Bioplant
Programa Inter-Universitário de Doutoramento
MAP

FUNDAÇÃO
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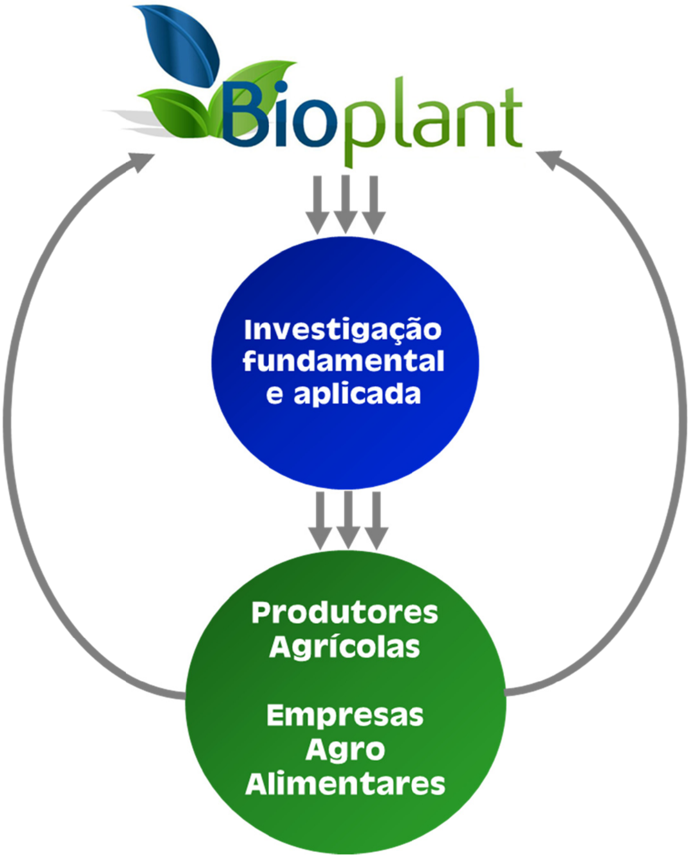
Programa Inter-Universitário de Doutoramento MAP em
Biologia de Plantas – Bioplant

MAP Joint Doctoral Programme in Plant Biology - Bioplant

4ª Workshop Anual Bioplant

18 e 19 Julho 2013



PROGRAMA / PROGRAMME

Livro de resumos / Book of abstracts

Universidade do Minho
universidade de aveiro
U.PORTO

Bem vindos *à 4ª* ***Workshop Anual BioPlant*** *organizada pela 4ª edição do Programa Inter-Universitário de Doutoramento em Biologia de Plantas – Bioplant, das Universidades do Minho, Aveiro e Porto (programa MAP).*

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).*

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.

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.

Organização da Workshop / *Organization of the workshop*

Mariana Sottomayor	Departamento de Biologia, FCUP
Paula Melo	Departamento de Biologia, FCUP
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Conceição Santos, UA
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Mariana Sottomayor UP, Diretora Bioplant 2013
Paula Melo, UP

Programa / *Programme*

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18 Julho / July 18th

09:00 **Abertura** / ***Opening***
José Luís Santos, Vice-Diretor da FCUP

09:30 **Conferência de abertura / *Opening lecture***
Excitation about sex in plants: merging biophysics with genetics on the pollen tube system CC1
José Feijó, FCUL / IGC

10:30 **Coffee Break - Posters**

11:00 **AGP6 and AGP11 biological mode of action in Arabidopsis pollen and pollen tube growth** CC2
Sílvia Coimbra, FCUP / BioFIG

11:30 **RNASeq analysis of the *Quercus suber* root response to drought** O1
Herlânder Azevedo, DB-UM / BioFIG

11:45 **SUMO proteases control development and stress responses in *Arabidopsis thaliana*** O2
Pedro Humberto Castro, DB-UM / BioFIG

12:00 **Posters**

13:00 **Almoço / Lunch**

14:00 **Legacy and Emerging Contaminants in Plants: selection and utility of Biomarkers** CC3
Conceição Santos, DB-UA / CESAM

14:30 **Anti-bacterial potential of silver nanoparticles green synthesised using *Withania somnifera* aqueous leaf extract** O3
Marslin Gregory, DB-UM / CITAB - Aluno Bioplant

