-1664) " *To understand complex biological systems requires the integration of experimental and computational research -- in other words a systems biology approach. Computational biology, through pragmatic modelling and theoretical exploration, provides a powerful foundation from which to address critical scientific questions head-on. The reviews in this Insight cover many different aspects of this energetic field, although all, in one way or another, illuminate the functioning of modular circuits, including their robustness, design and manipulation. Computational systems biology addresses questions fundamental to our understanding of life, yet progress here will lead to practical innovations in medicine, agriculture, drug discovery and engineering"*

Taking the example of human or animal diseases, comparing fingerprints of healthy and disease states, may reveal differences capable of definition of diagnostic disease or of biomarkers. Taking into account the similarities/differences, common denominators of disease / resistance might well lead to better understand the cause and effect elements of human and plant dis ases, thus creating the capacity of novel intervention strategies for the well-being of mankind through a better agriculture and agriculture practices. Such a drive can only be afforded by the creation of a permanent capability of research interaction and networking for a *Systems Biology* approach.

*Systems biology* being the iterative and integrative study of biological systems in response to perturbations is based on hypotheses built up from the results of global functional genomics analyses of the complexity of the genome, transcriptome, proteome, metabolome, etc. as previously considered. Its implementation by crossdisciplinary teams should allow accessing small variations in the large number of elements underlying functioning of biological systems. Systems biology may contribute to: Technology development; Advances in basic concepts of biology [Animal (including man), plant, microbial] ; World practical applications in predictive and preventive medicine for the well being of mankind; World practical applications in predictive, preventive and improved agriculture and forestry; World practical applications in environment preservation and quality rescue.

In this talk we will try to give an overview on the role of the Omic's on the integrative knowledge of plant systems biology, thus serving the biotechnology developments for a new agriculture more able to face the present challenges of a changing Global Climate and, at the end, saving the environment and contributing for the well being of mankind.

# Conferencia de Encerramento / *Closing Lecture*

## "GrapeBerryFactory" - Sugars, acids, phenolics and water on grape development and ripening

*Natacha Fontes[1]; Artur Conde[1]; Viviana Martins[1]; Henrique Noronha[1]; Richard Gonçalves[1]; Sandra Paiva[1]; Ana Regalado[2]; Alberto Dias[1]; Manuela Chaves[2]; François Chaumont[3]; Serge Delrot[4]; Ana Cunha[1]; Hernâni Gerós[1]*
*[1]Centro de Investigação e de Tecnologias Agro-Ambientais e Biológicas (CITAB), Departamento de Biologia, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal*
*[2]Instituto de Tecnologia Química e Biológica, Apartado 127, 2781-901 Oeiras, Portugal, [3]Institut des Sciences de la Vie, Université Catholique de Louvain, Croix du Sud 5-15, B-1348 Louvain-la-Neuve, Belgium, [4]UMR 1287, Ecophysiology and Grape Functional Genomics, University of Bordeaux, INRA, Institut des Sciences de la Vigne et du Vin, Domaine de la Grande Ferrade, 210 chemin de Leysotte, 33883 Villenave d'Ornon, France*

Photoassimilate transport and assimilation is of utmost importance for crop productivity. This is especially true in grape berry where the mesocarp and exocarp cells may store high concentrations of sugars, acids and phenolics in the vacuole. Several disaccharide and monosaccharide transporters were already identified in grape berry, such as VvHT1. Many research groups, including our own, have provided significant progresses in this research area, ultimately aiming the improvement of wine quality through better grapegrowing practices; however, unlocking the secrets behind grape berry development and ripening is still a major challenge. In the context of the former research project "Transport of monosaccharides in *Vitis vinifera*", developed in collaboration with the *Institut des Sciences de la Vigne et du Vin Bordeaux Aquitaine* (ISVV, France), special attention was paid to the transport of sugars and polyols in *V. vinifera* and *Olea europaea,* two economically important crop species whose productivity can be severely affect by several stress factors. In the recently approved project "GrapeBerryFactory" (PTDC/AGR-ALI/100636/2008) we are interested in the biochemical mechanisms and transport steps involved in the production and accumulation of sugars, organic acids and phenolics in grape berry, especially into the vacuole, and how these steps are coordinated during ripening and influenced by water limitation and changing temperatures. Also, the discovery of the functional role of a mannitol transporter during grape ripening, and how its expression is regulated by drought, will be a great challenge.

# Resumos das Comunicações Orais / *Abstracts of Oral Communications*

## O1

### Nitrogen Assimilation in the model legume *Medicago truncatula*

*Ana Rita Seabra; Liliana Santos Silva; Paula Melo; Ana Campilho and Helena Carvalho*
*Molecular Biology of Nitrogen Assimilation group; Instituto de Biologia Molecular e Celular. Rua do Campo Alegre, 823. 4150-280 Porto.*

Nitrogen is the major nutrient limiting plant growth and crop yield, it is therefore extremely important to understand the mechanisms by which it is taken up and utilized by plants. Legumes, unlike most other plant families, are able to form symbiosis with nitrogen-fixing bacteria (*Rhizobia*), enabling them to obtain nitrogen from the atmospheric $N_2$. These plants have a selective advantage in many natural environments, and they are valuable for sustainable agriculture. The group Molecular Biology of Nitrogen Assimilation is using the model legume *Medicago truncatula* to investigate the regulatory mechanisms that control the key enzyme Glutamine Synthetase (GS) and evaluate its involvement in the regulation of nitrogen use efficiency (NUE). GS catalyses the first step at which nitrogen is brought into cellular metabolism, and the enzyme is complex and precisely regulated, implying controls operating at several different levels. *M. truncatula* is particularly well suited for the study of GS because it contains a small GS gene family and is amenable to reverse genetics and functional genomics. We have therefore chosen this plant to obtain a holistic view of the mechanisms that regulate the enzyme and use this knowledge to modulate specific metabolic pathways. This presentation is intended to give an overview of the research performed by the group emphasizing the major scientific achievements on the structural, mechanistic and regulatory properties of the enzyme and its regulatory role in nitrogen metabolism.

## O2

### Flow Cytometry – an overview and potentialities

*Conceição Santos*
*CESAM, Dep. Biology, FUA, Aveiro, csantos@ua.pt*

Flow cytometry (FCM) allows multiparamteric analyses and separation of rare subpopulations within heterogenous populations. Plant FCM is far less developed than animal FCM. Several constrictions due to, e.g., the presence of cellulosic cells walls, richness in cytosolic compounds may interfere with a reliable plant FCM analysis and this field is currently under intense investigation. Plant FCM has mostly evolved in structural analyses (e.g. ploidy, DNA content), however functional plant FCM has unlimited interest providing a cytomic perspective of organized systems as a result of both genetic and environmental conditions. The advances performed in the last years concerning these two fields of plant structural and functional FCM will be covered and articulated with other plant science fields, such as genomics, biotechnology, phylogenetics, etc. In particular concerning, functional FCM, most studies are focused on cell cycle analyses, and other few available studies on cell viability and programmed cell death. This field is scarcely explored in plant sciences, but due to its potential, we predict that within the next decade a huge development will take place contributing to new insights on measuring plant cell parameters and plant research.

## O3

# Health Improving Effects of Plant Metabolites through Induction of Stress Responses

*Cristovao F. Lima*
*CITAB/Department of Biology, School of Sciences, University of Minho, Braga,Portugal.*

Epidemiological studies indicate a direct relationship between plant antioxidants intake and increased longevity as well as decreased mortality from chronic age-related diseases, such as cardiovascular diseases and cancer. However, due to their low bioavailability after intestinal absorption, plant bioactive compounds are likely to act not as direct antioxidants but rather through the modulation of proteins, gene expression and cell signaling pathways. As xenobiotics, we hypothesize that plant metabolites may induce stress responses and cellular antioxidant defenses by imposing mild stress to human cells.Our group is currently investigating the ability of some plant extracts and isolated phenolic compounds to induce cellular stress responses, namely the induction of heat shock proteins, the induction of cellular antioxidant defenses through Nrf2/ARE signaling, and induction of protein turnover pathways such as macroautophagy and proteasome activity.As an example, results from the ability of curcumin to induce cellular antioxidant defenses through induction of stress response in normal human skin fibroblasts will be shown. This work indicates that curcumin have hormetic effects, i.e. induce adaptive responses to a low or intermittent dose of otherwise harmful condition resulting in protection against subsequent stresses, which can be a useful approach towards aging intervention.

## O4

# Bacterial-plant symbiosis and the oxygen paradox: from a bad first date to a happily-ever-after finale

*Catarina L Santos[1]; Arlete Santos[1,2]; Anita Sellstedt[3]; Philippe Normand [4] & Fernando Tavares[1,2]*
*[1]Cellular and Applied Microbiology, Instituto de Biologia Molecular e Celular, Porto, Portugal*
*[2]Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Porto, Portugal*
*[3]Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, Umeå, Sweden*
*[4]Ecologie Microbienne, Université Lyon I, Villeurbanne, Lyon, France*

Oxidative stress, described as an unbalanced situation between the pro-oxidant and the anti-oxidant forces inside a cell, is a global stress situation characterized by an increase in the Reactive Oxygen Species (ROS) and able to damage virtually every molecule inside a living cell. Among other origins, ROS can be intentionally released by animals and plants upon the perception of a bacterial invader, playing both a cytotoxic and a regulatory role in the host innate immune responses. Interestingly, this process has been reported to happen in a number of mutualistic relationships, namely nitrogen-fixing symbiotic interactions. *Frankia* spp. are nitrogen-fixing soil Actinobacteria able to induce symbiotic root nodules in a wide range of plants. Comparative genomic analyses within *Frankia* strains have shown an unique kaleidoscopic organization of structural and regulatory genes engaged in the oxidative stress response, which suggests the existence of significantly different ways to sense and overcome ROS. In parallel with this *in silico* data, physiological analyses of the oxidative stress response in different frankiae strains revealed different patterns of regulation, transcription and activity of the ROS-scavenging enzymes. Comprehend how these different oxidative stress defence strategies were shaped along the evolution and if the symbiotic partners were part of the selective pressures is a fascinating evolutionary problem, and may contribute to the overall understanding of the oxygen-depending infecting mechanisms.

## O5

### Morphology, behaviour, ultrastructure and phylogeny of Dinophyta

*Antonio José Calado*

Dinophyta, or dinoflagellates, are a group of eukaryotic protists with an unusually high number of peculiar features. They are prominent members of marine and freshwater environments where they act both as important primary producers and as consumers. Some species are mixotrophic and therefore play a dual ecological role. Dinoflagellate species are essentially unicellular and therefore require a diversity of microscopical approaches to their study. I will illustrate work done for the elucidation of feeding mechanisms and behaviour, and the underlying cell features related to food uptake. The reevaluation of the phylogenetic relationships of some groups of dinoflagellates on the basis of a combination of morphology, external and internal fine structure, and DNA-based phylogenetic hypotheses will be exemplified.