14:45 **Antioxidant capacity of *Melia azedarach* under water deficit conditions** O4
Carla Azevedo, DB-UA / CESAM

15:00 **Reflorestação de zonas degradadas pela seca com amargoseira: I. crescimento e fotossíntese** O5
Maria da Costa, DB-UA / CESAM - Aluna Bioplant

15:15 Coffee Break - **Posters**

15:45 **Fungal diversity in chestnut orchards: a comparison between above- and below-ground diversity** CC4
Teresa Lino Neto, DB-UM / BioFIG

16:15 **Dot-blot validation of novel DNA markers for detection and genotyping of *Ralstonia solanacearum* species complex** O6
Pedro Albuquerque, FCUP / CIBIO

16:30 **Contribution of the plant rhizosphere system to the phytoremediation of cadmium in estuarine areas** O7
Cristina Almeida, FCUP / CIIMAR

16:45 **Deciphering intracellular protein trafficking pathways** CC5
José Pissarra, FCUP / BioFIG

17:15 **The Plant Specific Insert and Its Molecular Role in Protein Sorting** O8
Bruno Peixoto, DB-UP / BioFIG

17:30 Posters

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19 Julho / July 19th

09:30 **Purification and functional characterization of the grapevine aquaporin VvSIP1** CC6
Hernâni Gerós, DB-UM / CITAB

10:00 **Photosynthesis in grape berry tissues: an in vivo approach by imaging PAM fluorometry** O10
Ana Cunha, DB-UM / CITAB

10:15 **Unravelling the molecular network regulating zinc content in plants: from Arabidopsis to rice** CC7
Ana Assunção, CIBIO

10:45 Coffee Break - **Posters**

11:15 **Copper transporters in grapevine** O11
Viviana Martins, DB-UM / CITAB - Aluna Bioplant

11:30 **Identification of heavy metal transporters in Solanaceae plants** O13
Alberto Pessoa, FCUP / BioFIG

11:45 **A highly efficient leaf protoplast expression system for the study of anticancer alkaloid metabolism in *Catharanthus roseus*** O9
Patrícia Duarte, IBMC

12:00 Posters

13:00 Almoço / Lunch

14:00 **A molecular antagonism that shapes the flower** CC8
Manuela Costa, DB-UM / BioFIG

14:30 **Major Research Topics at CITAB** CC9
Francisco Manuel Pereira Peixoto, UTAD / CITAB

15:00 **Desenvolvimento Sustentável e Ecoinovação - ferramentas de combate à crise** O12
Joaquim Manuel Soares Guedes, Ecoinside

15:15 Coffee Break - Posters

15.45 **Unravelling the metabolism and transmembrane transport of the highly valuable medicinal alkaloids from *Catharanthus roseus* (L) G. Don.** CC10
Mariana Sottomayor, FCUP / IBMC

16:45 Atribuição de prémios aos Posters

Conferências Convidadas

Invited Lectures

CC1 – José Feijó

Excitation about sex in plants: merging biophysics with genetics on the pollen tube system

Instituto Gulbenkian de Ciência (IGC), Oeiras
Department of Plant Biology, Faculty of Sciences, University of Lisbon

CC2 – Sílvia Coimbra

AGP6 and AGP11 biological mode of action in Arabidopsis pollen and pollen tube growth

BioFIG, Department of Biology, Faculty of Sciences, University of Porto

Arabinogalactan proteins (AGPs) are cell wall proteoglycans that were shown to be important for pollen development. An Arabidopsis double null mutant for two pollen-specific AGPs (*agp6 agp11*) showed reduced pollen tube growth and compromised response to germination cues in vivo. So, in order to understand the mode of action of these AGPs, an Affymetrix ATH1 genome array in the *agp6 agp11* double null mutant pollen tube was performed. The lack of two specific AGPs induced a meaningful shift of the gene expression profile. Calcium- and signaling-related genes were found to be altered, which gives support to the known roles of such genes in pollen tube growth. The presumed involvement of AGPs in signaling cascades was also reinforced. Cysteine-rich proteins have been proposed to play a role in recognition and fertilization, and it was thus quite relevant that such genes were found to be differentially expressed. The putative involvement of AGPs in signaling cascades through calmodulin and protein degradation via ubiquitin was found. Also, stress related genes were found to be affected, which supports the recognized similarities between signaling pathways in both defense and pollen tube growth (Costa et al., 2013).
Yeast two-hybrid experiments gave further support to these signaling pathways and revealed putative AGP6 and AGP11 interactors implicated in the process of recycling by endocytosis of cell membrane components, through clathrin-mediated endosomes and multivesicular bodies. A model for AGP6 and AGP11 biological mode of action in pollen tube growth is presented.