## O6

### *HP6* function during development of root lateral organs in *Arabidopsis* thaliana and *Medicago truncatula*

*Ana Campilho and Helena Carvalho*
*IBMC, Porto, Portugal*

Adaptation of root architecture to nutrients and water availability in soils is crucial for optimized plant growth. The root system adapts its total absorptive surface through the coordinated development of lateral roots in response to environmental conditions. Additionally, roots from legumes (*Fabaceae*) have the unique ability to establish a symbiotic association with bacteria of the family *Rhizobiaceae* leading to the formation of a newly specialized root lateral organ: the root nodule. This organ provides the proper microenvironment for bacteria to fix atmospheric nitrogen.In the *Arabidopsis* root apical meristem, *AHP6* acts in a spatial specific way to inhibit cytokinin signaling*. Interestingly*, AHP6* is also expressed in *Arabidopsis* lateral root primordia and mature lateral roots. In the genome of the model-legume *Medicago truncatula*, i*n silico* analysis predicts the existence of two *AHP6* orthologues. One of them, *MtHP6a* is expressed in roots and interestingly, in the root nodules of *Medicago truncatula*. Therefore, *HP6* may play an important role during development of the two root lateral organs, lateral roots and root nodules. The presence of *HP6* s may limit the number of cells responding to cytokinin and thereby help to define and sharpen cell differentiation boundaries. The importance of this mechanism is being investigated during the development root lateral organs, using two plant models systems, *Arabidopsis thaliana* and *Medicago truncatula*.

## O7

### Stress fotooxidativo em diatomáceas bênticas

*Joao Ezequiel; CESAM/UA*

Marine biological productivity is largely based on the photosynthetic activity of diatoms, microalgae that account for 40% of global oceanic carbon fixation. In estuaries, up to 50% of the ecosystem primary productivity is due to microphytobenthos (MPB), the diatom-dominated benthic communities that form biofilms on intertidal flats. Exposure to high light causes photoinhibition, irreversible damages to the photosynthetic apparatus due to the accumulation of reactive oxygen species (ROS). Photooxidative stress is expected to represent a major cause of limitation of photosynthetic productivity. This project addresses the coupling between behavioural and physiological photoprotection against photooxidative stress in benthic diatoms.

## O8

### New insights on metabolomics and bioactivities of *Catharanthus roseus*

*David M. Pereira[a]; Federico Ferreres[b]; Mariana Sottomayor[c]; Patrícia Valentão[a]; Paula B. Andrade [a]*
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*Catharanthus roseus* is among the most important medicinal plants in the world, mainly due to the revolution that its anticancer alkaloids, vincristine and vinblastine, introduced in cancer chemotherapy. For many years the indolomonoterpene alkaloids were the main metabolites studied. Recently our group has been interested in this species' other metabolites (primary and secondary) and organic acids, aminoacids, phenolics and volatiles were characterized using different instrumental approaches, such as HPLC-DAD, HPLC-UV, HPLC-MS/ESI and GC-MS. The knowledge of this species chemistry resulted in further studies focusing other biological properties besides the anticancer properties already described. In particular, bioactivity-guided studies in several plant parts have revealed the high capacity of this plant's roots to inhibit the enzyme acetylcholinesterase, a key requirement for the development of new anti-Alzheimer drugs. Metabolomics–based studies led to the identification of the compounds responsible for this activity, which surpassed that of physostigmine, one of the reference acetylcholinesterase inhibitors. These compounds could constitute hit molecules for the development of new drugs to be applied in this neurological disorder. Moreover, a potent antioxidant activity was recently described, against both oxygen and nitrogen reactive species. In this talk, an update in *Catharanthus roseus* chemistry and bioactivity will be presented, thus showing how we still have much to learn with this unavoidable species.

## O9

### Effects of microcystins in plants

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The water bodies eutrophication increase the formation of harmful blooms of cyanobacteria (also called blue-green algae). Some genus of cyanobacteria such as the Anabaena or Nostoc synthesizes microcystins. Those toxins are cyclic heptapeptides with 7 amino acids [cyclo-(D-Ala-X-D-MeAsp-Z-Adda-D-Glu-MeDha), where X and Z are variable amino acids, D-MeAsp is D-erythro-β-methylaspartic acid, Medha is the N-methyldyhydroalanine and Adda is (2S, 3S, 8S, 9S)-3-amino-9-methoxy-10-phenyl-2 ,6,8-trimethyl-deca-4 (E), 6 (E)-dienoic acid]. When ingested by animals cause chronic or acute effects especially the disruption of the hepatocytes and eventually lead to animal death. Studies about their effects in plants are increasing but still not much is know about their effects at the cellular and metabolic levels. Probably, the irrigation of plants especially those with agronomic importance with contaminated water with cyanobacteria and/or microcystins may cause some physiological and/or cellular stress increasing the economic coats of agricultural explorations. However, since those toxins can accumulate in the plant cells a more serious a public heath problem can arise. In the present, the pteridophyte *Azolla* and the *Arabidopsis* can be used as models to study the effects at the molecular, cellular and physiological levels.

## O10

### Genetic diversity in *Olea europaea* L. cultivars assessed by microsatellites markers and pollen allergens

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Olive cultivation is one of the oldest agricultural activities in the Iberian Peninsula. The correct identification of the olive cultivars is very important for the conservation of genetic resources, and for the development of a competitive and sustainable olive production system. A number of Iberian olive cultivars have been so far identified by using either agronomical/morphological characters or different types of molecular markers. In this work Iberian olive cultivars were discriminated by 4 SSR markers.The increase in the area dedicated to olive tree growth is running in parallel to the increased use of this plant for ornamental purposes. Both factors are the major causes triggering the development of a relevant problem of public health like pollen allergy. The olive pollen is a major cause of allergy in Mediterranean countries, where up to 20% of the population of the areas with high density of olive plantations is affected of allergic symptoms. Ten out of the approximately 40-50 proteins causing this allergy have been isolated and characterized at the molecular level. Differences in the level of expression and sequence polymorphism of some allergenic proteins have been characterized.Since, some olive cultivars produce pollen displaying diverse allergenic loads and differential allergenic characteristics their knowledge is important for clinical and allergic patients.This work was supported by FCT-POCI 2010 and CSIC/Fundaçao Ciencia e Tecnología 2007PT0039.

## O11

### Proteomics for analysis of protein expression in plants

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Protein expression and activity, modulates the metabolism and biochemistry of plant cells. This has a strong effect in the behavior of plants allowing them to respond to environmental challenges. The analysis of protein expression and its activity is therefore a fundamental discipline towards a deeper knowledge in the physiology of plants. Proteomics has been regarded as a valuable methodology to undertake such studies allowing a high throughput protein characterization including post-translational modifications. A proteomics approach was used in previous research to identify bacterial protein markers and enolase as a plant response protein in the interaction between Olea europaea subsp. europaea and the bacteria Pseudomonas savastanoi pv. Savastanoi. Proteomics was also applied to characterize the expression and interaction of Zea mays L. plastidial transglutaminase with thylakoid membrane proteins. Ongoing research concerns the effects of natural toxins produced by cyanobacteria in plants and the identification of the pathways of toxin's up-take, bio-accumulation/transformation and toxicity. Molecular biology and proteomics are therefore valuable strategies to conduct the research and investigate key molecules involved in these pathways.

## O12

### The Laboratory of Plant Peroxidases and Secondary Metabolism

*Mariana Sottomayor*
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The Laboratory of Plant Peroxidases and Secondary Metabolism is a young and enthusiastic group of researchers with a strong collaborative and training ethos. Our work has developed around the interest on the biosynthesis of the anticancer terpenoid indole alkaloids (TIAs) of the medicinal plant *Catharanthus roseus*, which are produced in extremely low levels. We have characterized a class III peroxidase III (Prx) involved in a key biosynthetic step of the TIAs, and therefore we became also interested in this intriguing group of enzymes. Prxs are located at the wall and vacuoles of plant cells, where they can oxidate and cross-link a number of different secondary metabolites at the expense of $H_2O_2$. We have been investigating how Prxs reach either the vacuole or the cell wall and which may be the reactions they catalyze in vivo, and their physiological consequences, namely for plant-pathogen interactions, senescence and root growth. The work with Prxs has evolved from *C. roseus* to the model plant *Arabiodopsis thaliana*, with which we have now acquired significant expertise. Concerning *C. roseus*, we are also interested in the improvement of the biotechnological potential of this highly valuable plant, namely in what concerns unraveling the metabolism of their anticancer TIAs, and the characterization of new bioactivities and respective phytochemical basis. We are now implementing a powerful strategy involving omic approaches that should lead to the discovery of novel candidate genes involved in the biosynthesis, regulation and transport of the TIAs. We are also optimizing transient expression techniques for functional analyses of the candidate genes, and a protocol for the generation of *C. roseus* transgenic plants.

## O13

### Heavy metal environmental remediation by Solanum nigrum L.: the (metal-specific?) role of metallothioneins in heavy metal homeostasis

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The environmental pollution caused by heavy metals is, nowadays, a major ecological problem with disastrous future consequences to our planet. One of the emerging technologies to solve this problem is phytoremediation, the use of plants and their associated microbes for environmental cleanup. *Solanum nigrum* L. is a plant species that hyperaccumulates Cd and Zn, and is a potential candidate for the phytoremediation of other metals, such as chromium (III) and nickel. In this work, *S. nigrum* plants were grown hydroponically for 4 weeks in Hoagland solution under different situations: one set without Ni or Cr (III); two sets, one exposed to 7.5 μM Ni and another to 375 μM Cr; and other two sets consisted on a short shock treatment with 100 μM Ni or 1 mM Cr throughout the last week. Plant fresh and dry weight, shoot and root length, and the variation of water content were determined. Total RNA was extracted and used for RT-PCR reactions aimed at amplifying *S. nigrum*-specific cDNAs encoding metallothioneins (MTs). The preliminary results reveal that *S. nigrum* can tolerate very high concentrations of Cr (III) in the rhizosphere and that the exposure to Ni is detrimental to plant growth. The mRNA accumulation data obtained so far suggests that MTs may play specific roles in these heavy metals' homeostasis. Future studies will be performed to evaluate the degree of stress that plants are subjected when exposed to these Ni or Cr treatments, at the biochemical and molecular levels.

## O14

### Understanding the molecular basis of plant recalcitrance against Agrobacterium mediated transformation

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Plant recalcitrance is the major barrier in developing Agrobacterium-mediated transformation protocols for several important plant species including Hypericum perforatum (HP). Hence, we studied the response HP suspension cultures upon elicitation by Agrobacterium. When challenged with Agrobacterium, HP cells swiftly produced an intense oxidative burst, a typical reaction of plant defense. Agrobacterium viability started to decline and reached 99% mortality within 12 h, while the plant cells did not suffer apoptotic process. RNA blot analyses of HP cells co-cultivated with Agrobacterium have shown a rapid up-regulation of genes encoding important enzymes of the general phenylpropanoid pathway (PAL, phenylalanine ammonia lyase and 4CL, 4-coumarate:CoA ligase) and xanthone biosynthesis (BPS, benzophenone synthase). Analyses of HPLC chromatograms of methanolic extracts of control and elicited cells (HP cells that were co-cultivated for 24 h with A. tumefaciens) have revealed a 12-fold increase in total xanthone concentration and also the emergence of many xanthones after elicitation. Methanolic extract of elicited cells exhibited significantly higher antioxidant and antimicrobial competence than the equivalent extract of control HP cells indicating that these properties have been significantly increased in HP cells after elicitation. Four major de novo synthesized xanthones have been identified as 1,3,6,7-tetrahydroxy-8-prenyl xanthone, 1,3,6,7-tetrahydroxy-2-prenyl xanthone, 1,3,7-trihydroxy-6-methoxy-8-prenyl xanthone and paxanthone. Antioxidant and antimicrobial characterization of these de novo xanthones have revealed that xanthones play dual function in plant cells during biotic stress: (1) as antioxidants to protect the cells from oxidative damage and (2) as phytoalexins to impair the pathogen growth. From our study, it is clear that HP recognizes Agrobacterium as a potential pathogen and rapidly evokes its defense responses, leading to the drastic reduction of Agrobacterium viability. This could be one of the main reasons for the recalcitrance of HP against Agrobacterium infection. Unraveling the molecular mechanisms underlying Agrobacterium recognition and defense activation is in progress.

## O15

### Cardosins as tools to dissect vacuolar trafficking and sorting

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Cardosin A and B are two similar APs that accumulate in large amounts in the pistils of Cynara cardunculus and in germinating seeds. Cardosins expression seems to be developmental regulated and associated with organs under high metabolic activity despite a steady-state level have recently been detected. In cardoon pistils these two enzymes localize differently, being cardosin A vacuolar and cardosin B secreted, which led to the study of the different trafficking and sorting mechanism accounting for these specific accumulation. Cardosins biogenesis and trafficking pathways studied in Arabidopsis thaliana and Nicotiana tabacum heterologous systems showed that cardosins enter the secretory pathway and pass through the Golgi in their route to the vacuole or to the cell wall. In these systems, cardosin A is also found to accumulate in LVs or PSVs; despite cardosin B being secreted in A. thaliana seedlings, is vacuolar in N. tabacum leaves, which raised the hypothesis that secretion is a specific event occurring in some specialized tissues. Furthermore, we investigated which domains of cardosin A are responsible for its vacuolar accumulation. Constructions were obtained with the removal of the PSI and the C-terminal peptide and we found that vacuolar accumulation was not impaired by the lack of these regions separately. Moreover, the PSI and the C-terminal peptide were capable of directing a secreted mCherry to the vacuole, proving that both domains are effective in the sorting. It is not yet clear how these two regions work in the native protein and which one is commanding the sorting; but it is possible that the existence of these two signals reflects the existence of different routes to the vacuole. Cardosins expression and accumulation patterns, both in the native and in heterologous systems, seems to reflect a differentiation of the trafficking pathways according to specific cell needs.

## O16

### Functional genomics in the model plant *Arabidopsis thaliana*

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The *Arabidopsis thaliana* genome was fully sequenced in 2000, providing an important basis for functional discovery in plants. Despite the relevant increase in post-genomic tools, determining the function of all genes is still a tremendous challenge. Functional screening in the *A. thaliana* biological model makes use of phenotype-centred forward and reverse genetic approaches, usually using loss-of-function mutant analysis. Based on a reverse genetics approach, the Plant Functional Biology group at the University of Minho has been carrying out research that tries to unravel the molecular mechanisms of various aspects of plant physiology. These include the biology of abiotic stress resistance mechanisms (*e.g.* heat and drought), the physiology of flowering and the regulatory function of post-translational protein modification mechanisms.

## O17

# Ecological research applied to biodiversity conservation and monitoring

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The North of Portugal supports an exceptional diversity of ecosystems which are the support of a remarkable number of species of flora and fauna. Present landscapes are subjected to profound environmental changes, either by the modifications caused by the agricultural systems and forestry production, or by the increasing road network, the expansion of major urban areas, the invasion of ecosystems by alien species or the construction of new infrastructures for energy production. It is expected that the impacts caused by these environmental drivers of change continue to increase in the first decades of the present century, aggravated by the likely impacts of global environmental changes. In this context, biodiversity monitoring (understood as regular sampling of data on parameters and indicators selected according to specific objectives of monitoring) is a fundamental tool in the pursuit of conservation and to assess the progress made towards the target of reducing biodiversity loss by the year 2010 and beyond. As a contribution for such goals, an integrated information and monitoring system for biodiversity in the Northern region of Portugal is being developed, with the general purpose of regularly providing information on the status of regional biodiversity to support management actions, technical-political decisions, and international reporting on the status of national biodiversity.

## O18

# Allergenic potential nature of airborne tree pollen in Porto assayed by aerobiological, immunochemical and hospital admissions data

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Anemophilous trees release great amount pollen, being an important source of allergens. The aims of this work were to characterize the aerobiology of *Platanus*, *Acer*, *Salix*, *Quercus*, *Betula* and *Populus* pollen, linking it with monthly emergency hospital admissions and to identify the reactivity levels in sensitized patients. The study was conducted in Porto (2005-2009). Airborne pollen sampling was performed using a Hirst-type volumetric spore trap. The antigenic and allergenic properties of pollen collected from urban trees, were investigated using SDS-PAGE and immunological techniques using polysensitized-patient sera. Hospital admissions of asthma or dyspnea related with respiratory diseases were obtained from the Emergency Room database of Hospital Geral de Santo António. High levels of airborne tree pollen, considered moderate to high risk for allergenic reactions, and emergency hospital admissions were related. High binding affinity of specific IgE to pollen extracts of the most abundant tree pollen present in the atmosphere (*Acer*, *Salix* and *Platanus*) was observed. Patient sera revealed multiple similar allergenic bands shared by the different extracts which can indicate the existence of a similar pollen allergenic profile and possible cross-reactivity reactions. Studies combining aerobiological, immunochemical and clinical approaches can be useful in medical practice as well as in arquitectural landscape planning, avoiding the use of allergenic pollen producer species.

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## O19

### Agricultural Research for Development: a contribution of Eco-Bio/IICT

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The main mission of the Tropical Research Institute from Portugal (IICT) is to promote international cooperation with tropical countries, particularly with those from the Community of Portuguese-Speaking Countries (CPLP). Thus, within IICT, the different research units are committed to promote research and training for development. The Plant Ecophysiology, Biochemistry and Biotechnology Center (Eco-Bio) conducts research on plant-environment interactions, biodiversity, and food security, aiming at contributing to solve questions of agronomic, social, ecological or industrial relevance. Under this context, several partnerships with CPLP institutions have been established. The interdisciplinary ongoing projects combine the areas of physiology, biochemistry, genomics, proteomics, biotechnology, phytopathology and post-harvest technology, and include: i) fundamental and applied research; ii) rural community development actions; iii) training; and iv) civil society. During this presentation an overview of the Center will be presented with emphasis on four emblematic programs: abiotic stress in coffee (partnership with Brazil), agrobiotechnology for development (the Mozambique case study), biofuels (public-private partnership with Geocapital and action plan in Cape Verde, Guinea Bissau and Mozambique) and food security (key actions in Guinea Bissau and Mozambique).

## O20

### Diatoms as indicators of environmental change: from the cell to the community level –

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## O21

### Constructed Wetlands for domestic wastewater treatment – what do we know about its performances?

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Known in Portugal since 1980's, the Constructed Wetlands (CW) or reed beds began, only about ten years ago, to be a choice of some municipalities to solve sanitation problems of small communities, particularly those located in rural areas. At present, Águas do Ave, a company responsible for a Multimunicipal Sanitation System have six *FitoETARs* operating in the Vale do Ave region. These CW are generally designed according to Reinhold Kickuth, consisting of rooted emergent macrophytes (mainly, *Phragmites*) planted on an optimise substrate with a wastewater horizontal subsurface flow (HSSF). Microbial monitoring of these systems is generally scarce, despite the need of more knowledge about its biocenosis. The sanitation quality of a wastewater treated in a CW is a crucial aspect, mainly when the receiving water body is used as a swimming and/or recreation area. Beyond the information about the sanitation quality of the treated wastewater, knowledge on the dynamics of microbial communities established in such systems may give us valuable information in order to enhance its performances. The dynamics of microbial communities established in one of these systems was assessed using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). The results revealed (i) a high bacterial diversity within the system, (ii) no relevant differences in composition at the inlet and outlet of the reed bed and (iii) temporal differences in bacterial communities that seem be associated to different life stages of the reeds.

## O22

### Plant physiological performance studies

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The Laboratory of Biotechnology and Cytomic of the University of Aveiro/CESAM has several lines of investigation in the plant and animal fields. Her, we present two of this research lines in the plant area. These works focuses mainly on the study of the plant performance, mainly through the measurements of the photosynthesis (gas exchange and chlorophyll a fluorescence), carbohydrates and pigments contents, and on the oxidative stress through the measurements of the enzymes and metabolites of the antioxidant system (e.g. catalase, Gr, SOD). *Research line 1: Plant heavy metal stress:* Agriculture soils in many parts of the world are slightly to moderately contaminated by heavy metal (e.g. Cd, Pb and Hg). This is usually due to long-term use of fertilizers, contaminated sewage sludge application, dust from smelters, industrial waste and bad watering practices in agricultural lands. Metal toxicity in plants lead to a reduced growth and consequently to a loss of productivity. The effects of several cadmium concentrations on plant performance and on the antioxidant system are analysed in the model plant, *Lactuca sativa*.*Research line 2: Plant micropropagation – acclimatization*: The advantages of a rapid production of high quality plants by tissue culture draw the attention of its use in plant breeding programs. Presently, the benefits of integrating plant tissue culture (namely micropropagation) in agriculture and forestry large scale commercial production is irrefutable, namely on commercial plantation forestry. *In vitro* culture protocols are in general designed to provide the optimal culture conditions and the minimal stress environment for plant multiplication. However, due to the stress culture *in vitro* condition, when transferred to *ex vitro* conditions, plantlets are very susceptible to various types of stress. Plant physiological responses and plant stress defence mechanisms during the acclimatization of *Ulmus minor* to *ex vitro* conditions are analysed.

## O23

### Protect and manage Flora and Habitats with High Conservation Value through FSC

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The Forest Stewardship Council (FSC) is a certification system that provides accreditation for the sustainable management of forests, implying compliance with the principles and criteria that comprise the framework of the scheme. These include natural values such as flora, vegetation, fauna and landscape. Natural values should be identified, protected and monitored. Areas where rare, threatened or other exceptional natural values were identified constitute High Conservation Value Areas (HCVA). Thus, by using the Habitat Approach methodology – analysis of plant communities through Phytosociology (Braun-Blanquet, 1979), AmBioDiv has identified several species and habitats with conservation status in set-aside forest areas, providing important management guidelines for owners, farm and forest managers. In the present paper, RELAPE flora species (rare, endemic, localized, threatened and in risk of extinction) as well as the identified plant communities will be presented. The most relevant species and habitats are: *Narcissus jonquilla* in communities of *Ficario ranunculoides-Fraxinetum angustifoliae*; *Doronicum plantagineum* in communiites of *Arbuto unedonis-Quercetum pyrenaicae*; *Limodorum abortivum* in mosaic communities of *Oleo sylvestris-Quercetum suberis* and *Asparago aphylli-Quercetum suberis*; *Sphagnum squarrosum* in humid regions of *Cirsio filipenduli-Ericetum ciliaris*.

## O24

### From the lab to the market: exploring medicinal and aromatic plants

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***NaturalConcepts*** Lda. is a *spin-off* company devoted to the development, production and commercialization of medicinal and aromatic plant (MAP) products. It was formed in the Biology Department of the University of Minho and comprises graduate researchers in phytochemistry and health sciences. Few among its products are teas/infusions (**Folha d'Água - chá biológico®**), extracts for the food industry and dietary supplements (**BioTeknics - Natural Concepts®**). Additionally, ***NaturalConcepts*** Lda. is also committed to the research and development of new products, production methods and innovative solutions. Portugal has a very rich flora that has been used as a source of natural products for a long time. Allying the most recent techniques and scientific knowledge to the empirics gathered by the folk use of these medicinal plants, we are able to create new value-added products. Simultaneously, the creation of companies such as ours increases the demand for raw-materials, driving the emergence of new MAP producers. Hence, the establishment of networks comprising researchers, producers and industrial partners seems the adequate framework to encourage the formation of innovative and competitive new companies in this field in Portugal.