Costa, M., Nobre, S., Becker, J., Masiero, S., Amorim, M.I., Pereira, L.G. and Coimbra, S. 2013. On hand, putative ligands for arabinogalactan proteins in Arabidopsis pollen development. BMC Plant Biology, 13:7.

CC3 – Conceição Santos

Legacy and Emerging Contaminants in Plants: selection and utility of Biomarkers

Department of Biology & CESAM, University Aveiro 3810 193 Aveiro

The selection of adequate plant endpoints, having the sensitivity and specificity that are required in modern plant toxicology, is being supported by complementary fields of e.g., molecular biology, -omics, systems biology, etc. However, other challenges emerge during selection, including the demand for a high predictive ability of any putative endpoint. Moreover, biomarkers properties are often affected by experimental variables, which may jeopardize their validation as reliable endpoints in plant (eco)toxicological studies. Some case studies will be explored concerning selected biomarkers (and related techniques) that are gaining importance in assessing plant responses to legacy and emerging contaminants. In particular, examples of metals and metal-based nanoparticles effects on the plant genome structure and function and on the cell functioning will be discussed. Some techniques used in the multiparametric approaches explored here include gene expression (qRT-PCR), metabonomics, cytomics (FCM), COMETS, catastrophe mitosis, electron paramagnetic resonance (EPR) and others. The combination of this multiple information aims at providing, as most complete as possible, a functional network of the plant cell's response. Besides using more innovative approaches, there is also a need to refocus strategies and methodologies, including, for example risk-benefit balance analyses. This comprehensive overview is an example of the changes of paradigm within modern plant toxicology. Interestingly, and despite plant toxicologists have today more tools and knowledge to study multiple mechanisms of functional effects of chemicals on plant cells, many challenges remain in modern toxicology, i.e, "how to use this plethora of information?" or "how to define best endpoints?".

CC4 – Teresa Lino Neto

Fungal diversity in chestnut orchards: a comparison between above -and below-ground diversity

Baptista P[1], Reis F[2], Pereira E[1], Tavares R[2], Lino-Neto T[2]

[1]CIMO / School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-854 Bragança, Portugal.
[2]Centre for Biodiversity Functional and Integrative Genomics (BioFIG), Plant Functional Biology Centre, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.

Chestnut (*Castanea sativa*) groves have a significant impact in the Portuguese economy, due to the production of fruits and wood, as well as to related activities, such as hunt and mushroom collection. The fungal community present in chestnut orchards was studied by a traditional survey of above-ground mushrooms and by a metabarcoding approach using 454-pyrosequencing of soil DNA samples. The fungal diversity present in chestnut orchards found by both analyses will be compared and reliability of both approaches discussed. Results will be also discussed taking into account the Implications of specific fungal interactions for chestnut orchard sustainability.

CC5 – José Pissarra

Deciphering intracellular protein trafficking pathways

BioFIG, Department of Biology, Faculty of Sciences, University of Porto

CC6 – Hernâni Gerós

Purification and functional characterization of the grapevine aquaporin VvSIP1

Henrique Noronha[1,2], Ana Paula Martins[3], Graça Soveral[3], François Chaumont[4] and Hernâni Gerós[1,2]

[1] Centro de Investigação e de Tecnologias Agro-ambientais e Biológicas CITAB-UM, Portugal
[2] Grupo de Investigação em Biologia Vegetal Aplicada e Inovação Agroalimentar (AgroBioPlant), Departamento de Biologia, Escola de Ciências Universidade do Minho, Braga, Portugal
[3] Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL) and Department of Biochemistry and Human Biology, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal
[4] Unité de Biochimie Physiologique, Institut des Science de la Vie, Université Catholique de Louvain, Croix du Sud 2-20, B-1348 Louvain-la-Neuve, Belgium