## O25

### Agricultura Biológica – Uma oportunidade com futuro

*Hélder Almeida*
*Mercatu – Alimentos Autênticos*

A perda da biodiversidade agrícola associada à agricultura intensiva é da ordem dos 75%, segundo estudo da FAO 1984. Actualmente, cultivam-se as mesmas variedades por todo o mundo, não se adaptando estas a todos os climas e tipos de solos existentes. Um dos princípios do modo de produção biológico é a selecção de variedades adequadas ao meio, como forma de luta contra pragas e doenças. Esta prática tem permitido contrariar a tendência de uniformização de variedades, abrindo uma janela para a manutenção do património vegetal herdado ao longo de inúmeras gerações. Segundo o organismo internacional Organic Monitor o sector da Agricultura Biológica cresce 5 000 milhões de dólares ao ano, caracterizando-se por taxas de crescimento de 15% ao ano. Este crescimento é justificado por estilos de vida mais saudáveis e pelo incremento da consciência ecológica. Os consumidores já não restringem a sua procura a produtos alimentares biológicos, procurando também bens não alimentares como cosméticos, produtos de higiene pessoal e de limpeza com certificação Bio. Toda esta evolução é sustentada por investigações relativas às potencialidades e aplicações na indústria das variedades regionais em declínio, permitindo o seu desenvolvimento na paisagem agrícola assim como o surgimento de novas áreas de negócio.

## O26

### Botânica Jardins

*Hernâni Madail*

Botânica Jardins brings together stunning gardens to create the ideal landscape to suit your lifestyle. We offer the highest standards in prestige, traditional and contemporary landscaping. Our company's main core has the highest qualifications and extensive experience in landscape construction. Hernani Madail is the Botânica Jardins' landscaper; he has a degree in Agronomical Engineering and he's regarded as leader in his field with a combined industry experience of more than ten years. With our acknowledged expertise, we have the right landscaping services and skills to design and build the best outdoor living solutions. Our services include all forms of hard landscaping, horticultural expertise, water feature construction and garden lighting. We also have a large experience in irrigation systems complementing the overall project for our clients.

## O27

# First line challenges for young entrepreneurs

*Luís Pontes; Maria J. Sousa*

Keywords: plant tissue culture, comercial research, market aplication, implementation, cost efectiveness

The aim of our presentation in representation of the University of Aveiro Peak Plants spin off project, is mainly the display of the general guidelines that should orientate such biotechnology ventures, with a special enphasis in encouraging younger entrepreneurs. Our mission with this presentation is to open the minds of young researchers for an ongoing global atempt, to turn kitchen based research work, into successfull business of not less chalenging scientific value upgrade. With this presentation we want to open a window of enthusiasm with specific examples, without smoothing up any of the risks envolved in a market currently filled up with a miriad of services and strategies to take advantage of the natural lack of experience o most reseachers in the Portuguese academic based scientific teams.

## O28

# Salt effects on growth, nutrient and secondary compound contents of Diplotaxis tenuifolia

*Cátia Carina Guerra; Arjen de Vos; Conceição Santos*
*Universdidade de Aveiro*

This work focuses on growth responses of *Diplotaxis tenuifolia* L. grown under salt stress conditions. Plants were grown in water culture at 0, 50, 100, 200 and 300 mM NaCl, survived, grew and reproduced in all these salinity treatments. Signs of growth inhibition were noticed for the highest salt concentrations. Plant dry matter content and leaf area were successfully measured and relative growth rate (RGR) and its components, Unit Leaf Rate (ULR, usually known as NAR) and Leaf Area Rate (LAR) were calculated. Expansion of plant leaf area reduced with increasing salinity, with the highest plant productivity (ULR) obtained at 100 mM NaCL. The slight increase in RGR values was probably due to higher values of LAR, while values for ULR presented little variation. Accumulation of cations decreased with increasing salinity but $Na^+$ increased to very high values. Na/K ratio increased significantly with increasing salinity; old leaves showed much lower values for this ratio than the new leaves, which may indicate a compartmentation of $Na^+$ towards the old leaves. Total polyphenol content and quercitine maxed at 50 mM NaCl. Nitrogen decreased with increasing salinity although not as drastically as other ions ($Ca^{2+}$, $Mg^{2+}$, $K^+$). Results show optimal growth at moderate salinities and maximum productivity at 100 mM. This works' findings will help agricultural sustainability programs in the selection for halophytic species for their economical potential

## O29

### Presence of atypical flowers in Aragonez (V. vinifera L.) cv.

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In a vineyard, located in Douro region (North of Portugal) some plants of the cv Aragonez presented different floral morphological characteristics of this and other cultivars of the same genus. The aim of this work was to study comparatively the plants with atypical and normal flowers. Flower and pollen morphology was examined by light and scanning electron microscope. The atypical flowers presented short pedicel, almost sessile; the corolla at anthesis did not form a calyptra (apical dehiscence), the filaments were short, the stigma was sessile, the ovary was globose, and the nectaries were underdeveloped. Pollen from both flowers was usually radially symmetrical, isopolar, tricolporate presenting sexine much thicker than nexine and reticulate tectum. DNA analysis revealed no polymorphism among these plants. The atypical flowers showed low fruit-set and productivity. Also, the berries were smaller with no or few seeds. The analysis of chemical parameters of the berries showed some differences between them. During the pruning the plants that produced atypical flowers showed greater vegetative growth. This work was supported by FCT-POCI 2010.

## O30

### Identification and analysis of second site mutations in the *Arabidopsis thaliana dry2* mutant by Map-Based Cloning

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In a search for genes determinant in plant drought tolerance we identified the *Arabidopsis thaliana* EMS recessive *dry2* mutant that is drought hypersensitive. The *dry2* mutant was identified using map-based cloning as being a *Squalene Epoxidase Gene-1* (*SQE1*) mutant allele. Mutations in SQE1 cause reduced root and hypocotyl elongation, diminished stature and unviable seeds, and an altered production of Reactive Oxygen Species (ROS) indicating an essential role for this gene in plant development. To further investigate this possibility we set out to identify second-site mutations induced by EMS that abrogated the drought hypersensitivity phenotype observed in *dry2,* as this phenotype is easily scored. Second site mutations in *dry2* background suppress the defective shoot growth and ROS accumulation. We are now in the process of identifying those second-site mutations using map-based cloning.

## O31

# Biopesticides made from MAP extracts: antinematode effects of *Hypericum* sp.

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Root-knot nematodes, *Meloidogyne* spp., are among the most wide-spread and economically important plant-parasitic nematodes in the world. They are known as damaging a wide variety of crops, especially in tropical and sub-tropical regions. Given the environmental concerns on the use of artificial chemical pesticides, various have been the attempts to find clean and green alternatives to control of such nematodes and the use of various plant species with antagonistic or even toxic effects, commonly known as medicinal and aromatic plants, has been subjected to research with the objective to find new applications of their essential oils and extracts as biopesticides effectives against several plagues, fungi and nematodes. In this work we performed the study of the essential oils and water extracts composition of *Hypericum* sp. and researched their antinematode effects. Eggs of *Meloidogyne javanica*, obtained directly from egg masses isolated from the tomato infected roots, were used for hatching studies. The egg masses were placed in a 5 cm-diam. Petri dish with distilled water and then, all eggs containing juveniles in the same second stage (J2), were separated under a stereomicroscope and transferred (20/ well) one by one or in small amounts with the aid of a dissection needle and/or a feather-stitch, to each of the wells of Enzyme Linked Immuno Sorbent Assay - ELISA (6 wells) plates. All tests were conducted in the wells of the ELISA plates containing 5 ml of the *Hypericum* water extracts at different concentrations: 4, 6, 8 and 10 g/100 ml. Distilled water was used as control with five replicates of each extract concentration. The plates were placed in the dark at 22 ± 2 ºC. Cumulative juveniles eclosion was calculated separating, and adding to the anterior ones, the juveniles ecloded from the eggs, every 24 hours till 360 h. Homogeneity of variance was verified for cumulative J2 eclosion by Bartlett's test. When it was not verified, data were transformed to log 10 (x+1) prior to ANOVA. Significant differences were analyzed by Tukey's or Dunn's tests ($P < 0.05$). For mortality studies egg masses of *M. javanica* were placed on a small square piece of muslin with 30 µm openings supported as a small sieve (2.5 cm ∅, 1 cm high) in a Syracuse (2 ml cap.) glass block; about 1 ml tap water was added, until the muslin was just submerged. Juveniles collected during the first 24 hr were discarded and the subsequent J2 collected were used. Twenty J2 were exposed to 2 ml of each of the same as anterior concentrations of the lyophilized extract: 4, 6, 8 and 10 g/100 ml. As before, distilled water served as control with five replicates of each extract concentration and the tests were conducted in the wells of ELISA plates maintained in the dark at 22 ± 2 ºC. A relation between the concentration of the extract and hatching of *M. javanica* was found. The effect on eclosion inhibition was higher for 10 mg/ml than for 4 mg/ml. However, in the first 24 h apparently there was no eclosion. The mortality of J2 of *M. javanica* was directly dependent on the concentration of the extract. being observed in the first 24 hours for 6, 8 and 10 mg/ml but only after 72 h with 4 mg/ml.

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# Resumos dos Posters / Abstracts of Posters

## P1

### Prevention and treatment of diabetic retinopathy by natural antioxidant containing in chitosan-based nanomedicines

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Oxidative processes are critical factors in ocular conditions that may lead to pathologies such as Diabetic Retinopahty due to structural and functional modification of the tissues, apoptosis of capillary cells and retinal microvascular changes, making the loss of vision inevitable. Application of antioxidants may reduce apoptosis and restores partially functional tissues. Considering the multiple benefits of antioxidants in DR and that conjunctival drug permeability improvement is one of the major challenges in ocular drug delivery, the major topic of this work is to study the absorption capacity and consequent bioavailability of natural antioxidants incorporated in chitosan-based nanoparticles to prevent and treat DR. In this regards, it is proposed to characterize, quantify and compare the antioxidant potential of *Salvia* sp. and *Satureja montana* extracts and their main pure antioxidants: rutin, caffeic and clorogenic acid. Then, antioxidant compounds are encapsulated into different chitosan-based nanoparticles, nanoparticles and release profile characterized and ocular permeation evaluated using *in vitro* cell models. Finally, the effect of antioxidant nanomedicines across human ocular is evaluated in diabetic animal after topical administration *in vivo*.

## P2

### Induction of Insulin Secretion and Antioxidant Protection in β-cells: Effects of *Catharanthus roseus* and compounds alone

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Diabetes mellitus type 2 is characterized by peripheral insulin resistance and impairment of insulin secretion. Beta-cells possess reduced levels of antioxidant defenses and, therefore, are more vulnerable to oxidative stress, which affects insulin secretion and ultimately results in cell death and loss of β-cell mass. HIT-T15 cells (hamster β-cells) were used here to study the potential of *Catharanthus roseus* (a plant traditionally used for its hypoglycemic potential) extracts and compounds to induce insulin secretion and protection against imposed oxidative stress. With this model we were able to reproduce the insulin secretion stimulatory effect of glibenclamide as measured by ELISA. In addition, oxidative stress induced by both *tert*-butyl hydroperoxide and 2-deoxy-D-ribose (dRib) negatively affected insulin secretion, which were prevented by the antioxidant NAC. Water extracts of *C. roseus* did not induced insulin secretion, contrarily to what have happened with some of its main flavonoinoids (aglycone) quercetin (Q) and kaempferol (K). Q also induced the levels of the intracellular antioxidant glutathione. Both flavonoids also protected against cell death induced by the sugar oxidant dRib. We conclude that HIT-T15 cells are a good model to study different types of damage in β-cells related with diabetes pathology, and possible protective effects of plant extracts/compounds.
*Acknowledgments:* This work was supported by the Mecenato Científico Jerónimo Martins SGPS 2009-2011.

## P3

### Chemoprevention and antitumor effects of *Salvia officinalis* extract on colon cancer: involvement of the MAPK/ERK pathway

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Epidemiologic studies have shown that diet and nutrition are key factors associated with colorectal cancer (CRC). PI3K/Akt and MAPK/ERK signaling pathways play a critical role in cell proliferation and survival and components of these pathways are found frequently altered in CRC. Plants from the genus *Salvia* (sage) are known for their wide range of medicinal properties, including anticancer activity. This study aimed to evaluate the effects of *Salvia fruticosa* (SF) and *Salvia officinalis* (SO) water extracts in two human colon carcinoma-derived cell lines, HCT15 and CO115, which have different profiles of constitutive activation of PI3K/Akt and MAPK/ERK pathways. Effects of SO in the prevention of ACF formation in rats were also evaluated. Our results showed that SF and SO were able to induce apoptosis and inhibit cell proliferation (mainly on HCT15 cells), measured by TUNEL and BrdU incorporation assays, respectively. Although both sage extracts and RA did not inhibit Akt phosphorylation measured by western blot, they were able to inhibit ERK phosphorylation in HCT15 cells, which have KRAS mutation. *In vivo*, SO significantly decreased DNA damage induced by $H_2O_2$ in colonocytes (measured by comet assay), and also decreased the number of ACF in rats. Our findings suggest a chemopreventive role of sage extracts in CRC. *Acknowledgements:* FCT supported CPRX (SFRH/BD/27524/2006) AAR (SFRH/BD/35672/2007) and DP (SFRH/BD/64817/2009), as well as the work (POCI/AGR/62040/2004).

## P4

### Sage drinking improves plasma lipid profile, erythrocyte antioxidant defences and increases lymphocyte Hsp70

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*Salvia officinalis* (common sage) is a medicinal plant to which antioxidant, anti-inflammatory and antimutagenic properties have been attributed. In order to test possible beneficial preventive effects to diabetes in humans, we performed a pilot trial with six healthy female volunteers. The trial was carried out in three phases, which includes two weeks of baseline, four weeks of sage treatment (drinking of a sage infusion twice a day) and two weeks of wash-out. Sage treatment positively affected the erythrocyte antioxidant status as shown by increased SOD and CAT activities. Cholesterol and LDL levels significantly decreased and HDL levels significantly increased after treatment, indicating benefits also in lipid metabolism. However, no changes in glucose clearance were observed in the oral glucose tolerance tests at the end of treatment period. In addition, a reduction of in vitro lymphocyte DNA damage induced by $H_2O_2$ was observed during the treatment period, which was maintained through the wash-out period. During the *S. officinalis* drinking period, lymphocyte Hsp70 protein expression was significantly increased (about 2.25 times) and decreased to baseline following the wash-out period. Overall these results confirm the health improving potential of sage infusion drinking. *Acknowledgements:* FCT supported CMS (SFRH/BD/42566/2007) and AAR (SFRH/BD/35672/2007), as well as the work (POCI/AGR/62040/2004).

## P5

### Degradation of Metalaxyl by Chemical and Biological Routes

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The photocatalytic treatment of pollutants has shown a huge potential in environmental protection, in particular for the treatment of water-soluble pesticides with limited biodegradability. In this work, the efficiency of the photo-Fenton process was investigated under different operating conditions. In this process •OH radicals are formed homogeneously from a mixture of iron ($Fe^{2+}$) salts and hydrogen peroxide ($H_2O_2$) in the presence of light. An actual persistent pesticide, mainly consisting of metalaxyl, was selected as model pollutant. The experiments were performed in a batch photoreactor with a UV-vis lamp as light source and a glass jacket which cuts-off UVB and UVC lights, with the aim to investigate the process efficiency under visible radiation. In a typical run, the pH of a metalaxyl solution (151 mg/L) was adjusted to 2.8 and different concentrations of $H_2O_2$ were studied. Samples periodically withdrawn were analyzed by HPLC and TOC. Among the results obtained, it was possible to show that metalaxyl is poorly degraded by direct photolysis. However, when $H_2O_2$ is added to the reaction a pronounced increase in the degradation rate of metalaxyl is observed. The integration of the solar-driven engineering approach with a biological route is currently being studied in order to analyze the effect of intermediates formed from the chemical process on *Solanum nigrum* L. plants and to evaluate the efficiency of the designed integrated methodology.

## P6

### Metabolic and biological activities of *Ficus carica* materials

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*Ficus carica* L. is one of the first plants cultivated by humans as a seasonal food and it is an important constituent of the Mediterranean diet. Metabolic and biological screenings were performed on leaves, pulps, peels and latex of two *F. carica* Portuguese white varieties ("Pingo de Mel" and "Branca Tradicional"). Phenolics and organic acids profiles were determined by HPLC/DAD and HPLC/UV, respectively. Leaves, pulps and peels presented a similar phenolic composition, including three hydroxycinnamic acids, two flavonoid glycosides and two furanocoumarins. The organic acids profile of the several matrices presented mono, di and tricarboxilic acids. Differences in the qualitative and quantitative composition were observed between the several matrices. The antioxidant potential of the different plant materials was checked by distinct *in vitro* chemical assays. All materials exhibited activity against DPPH and nitric oxide radicals. However, only leaves and latex presented capacity to scavenge superoxide radical. Leaves were always the most effective matrix, which seems to be related with the phenolics composition. Additionally, acetylcholinesterase inhibitory capacity was evaluated, but only with latex a weak effect was observed. This species is a good source of bioactive compounds, especially phenolics, and may contribute to the prevention of diseases in which homeostasis is impared by oxidative features. **Acknowledgments:** Andreia P. Oliveira (SFRH/BD/47620/2008) is indebted to Fundação para a Ciência e a Tecnologia (FCT) for the grant.

## P7

### *Pieris brassicae vs Brassica oleracea* L. var. *acephala*: Metabolic profiling and biological activity

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It is well known that Brassicaceae, namely *Brassica oleracea* varieties, are an important source of bioactive compounds. *Pieris brassicae* constitutes a common pest of *Brassica oleracea* var. *acephala* (kale). *P. brassicae*/kale ecological duo was studied.The phenolics of *P. brassicae* (larvae, butterfly and excrements) and host plant were determined by HPLC/UV-DAD/$MS^n$-ES. Biological activity of kale and *P. brassicae* materials was evaluated using cell free systems and cellular assays. Thus, the antioxidant capacity of these matrices against DPPH, $O_2^{\bullet-}$ and $^{\bullet}NO$ and their acetylcholinesterase (AChE) inhibitory activity were determined. Additionally, the effects of these matrices on V79 cells under quiescent conditions, as well as after an oxidative insult were assessed. The characterized phenolics included acylated and nonacylated flavonoid glycosides, hydroxycinnamic acyl gentiobiosides and sulphated flavonols. It was verified that the insect sequesters, metabolizes and excretes kale's phenolics. All extracts exhibited antioxidant and AChE inhibitory potential in a concentration-dependent way. However, the results obtained on V79 cells demonstrate that all extracts potentiate the toxicity induced by $H_2O_2$. These results emphasize that the claimed antioxidant capacity observed in cell free systems is not confirmed in cellular models. **Acknowledgments:** The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT) for financial support of this work (PTDC/AGR-AAM/64150/2006). Fátima Fernandes is indebted to FCT for the grant (SFRH/BD/37963/2007).

## P8

### Extending the potential of *Capsella bursa-pastoris* L.

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*Capsella bursa-pastoris* L. (Cruciferae), commonly called shepherd's purse, is a medicinal species indicated for several human health treatments, such as bleeding stopper, anticancer and wound-healing agents. Phenolic compounds, namely flavonoids, are well known bioactive compounds. Thus, the plant aerial parts were subjected to different extractive conditions, in order to obtain an extract rich in flavonoids. Methanol (MeOH) and methanol:water (MeOH/$H_2O$) proved to be more efficient than hexane, chloroform or diethyl ether. Five compounds were identified by HPLC-DAD in MeOH and MeOH/$H_2O$ extracts: quercetin-6-*C*-glucoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, quercetin and kaempferol. The radical scavenging capacity (DPPH assay) and acetylcholinesterase (AChE) inhibitory activity of both extracts were evaluated. MeOH and MeOH/$H_2O$ extracts presented similar antiradical potential ($EC_{50}$=0.71 mg/mL and 0.78 mg/mL, respectively). However, concerning AChE inhibition, the MeOH extract presented an $EC_{25}$ (0.15 mg/mL) tenfold higher than MeOH/$H_2O$ one. These preliminary results are the first report on the scavenging activity and AChE inhibitory potential of this species. **Acknowlegments:** Clara Grosso thanks the Fundação Para a Ciência e a Tecnologia for the Post-Doc fellowship (SFRH/BPD/63922/2009).

## P9

## *Diospyros chamaethamnus*: Naphthoquinones' bioactivity in inflammation

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Naphthoquinones are important compounds in all organisms, where they play important roles in the biochemistry of energy production or in defence against invading pathogens. The genus *Diospyros* consists of woody shrubs and trees distributed in the tropical and sub-tropical regions of the world. *Diospyros* species are characterized by the presence of naphthoquinones, which often appear as dimmers, trimmers and, more seldom, tetramers. In this work, several naphthoquinones (plumbagin, diospyrin, naphthazarin, mamegaquinone and diosquinone) isolated from root barks of *Diospyros chamaethamnus* were studied for their anti-inflammatory activity. The bioactivity in inflammation was evaluated by measuring nitric oxide produced by RAW 264.7 macrophages exposed to 1 µg/mL lipopolisacharide (LPS). Dexamethasone was used as a positive control. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay and extracellular lactate dehydrogenase (LDH) determination afforded macrophages viability. Naphthazarin, diosquinone and plumbagin were the only naphthoquinones revealing a significant anti-inflammatory activity. Plumbagin was the most potent compound, with an $IC_{50}$ of 6.575 µM. Naphthoquinones could be a promising class of compounds in the treatment of inflammation, specially plumbagin. **Acknowledgements:** B. Pinho (SFRH/BD/63852/2009) is indebted to Fundação para a Ciência e a Tecnologia (FCT) for the grant.

## P10

## Hormonal regulation of the basic peroxidase isoenzyme from *Zinnia elegans*, an enzyme involved in lignin biosynthesis

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Xylem differentiation in plants is under strict hormonal regulation. Auxins and cytokinins, together with brassinosteroids (BRs), appear to be the main hormones controlling vascular differentiation. Here, we study the effect of these hormones on the basic peroxidase isoenzyme from *Zinnia elegans* (ZePrx), an enzyme involved in lignin biosynthesis. Results showed that an auxin and cytokinins induce ZePrx, in a similar way to their capacity to induce secondary growth (metaxylem differentiation). Likewise, the exogenous application of BR reduces the levels of ZePrx, in a similar way to their capacity to inhibit secondary growth. Consistent with this notion, the exogenous application of BR reverses the auxin/cytokinin-induced ZePrx expression, but, nonetheless, has no effect on the auxin/cytokinin-induced secondary growth. The study of gene expression by qPCR showed that ZePrx gene was indeed upregulated by exogenous auxins and cytokinins, but repressed by BR. This differential hormonal response is explained by the analysis of the ZePrx promoter, which contains i) cis-elements directly responsive to these hormones and ii) cis-elements targets of the plethora of transcription factors which are up regulated during the auxin- and cytokinin-induced secondary growth. Taken together, these results suggest that ZePrx is directly and indirectly regulated by the plethora of hormones that control xylem differentiation, supporting the role of ZePrx in xylem lignification. Acknowledgements. This work was supported by a grant from the MEC (BFU2006-11577/BFI)-FEDER. JG holds a fellowship (FPI) from the MCYT.