Water is transported through biological membranes by aquaporins, members of the widespread Major Intrinsic Proteins (MIPs). In plants, aquaporins are grouped in five sub-families, PIPs (plasma membrane intrinsic proteins), TIPs (tonoplast intrinsic proteins), NIPs (nodulin26-like intrinsic proteins), XIPs (X intrinsic Proteins) and SIPs (small and basic intrinsic proteins). In the present study the localization, expression and functional characterization of a VvSIP from the grape berry were performed. VvSIP1 is expressed in leaves and berries, with higher number of transcripts in the mature stage, and colocalizes at the ER in transformed yeast cells with *VvSIP1-GFP*. ER membrane vesicles purified from yeast overexpressing *VvSIP1* were characterized by stopped flow technique for their capacity to transport water. The protein was purified to homogeneity after VvSIP1-his tag heterologous expression in yeast followed by ER purification, membrane solubilization and Ni-NTA affinity chromatography. Water transport was confirmed after reconstitution of the purified protein in phosphatidylethanolamine liposomes. *VvSIP1* expression is remarkably up regulated by heat, as shown by Real-time PCR, suggesting a role in stress response in grapevine.

Acknowledgments: This work was supported by European Union Funds (FEDER/COMPETE-Operational Competitiveness Programme) and by national funds (FCT-Portuguese Foundation for Science and Technology) under the projects FCOMP-01-0124-FEDER-022692 and European COST action FA 1106 and the research projects PTDC/AGR-ALI/100636/2008 and PTDC/AGR-AAM/099154/2008. HN was supported by the PhD grant no. SFRH/BD/75257/2010 and APM by the PhD grant no. SFRH/BD/65046/2009

CC7 – Ana Assunção

Unravelling the molecular network regulating zinc content in plants: from Arabidopsis to rice

Joana G. Guedes[1], Diego Almeida[2], Mark G.M. Aarts[3], Nelson J. M. Saibo[2], Ana G.L. Assunção[1]

[1]Plant Evolution Group, CIBIO/InBIO, Universidade do Porto, Portugal
[2]Genomics of Plant Stress Laboratory, ITQB, Universidade Nova de Lisboa, Portugal
[3]Laboratory of Genetics, Wageningen University, The Netherlands

As a plant micronutrient, zinc is of major interest in agronomy and for human and cattle nutrition. Zinc deficient soils are widespread all over the world and the risk of inadequate diet and zinc malnutrition is estimated to affect one third of the global human population, i.e. around 2 billion people. Developing countries, where people depend on cereal-rich diets for sustenance, are the most affected. Improvement of crop efficiency in zinc deficient environments, and bio-fortification to alleviate human nutrition are plant-based solutions as source of micronutrients. An incomplete understanding of zinc homeostasis mechanisms in plants is however a limitation. Recently the first transcription factors regulating zinc homeostasis in Arabidopsis were identified (Assunção et al. 2010). The AtbZIP19 and AtbZIP23 were shown to be essential for the adaptation to zinc deficiency. Transcript profiling revealed only a small set of genes, to be induced in wild-type plants in response to zinc deficiency, but not in the *bzip19bzip23* Arabidopsis double mutant line. A suggested conservation of the zinc deficiency response regulatory network within the plant kingdom makes it very attractive to explore the molecular basis of such network in rice (*Oriza sativa* L.). Rice is one of the most important crops worldwide and it is the staple food for more than half of the world's population. Additionally it is a very relevant crop in Portugal. Transferring the knowledge from Arabidopsis to rice is of major importance. We present our preliminary results in the identification and analysis of the bZIP19/bZIP23 rice orthologs.

CC8 – Manuela Costa

A molecular antagonism that shapes the flower

BioFIG, Department of Biology, Faculty of Sciences, University of Porto

The action of antagonistic proteins on the transcriptional activity of developmental key genetic programmes is an important regulatory mechanism in the generation of different morphologies that characterise multicellular organisms.

In *Antirrhinum majus*, the dorsoventral asymmetry of the flowers is established through a molecular antagonism involving two MYB-like transcription factors, RADIALIS (RAD), and DIVARICATA (DIV). *RAD* is expressed dorsally in the floral primordia and promotes dorsal petal identity, whilst *DIV* is expressed in the whole floral primordia, despite having a phenotypic effect only in more ventral regions of the flower. Genetic and molecular studies have revealed that RAD antagonises the activity of DIV through an indirect interaction with another MYB-like protein identified on a yeast two-hybrid screen using RAD as bait (DRIFs, DIV and RAD-interacting proteins).