## P11

### Multifunctional roles of two class III peroxidases from *Arabidopsis thaliana*

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In order to approach the functions of specific Prx isoenzymes we set Arabidopsis leaf expressed Prxs as a target. *In silico* transcriptomic analysis identified respectively Prx 42 and Prx 33/34 as the main cell wall and vacuolar Prxs. Analysis of leaf Prx activities confirmed a simple isoenzyme profile, and the purification of the main leaf Prx activity enabled the identification of Prx 34 by PMF. qRT-PCR confirmed the high expression of these genes. Our results show that the expression of Prx42 is inhibited during host-pathogen interactions andits underexpression leads to an earlier and more intense response during avirulent resistance, indicating that Prx42 is adverse to host-pathogen interaction. On the contrary, the expression of Prx34 is stimulated and its underexpression leads to a delay and less intense response during avirulent resistance, indicating that Prx 34 contributes to plant defense. Leaves with underexpression of Prx34, but not of Prx42, become much more sensitive to toxicity of exogenously applied H2O2 than wt.Prx34 has been previously associated with root elongation. We observed that underexpression of Prx34 led to a much higher root branching at an early stage of root development, and to an increase in root length, contrary to what has been previously observed.
The production of overexpression Arabidopsis lines for Prx 34 and 42 is now underway and further thorough characterization of under and over expression lines will be performed.

## P12

### Sorting of class III peroxidases to the plant vacuole

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Vacuoles occupy most of the volume of plant cells and play key roles in plant physiology: they maintain the turgor pressure needed for support of the plant body and cell growth, perform lytic functions, sequester toxic compounds, participate in programmed cell death, accumulate defense proteins and thousands of secondary metabolites, participate in pH regulation and ion homeostasis, and store proteins to be used as a source of amino acids or energy. Most of these functions depend on the correct vacuolar sorting of a variety of proteins which ultimately determine the characteristics and properties of the vacuole. Moreover, knowledge on the determination of vacuolar sorting is essential for the improvement of the protein content of seeds used as food, and for the development of highly productive plants and cell cultures as green factories for the production of medicinal phytochemicals, vaccines, antibodies, etc. In our laboratory, we have worked for a long time with vacuolar class III peroxidases (Prxs) and their involvement in the biosynthesis of medicinal indole alkaloids in the plant *Catharanthus roseus*. Prxs form a large multigene family typical of plants that catalyzes the oxidation of small molecules or chemical groups at the expense of $H_2O_2$, and they may be localized in the cell wall or the vacuole. The determination of the vacuolar sorting of Prxs was uncharacterized. Here, we show that the C-terminal amino acid sequence of the main leaf vacuolar Prx from *C. roseus*, CrPrx1, is necessary and sufficient for the vacuolar sorting of GFP-CrPrx1 fusions, indicating that it constitutes a vacuolar sorting signal. The presence of a C-terminal vacuolar sorting signal directing the protein to a lytic vacuole, as is the case for CrPrx1, is unusual in plant cells, and indicates that the characterization of the Prx sorting pathway should add important knowledge concerning the general model of vacuolar sorting in plant cells.

## P13

### Structural features of glutamine synthetase from *Medicago truncatula*

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In depth understanding of the molecular details of glutamine synthetase (GS) activity in plants is of crucial importance, given the enzyme's key role in nitrogen metabolism. Although a significant effort has been devoted to understanding the mechanisms controlling GS activity in many different plant species, the structural features of plant GS have been mostly inferred from crystallographic models of the better studied bacterial enzymes. In order to unambiguously elucidate the three dimensional structure of plant GS, we decided to determine the crystallographic structure of the cytosolic GS isoform GS1a from the model legume *Medicago truncatula*. The fully active, N-terminal His-tagged recombinant enzyme was purified to homogeneity by immobilized metal-affinity and size exclusion chromatography. Single crystals suitable for X-ray crystallography were obtained by vapour diffusion techniques. The crystals belong to space group P21 with cell dimensions a=99Å, b=102Å, c=188Å, □=104º. Analysis of the self-rotation function for the diffraction data, as well as single-particle reconstruction from electron microscopy micrographs of GS1a, revealed that the enzyme is a decameric protein composed by two superposed homopentameric rings, and in clear contrast with the generally accepted octameric architecture of the complex. Crystallographic structure refinement is currently underway.This work was supported by project POCI/AGR/61025/2004.

## P14

### Overexpression of glutamine synthetase in root nodules of *Medicago truncatula* leads to enhanced nitrogen utilization efficiency

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Glutamine synthetase (GS) is a vital enzyme for the assimilation of ammonia into amino acids in higher plants and the enhancement of its activity has the potential to increase nitrogen utilization efficiency. In legume root nodules, GS is responsible for the assimilation of the ammonium released by symbiotic nitrogen fixation. To investigate how nodule GS activity affects plant performance, we have previously overexpressed GS1a cDNA specifically in root nodules of *Medicago truncatula* under the direction of a native leghemoglobin promoter.
In this study we have used these plants to examine the effects of increased nodule GS activity on phenotypic development, biomass production, rhizobial nitrogen fixation and nitrogen utilization efficiency and have used the tools of trancriptomics and metabolomics to identify the major transcript and metabolite changes associated with the altered nodule metabolism. Overall, the results indicate that *M. truncatula* overexpressing GS display enhanced growth phenotype as quantified by increases in biomass and seed production. Nodule GS activity was positively correlated with symbiotic nitrogen fixation activity and plant nitrogen utilization efficiency. These studies provide further support to the notion that it may be possible to increase nitrogen use efficiency by the manipulation of specific GS isoenzymes in transgenic crop plants.
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## P15

### Glutamine synthetase from *Medicago Truncatula* is regulated by tyrosine nitration

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Glutamine Synthetase (GS), is a crucial enzyme in nitrogen metabolism as it catalyses the first step at which nitrogen is brought into cellular metabolism and thus the enzyme must be precisely regulated. Many studies have been devoted to understand how GS is regulated in plants, which have shown that the enzyme is subjected to tight controls operating at many different levels. Although the regulation of GS at the transcriptional level has been well studied, there is clearly a lack of information concerning its posttranslational regulation. Nitric oxide (NO) and its related species can induce important posttranslational protein modifications through S-Nytrosylation and nitration. Is this study we have evaluated the effect of NO on GS activity. I*n vitro* incubation of the enzyme with reactive nitrogen species producers (peroxynitrite or tetranitromethane) induced a dose-dependent loss of GS activity that could be related to an increase in nitrotyrosine immunoreactivity. Incubation of the enzyme with epicathechin, a selective nitration inhibitor, prevented both GS inactivation induced by reactive nitrogen species and tyrosine immunoreactivity. These results strongly suggest that the inhibition of GS activity is due to tyrosine nitration. Mass spectrometry analysis of in vitro nitrated purified GS1a, identified TYR 341 as a nitrated residue. The data point to a potential post-translational regulation of GS from *M. truncatula* by tyrosine nitration. Further experiments are underway to investigate whether GS nitration is reversible and the physiological significance of this posttranslational modification for plant nitrogen metabolism.

## P16

### Identification of bacteria associated with different strains of *Bursaphelechus xylophilus*

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The etiology of pine wilt disease has not been well understood, however it is known that it's caused by the pine wood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner and Buhrer) Niclke. It is suspected that other microbes might be involved in the pathological processes. The pine sawyer *Monochamus galloprovincialis* (Olivier) (long-horned beetle) is the vector of the introduced nematode in Portugal, and it has also been reported that some bacteria participate in the pathogenesis, causing death of the diseased pine trees.The aim of this study was to identify the bacteria associated with three different virulent isolates of PWN (HF, 8A and 20) and with one avirulent strain, C14-5. Strain HF and 20 were isolated from Setubal region; 8A, isolated from the Portuguese central region; and C14-5, an avirulent strain from Japan. One nematode of each strain was cultivated in Nutrient Agar (NA) media and the bacteria colonies found along the trails of the nematode were isolated and identified using API 20 E and other tests, such as Gram staining, cytochrome C oxidase and catalase tests. Amongst the bacterial species identified, the *Pseudomonas* spp. was one of the most commonly found, as it is reported to help in the infection process.

## P17

### Fluorescence Activation Cell Sorting of *Catharanthus roseus* leaf protoplasts for isolation of cells accumulating medicinal indole alkaloids

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*Catharanthus roseus* accumulates in low levels the terpenoid indole alkaloids (TIAs) vinblastine and vincristine, used in cancer therapy, ajmalicine, used as an antihypertensive, and serpentine, used as sedative. Although much is known about the biosynthesis and regulation of TIAs, a number of biosynthetic steps remain unknown, and TIAs transport and accumulation inside the cells is largely uncharacterized, even if this information is crucial for any manipulation strategy to increase TIA levels. We aim to characterize the transport and accumulation of TIAs in *C. roseus* cells and vacuoles through several strategies, including the differential characterization of alkaloid accumulating cells (idioblasts) in comparison with common mesophyll cells. This strategy involves the isolation of protoplasts from leaves and the separation of idioblast protoplasts from those of common mesophyll cells by Fluorescence Activation Cell Sorting (FACS), followed by differential proteomic, transcriptomic and metabolomic analysis.Here, we show the results of protoplast isolation from *C. roseus* leaves and the optimization of FACS conditions leading to the isolation of two populations of mesophyll cells presenting different alkaloid accumulation (UV fluorescence). This achievement represents a crucial step for the success of our strategy.

## P18

### Assessment of plant species suitability for seeding on degraded soils Development of assessment protocol using *Cistus ladanifer* as case study

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The practice of seeding following major perturbances like high-severity wildfires is not widely implemented in Portugal, and therefore has received little research attention. The present study focuses on the development and testing of protocols that allow assessing the suitability of autochthonous species for seeding under extreme soil conditions. Suitability is understood in terms of germination and early establishment. Special attention is given to the relevance of using seeds from different provenances, i.e. populations from different parts of the species' distribution range in Portugal. The work is being carried in the framework of the PhD study of the first author (SFRH/BD/47522/2008). The presentation will emphasize the ongoing development of the protocols and the initial test results obtained with *Cistus ladanifer*. *C. ladanifer* has been studied for ecological restoration of abandoned mine areas. Nonetheless, its suitability for seeding will often be questionable because of its high inflammability and, thus, elevated wildfire hazard. There are also other species being studied in this first phase that are especially indicated for use in ecological restoration. After the germination experiments there were found significant differences between provenances. Nutrition status, photosynthesis, pigments concentration, carbohydrate content and osmolarity will be used to study physiological performance of the seedlings. Finally, flow cytometry will be used to test the genetic material differences between provenances.

## P19

### Chloroplasts structural and functional assessment by flow cytometry

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Flow cytometry (FCM), a technique used to measure physical-chemical proprieties of particles flowing in a fluid stream has been used with some success to analyze chloroplast, allaying high speed (up to thousands of events per second) and accuracy to the possibility of performing multiparametric assays. In order to assess chloroplast functionality, *Pisum sativum* plants were exposed to paraquat, a photosynthesis acting herbicide, at maximum permitted concentration. Analyses were performed after 3h, 6h, 12h, 15h, 18h, 21 and 24h of exposure. Chloroplasts were characterized by FCM with respect to forward angle and side angle light scatters (reflecting volume and complexity of the particle) and autofluorescence. This data was complemented with information collected using pulse amplitude modulation (PAM) fluorometry and chlorophyll a and b quantification. FCM results were in agreement and highly correlated with Fv/Fm ratio, proving that paraquat exposure significatively affected fluorescence mainly after 12h of exposure, but not the quantity of chlorophyll *a* or *b*. More importantly, significant variations of the morphological status of the chloroplast, as measured by FCM, were detected before any significant effect in fluorescence was observed. Taken all together, FCM proved to be an effective tool for chloroplast functionality assessment and is expected that the data presented here will encourage the application of FCM in this area of research.

## P20

### Development of Regeneration and Transformation Protocols for the Genetic Improvement of the Medicinal Plant *Catharanthus roseus*

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*Catharanthus roseus* is an important medicinal plant due to the accumulation in the leaves of terpenoid indole alkaloids with anticancer activities. The high pharmaceutical value of these metabolites allied to their low levels in the plant made of *C. roseus* one of the most studied medicinal species, at the biochemical and molecular levels. However, no efficient protocol for transformation and regeneration is available, a fundamental tool that would potentiate basic research on *C. roseus*, and enable the development of strategies for genetic improvement.
Thus, the aim of this work was to optimize an efficient protocol for regeneration of *C. roseus* through somatic embryogenesis, and to develop an associated protocol of transformation mediated by *Agrobacterium tumefaciens*, using fusions between a class III peroxidase and RFP, in order to generate *C. roseus* transgenic plants. This work showed that mature embryos extracted from *C. roseus* seeds are an excellent *explant* for regeneration of whole plants via somatic embryogenesis. Additionally, it was proven that *C. roseus* mature embryos respond positively to *A. tumefaciens* infection and thus, production of transgenic plants of this species should soon be possible. This will constitute a key tool for the genetic improvement of a highly important medicinal species, with potential impact in the pharmaceutical industry.

## P21

### Study of specific genes of infection by *Bursaphelenchus xylophilus* in Portuguese pine trees

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The Pine Wood Nematode (PWN) *Bursaphelenchus xylophilus* (Steiner and Buhrer) Niclke is the cause of Pine Wilt Disease (PWD). Symptoms consist in a decrease in photosynthesis, chlorosis of needles, denaturation of xylem and cortex parenchyma cells, traumatic resin canal formation, production of phytotoxic substances and enhanced respiration and ethylene production, thus threatening worldwide pine forests and related economy. Particularly in Portugal, this has been a great problem distributed throughout a large segment of our pine productry areas.We have recently confirmed that peroxidase is very important in the early response to the infection and also the already referred ethylene, whose synthesis is over-expressed by the plant under stress induced conditions.A targeted gene expression approach was taken out in order to verify the infection mechanisms by *B. xylophilus* in *Pinus pinaster* and *Pinus pinea* and to enlighten the molecular mechanisms of tree resistance and susceptibility. The main goal of this work was to identify genes expressed after the infection with virulent (HF isolated from Setubal region) and avirulent (C14-5 isolated from Japan) strains, to find an effective way to induce resistance to the nematode in pine trees.The methods were based in RNA extraction from inoculated trees and semi-quantitative RT-PCR to identify specific genes expressed by the plant and nematode in a time course trial.

## P22

### Evaluation of prevention of DNA damage and induction of DNA repair in *Saccharomyces cerevisiae* by *Ginkgo biloba* leaf extracts

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Extracts of *Ginkgo biloba* leafs (GBE) have been used for centuries in traditional oriental medicine to treat a very wide range of ailments. These extracts contain flavone glycosides, terpene trilactones (ginkgolides and bilobalide), ginkgolic acids, proanthocyanides and other uncharacterized compounds. The flavone glycosides and terpene trilactones fractions are believed to be responsible for the pharmacological properties of GBE, which are very popular, making it as one of the best-selling herbal medications worldwide. In this work we investigated the DNA protective effect of GBE in the model organism *Saccharomyces cerevisiae*. Typical experiments involved incubation of yeast cells with GBE before and during oxidative shock by hydrogen peroxide. Our results obtained with the comet assay show that DNA damage is significantly decreased upon GBE treatment (before and during $H_2O_2$ incubation) in a dose-dependent manner. In addition, DNA repair is significantly improved in cells pre-treated with GBE. As expected, GBE treatment improved survival of yeast cells when challenged with oxidative shock with $H_2O_2$, which is in accordance with decreased intracellular oxidation upon pre-treatment with GBE as revealed by flow cytometry with the redox sensitive probe dichlorofluorescein diacetate. Even in the absence of $H_2O_2$, yeast cells showed a decreased intracellular oxidation state, suggesting that GBE can protect cells from endogenous reactive oxygen species.

## P23

### Chromium (VI) toxicity in crops: comparative endpoints for oxidative stress and genotoxicity

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Chromium is extensively used in industries and its resulting accumulation in soils/water raises several environmental concerns. Some stable states of Cr are: the essentially micronutrient in human diet Cr(III), and the highly toxic Cr(VI). Contradictory effects were described for chromium phytotoxicity. Chromium is an oxidant agent, damaging biomolecules such as lipids, proteins, pigments and nucleic acids. We focus here on the differential effects of Cr(VI) on the antioxidant defence system of the crop lettuce (*Lactuca sativa* L. cv. Povoa). In leaves, CAT and APX activities increased with Cr(VI) exposure from 200 mg/L. Contrarily, POX activity decreased significantly in treated leaves, though at 300 mg/L there were no significant differences from control. GR activity decreased significantly at 50 mg/L and no other statistic differences were noted comparing to control. SOD increased significantly its activity with Cr(VI).For roots, APX activity increased significantly and continuously with Cr(VI), however was observed a reduction at the lowest concentration, 50 mg/L. There was a significant increase of POX activity in roots, from 200 mg/L, although it decreased at 50 mg/L. CAT activity increased from 200 mg/L. In GR only 300 mg/L was statistically different from the control, showing an increase in activity. SOD showed its significant increase from 200 mg/L, with the higher value at this concentration. Simultaneously, genotoxicity and mitogenicity induced by Cr(VI) were evaluated by flow cytometry, and differences were found. Data supports the use of some antioxidative enzymes, and flow cytometric analyses are putative endpoints to assess Cr toxicity in plants.

## P24

### Regulation of the QsMyb68 Transcription Factor from cork oak – evidence of alternative splicing

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The cork oak *Quercus suber (Qs)* forest is a unique and emblematic resource for Portugal, being the source of a renewable and sustainable natural product, the cork. Each year, the cork oak tree produces a layer of suberized phellem cells that accumulate in form of annual rings around the trunk, as a result of cork cambium activity. Despite its importance, little is known about the molecular processes underlying cork biosynthesis and differentiation. A previous molecular approach identified a list of candidate genes implicated in cork formation. One of these genes codes for an R2R3Myb transcription factor (QsMyb68). The *R2R3Myb* gene sub-family has been described as involved in the phenylpropanoid and lignin pathways, both involved in cork biosynthesis. RT-PCR studies of *QsMyb68* transcript revealed expression in cork samples (virgin, second and reproduction), stem and branches whereas in leaves and fruit no expression was detected. RT-PCR also showed that two alternative spliced (AS) transcripts are present in tissues where *QsMyb68* is expressed. Sequence analysis revealed that in one of the AS variants, an intron with a simple sequence repeat (SSR) is retained within the 5' untranslated region. Results of real-time quantification of both AS variants in *Qs* tissues are also discussed. The expression of *QsMyb68* observed only in suberized tissues points to its putative involvement in secondary growth and the associated AS mechanism supports the regulatory role of QsMyb68 on cork biosynthesis.

## P25

### *Lycopersicon esculentum* seeds: byproduct valorization

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In recent times, the study of seeds has assumed increasing importance. This vegetal material constitutes a source of specific compounds in high concentration. Although *Lycopersicon esculentum* (tomato) fruit is widely studied, only a few works focus their attention on the seeds. In this study tomato seeds (Bull's heart and Cherry varieties) were analyzed. Phenolics and organic acids profiles were achieved by HPLC/UV-PAD/$MS^n$-ESI and HPLC-UV, respectively. Qualitative and quantitative differences were found between the two studied varieties. Although tomato seeds are considered as waste in the production of tomato derivatives and some consumers remove them from the fruit before use, this matrix revealed interesting biological potentialities. Antibacterial activity was tested against several bacteria and the antioxidant capacity was evaluated against DPPH, superoxide and nitric oxide radicals. Concerning these parameters "Bull's heart" variety was the most promising matrix. Additionally, preliminary results point to potential antifungal activity against yeast and moulds.Thus, tomato seeds extracts can be regarded as an alternative to synthetic preservatives in the food industry and also potentially used as food supplement or source of bioactive compounds. In this way, we can give a profitable use to this material usually considered an industrial byproduct. **Acknowledgements:** Marcos Taveira (SFRH/BD/62662/2009) and Luís Vale-Silva (SFRH/BPD/29112/2006) are indebted to FCT for the grants.

## P26

### Can *Solanum nigrum* L. be use to phytoremediate nickel-polluted sites? The first steps…

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Phytoremediation - the use of plants and their associated microbes to remove or immobilize contaminants - may offer a low cost method for the remediation of heavy metals-contaminated soils. *Solanum nigrum* L. is a plant species that has been reported to hyperaccumulate heavy metals such as Cd and Zn, and is a potential candidate for phytoremediation and for the accumulation of Ni. Thus, to assess the effect of Ni on *S. nigrum*, seeds were allowed to germinate and grow for 4 weeks in sterile Hoagland solution supplemented with increasing concentrations of Ni: 0 µM, 0.5 µM, 7.5 µM, 30 µM, 50 µM and 100 µM. Significant decreases on fresh weight and root length could be observed starting from the 7.5 µM treatment, whilst the shoot length significantly decreased only in the 100 µM treatment. Subsequently, plants were grown hydroponically for four weeks in Hoagland solution under 3 different situations: one set without Ni; one exposed to 7.5 µM Ni; and a third consisted on a 100 µM Ni shock treatment throughout the last week. A significant decrease in root and shoot fresh weight and length, in shoot dry weight, and a significant parallel increase in water content, was observed for both treatments. These preliminary results reveal that the exposure to Ni causes deleterious effects to the plants. Future studies will evaluate the degree of stress plants exposed to these Ni concentrations, both at the biochemical and molecular levels.

## P27

### Is chromium (III) possible to be phytoremediated by *Solanum nigrum* L.? The beginning…

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One of the emerging technologies to solve the environmental pollution problem is phytoremediation, the use of plants and their associated microbes for environmental cleanup. *Solanum nigrum* L. is a plant species that has been reported to hyperaccumulate heavy metals such as cadmium and zinc, and is a potential candidate for phytoremediation and for the accumulation of other metals, such as chromium (III), which is a highly toxic environmental pollutant. Thus, to verify the effect of Cr (III) on *S. nigrum* development, seeds were allowed to germinate and grow for 4 weeks in Hoagland solution supplemented with increasing concentrations of Cr (III): 0 µM, 125 µM, 250 µM, 375 µM and 500 µM. Significant decreases on seedling fresh weight and root length could be observed starting from the 375µM treatment, whilst the shoot length significantly decrease only in the 500 µM treatment. Control-derived seedlings were grown hydroponically for 4 weeks in Hoagland solution under 3 different situations: one set without Cr (III); one exposed to 375 µM Cr (III); and the third consisted on a short shock treatment with 1 mM Cr (III) throughout the last week. It was possible to observe that there were no significant variations in root and shoot fresh and dry weight and length and in water content for both Cr (III) treatments. These preliminary results reveal that *S. nigrum* can tolerate very high concentrations of Cr (III) in the rhizosphere.

## P28

### Phlorotannins in edible macro algae purified extracts

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The Portuguese coast is characterized by the presence of several macro algae species which constitute a rich source of bioactive compounds. Among them we can highlight phlorotannins, which are polyphenols restricted to brown seaweeds (Phaeophyta). Phlorotannins have primarily been regarded as defense chemical agents against herbivores. Due to their protein precipitating capacity, these compounds are capable to deter feeding, especially by fish. Nowadays, diverse effects of phlorotannins are reported on biological systems, namely anti-inflammatory, anti-cancer, bactericide, anti-oxidant and anti-diabetic activities. These compounds are formed by the polymerization of phloroglucinol (1,3,5-trihydroxybenzene) units. For the present study, our interest was focused in the quantification of phlorotannins in ten edible Phaeophyta collected along the Portuguese west coast. The methodology applied for the quantification consists in a colorimetric assay, using 2,4-dimethoxybenzaldehyde (DMBA), specific for hydroxyl groups in the positions 1' and 3' and 1', 3' and 5', characteristic of phlorotannins. The extracts used were previously purified with n-hexane to remove lipids and with toluene to remove pigments. The determination of the anti-inflammatory activity of phlorotannin purified extracts from the same species is also under study, and seems to be dose dependent. **Acknowledgements**: G. Lopes (SFRH/BD/61565/2009) and J. Bernardo (BII) are indebted to Fundação para a Ciência e a Tecnologia (FCT) for their grants.

## P29

### Early Indicators of Coastal Dynamics in Climate Change Vulnerability Assessment

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Global warming and other climate change related processes are expected to impact significantly on coastal dynamics, which may induce important ecological and economical consequences. Therefore early indicators of dynamic shifts are needed for vulnerability assessments, for monitoring protocols and for restoration processes. In recent years, we have developed intensive studies on the relationship between coastal dynamics, dune morphology, and vegetation assembly. From general patterns of ecosystem structure to specific features of morphology and vegetation, these studies have provided strong evidence on the potential of this relationship for evaluation and monitoring purposes. Recently, we have been assessing the fine-scale assembly of dune vegetation under different coastal dynamics as recognised from previous studies and from quantitative features of local geomorphology. These detailed assessments have allowed the identification of a set of promising indicators that can be evaluated through cost-effective methodologies and are may thus prove to be suitable to support monitoring protocols. These indicators range from foredune morphology and spatial patterns of plant species to numerical indicators of diversity for entire vegetation profiles. Even though the effectiveness of these indicators still has to be further validated, we believe they can provide the baseline for consistent assessments of local dynamics, for the development of predictive models to forecast climate change impacts, for detailed assessments of vulnerability and resilience of dune ecosystems to shifts in dynamics (e.g. driven by climate changes), and for restoration protocols directed towards the development of more resistant dune systems.

## P30

### Cyanobacteria in the intertidal zones of the Portuguese coast

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Cyanobacteria are photosynthetic prokaryotes with a wide geographical distribution that are present in a broad spectrum of environmental conditions. Benthic cyanobacteria grow along the shore, mainly in the intertidal zone, forming cohesive mats. In these habitats, cyanobacteria are exposed to a range of daily stresses such as nutrient limitation, high UV-radiation, and desiccation [1]. Cyanobacteria play a major role in the global carbon cycle as important primary producers, and the diazotrophic taxa are fundamental to the nitrogen cycle, particularly in oceans [1]. They are also recognized as being a rich source of biologically active natural products. Some of these compounds are toxic to a wide array of organisms [2] and some are potentially useful in several fields, namely as pharmaceuticals [3]. Despite their important role, little is known about the diversity of these organisms along the Portuguese coast. To evaluate the diversity of cyanobacteria in the intertidal zones of the Portuguese coast, nine beaches were sampled, approximately 100 cyanobacterial isolates were retrieved and are being characterized by a polyphasic approach. Phylogenetic analyses were carried out and a screening for putative diazotrophs and toxins producers was also performed.

## P31

### Research at Laboratory of Biotechnology and Cytometry

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The Laboratory of Biotechnology and Cytomics belongs to the Associated Laboratory CESAm at University of Aveiro. It has presently two main research lines, each with a solid and specialised team, and coordinated by senior PhD researchers: 1) cytotoxicity and developmental biology of plant cells; 2) cytotoxicity and developmental cell cycle in animal/human cells. The cytotoxicity and developmental biology of plant cells has started as result of the initial background of the group's leader, and applied and basic research in plant biotechnology (e.g. micropropagation and use of in vitro systems to better understand stress condition interactions) has been developed in the last 20 years. One Agronomic engineer, eight PhD students, three PhD researchers and one Assistant Researcher have been working in this plant team for the last decade. The team covers techniques in cell culture, quantitative cytometry (applied to cytotoxicity studies), and PCR/RT-PCR (techniques that are transversal to both animal and plant cells), covering several research projects in plant biotechnology and –omics. The second research field has been established since 2006, and the team has two PhD researchers of animal sciences, two PhD students, and three master students. This research field resulted from the several challenges addressed to this laboratory by other groups requiring the team expertise in cytotoxicity and cyto-genomics. It is presently using in vitro human cells to evaluate metals (from environment and/or nanoparticles interaction) in cytotoxicity endpoints (e.g. (viability/apoptosis, oxidative stress and genotoxicity).

## P32

### CEBAL – Biotecnologia@BaixoAlentejo

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Biotechnology is one of the most important sectors of the new knowledge-based economy. In Baixo Alentejo, agriculture, in its diverse areas (agro-forestry and livestock), was considered essential for the development of this region. In this context, the Centro de Biotecnologia Agrícola e Agro-alimentar do Baixo Alentejo e Litoral (CEBAL), a private, non-profit research and development unit located in Beja was created. CEBAL aims to become a strategic infrastructure for the development of high quality research, technology transfer and innovation in the fields of Biotechnology. CEBAL activities privilege the development of applied research trying to focus on questions with potential impact on local economy and to propose biotechnological solutions for problem solving, production optimization or product certification. CEBAL also emphasizes the identification and development of new opportunities for possible application in the region. Activity areas involve plant production on its dual approach, agriculture and forestry (agro-forestry biotechnology), animal production (animal production biotechnology), processing and improvement of agricultural food products (agro-food biotechnology) and implementation of added value products from sub-products and wastes and new ways to relaunch the use of traditional raw materials (valorization of wastes and raw materials). CEBAL combines R&D activities with postgraduate teaching and training, consulting, services and incubation of technology-based companies.

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## P 33

### Bright Yellow-2 (BY-2) cell line: a model for intracellular sorting studies

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In the past years, plant cell cultures have emerged as models for the study of several intracellular phenomena, given their high growth rates, amenability to genetic transformation and suitability for a series of drug assays otherwise difficult to assess in a whole plant or an organ. Propagation of plant cells was developed sixty years ago, and cultures have been established from different plant species trying to answer to different research goals. BY-2 is a well characterized cell line isolated from the cultivar Bright Yellow-2 of *Nicotiana tabacum*. These cells present numerous advantages for laboratory work, they grow in uniform filaments and are about the double size of the Arabidopsis cells, facilitating the observation and identification of their intracellular content. BY-2 are known as the plant "HeLa" cells, exhibiting a marked growth rate and acquiring rapid cell cycle synchronization. Their relatively low autofluorescence and easy transformation make them an even more interesting and useful plant cell model system. All these advantages led to an increase in the use of these cells by researchers in a wide variety of studies. In our laboratory we are exploiting the use of BY-2 cell line for the study of protein intracellular trafficking, with special emphasis in cardosin A and cardosin B biogenesis and sorting. We were successful to establish in our laboratory BY-2 cell lines expressing cardosin A fused both with Kaede and mCherry. Preliminary results showed a different accumulation pattern when cardosin A was fused to mCherry and Kaede, being identified in the vacuoles and Golgi apparatus, respectively. This expression system has an important potential in the study of cardosin A biogenesis and sorting.

## P34

### Cardosin A accumulation pattern in transgenic lines of *Arabidopsis thaliana* flowers

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Cardosins are aspartic proteases abundant in *Cynara cardunculus* (cardoon) flowers. Cardosins A and B have been extensively characterized, as they are considered to be good models to study plant aspartic proteases and intracellular sorting and trafficking. Though cardosins A and B are very similar, they have different localizations in the cardoon flower: cardosin A is vacuolar and has been localized in stigmatic papillae, while cardosin B is secreted and has been detected in the transmitting tissue. *Arabidopsis thaliana* was already used as an heterologous system for the study of cardosins A and B expression and accumulation in seeds and proved to be effective and trustworthy reliable. In this work, transformed *A. thaliana* stable lines (expressing cardosin A) were used to study its expression and accumulation in flowers by immunolocalization. Preliminary results showed that cardosin A has been expressed in the whole flower with more particular evidence in the nucellus of the ovules and in the remnants of the tapetum. These results support the previous work developed in this system, confirming *A. thaliana* as a good model to study the localization and intracellular trafficking of cardosin and pointing to the possible involvement of this aspartic protease in the reproduction process. Further studies involving cardosin A and B detection by electron microscopy will complement and give new insights to this work.

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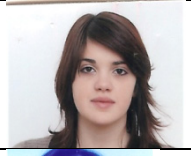
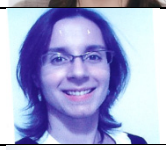
| **Cardoso**, Luísa |  | | DB-FCUP e IBMC – Instituto de Biologia Molecular e Celular, Rua do Campo Alegre, 823, 4150-180 Porto. luisa.cardoso@ibmc.up.pt |
| **Ferreira**, Elsa |  | | DB-FCUP e CGUP. Rua do Campo Alegre, s/n.º, 4169-007 Porto. elsalex.ferreir@gmail.com |
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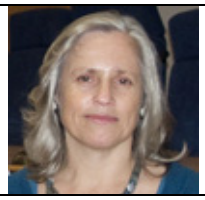
| **Mina**, Isabel |  | O21 | CITAB – UM, Departamento de Biologia, Escola de Ciências – Universidade do Minho, Campus de Gualtar 4710-057 Braga, Portugal, icapmina@bio.uminho.pt |
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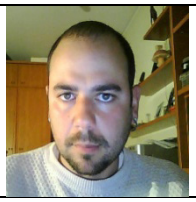
| **Pontes**, Luís |  | O27 | Peak Plants luispontes@peakplants.com |
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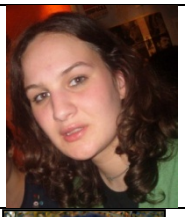
| **Seabra**, Ana Rita |  | O1<br>P13<br>P14<br>P15 | Molecular Biology of Nitrogen Assimilation,Instituto de biologia molecular e celular – Universidade do Porto,Rua do Campo Alegre, 823 4150-180 Porto, ars@ibmc.up.pt |
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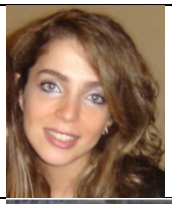
| **Silva**, Sara Baptista da |  | P1 | Faculdade de Farmácia da Universidade do Porto, Rua Aníbal Cunha 164 4050-047 Porto Portugal, dcf09018@ff.up.pt |
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