To better understand the role of the DRIF proteins in the molecular antagonism that RAD exerts over DIV function, the DNA-binding activity of DIV was analysed by gel-shift assays in the presence of the DRIF and RAD proteins. The subcellular co-localisation of these three proteins was determined using fluorescent tagged proteins in transient assays in tobacco epidermal cells. The expression pattern of the *DRIF* genes was analysed by RT-PCR and the evolutionary history of these new MYB-like proteins studied by phylogenetic analysis.

Our results suggest that the molecular antagonism that RAD has over DIV is mediated by the DRIF proteins. In the ventral domain of the flower DIV and DRIFs interact inside the nucleus in order to determine the ventral identity. However, in the dorsal domain, where RAD is present, RAD interacts with the DRIF in the cytoplasm and prevents their nuclear localisation, preventing DIV from interacting with the RIPs, which results in flower dorsal identity. These results along with the fact that the DRIF, RAD and DIV proteins are present in such a variety of land plant species, suggest that this module of interactions may have been implicated in a broader set of functions throughout plant evolution.

CC9 – Francisco Peixoto

Major Research Topics at CITAB

CITAB/UTAD

CITAB research is focused on the agro-food and forestry systems using the production-chain approach as a whole. This Research Unit is composed by a multidisciplinary team with expertise ranging from fundamental sciences such as biology and chemistry to agronomists, forestry engineers and ecologists.

CITAB was organized with the objective of moving a step forward in the research and development of the agricultural sciences bringing innovation to the conventional production chains in the fields of agriculture and forestry and respective relations to environment.

CITAB is divided in three areas; Ecointegrity, Sustainable Agro-food Chains and Biosystems Engineering. Each of these groups is structured in different lines of research and the main leading areas are: Increasing resource efficiency; biodiversity, sustainable ecosystems and ecosystem services. Added value of agri-food and forestry co-products. Optimization of agri-food chains. Innovative technologies; biobased materials, agri-food and forestry. It will be presented some of the main results achieved by the projects currently under development in CITAB.

CC10 – Mariana Sottomayor

Unravelling the metabolism and transmembrane transport of the highly valuable medicinal alkaloids from *Catharanthus roseus* (L) G. Don.

IBMC, Department of Biology, Faculty of Sciences, University of Porto

Comunicações Orais
Oral Communications

O1 RNASeq analysis of the *Quercus suber* root response to drought

***Azevedo H**, Magalhães A, Martins I, Lino-Neto T, Tavares RM*

Center for Biodiversity, Functional & Integrative Genomics (BioFIG), Plant Functional Biology Center, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Cork oak (*Quercus suber* L.) is a typical species from the Portuguese Mediterranean forest, usually disseminated in dry environments. Mainly due to its high economic value, cork oak has been considered a protected plant species and a national species of interest. Climate alterations, in particular, increased temperature, high light intensity, drought and air pollution are factors that directly affect the development of plants and reduce their productivity. In the present work, the effect of soil water content was studied in *Q. suber* seedlings, as two-month-old plants were subject to different water stress regimes to impose drought stress. Photosynthetic activity measured by PAM fluorometry and photosynthetic pigment content were used as physiological indicators of stress. Subsequently, the transcriptome of *Q. suber* roots in response to moderate and severe drought stress was analysed by Next Generation Sequencing using 454 (Roche) technology. Data was then mapped against the recently established *Q. suber* transcriptome, and RNASeq analysis was performed to establish differential expression. This allowed us to identify over 300 effector and regulatory proteins that are likely to play a key role in *Q. suber* drought tolerance at root level, establishing a fundamental basis for new functional studies.

Project funding by FCT (SOBREIRO/0033/2009 and PTDC/AGR-GPL/118505/2010).

O2 SUMO proteases control development and stress responses in *Arabidopsis thaliana*

***Castro PH**, Couto D, Freitas S, Ruiz-Albert J, Tavares RM, Bejarano ER, Azevedo H*

Center for Biodiversity, Functional & Integrative Genomics (BioFIG), Plant Functional Biology Center, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal (P.H.C., D.C., S.F., R.M.T., H.A.)
Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Dept. Biología Celular, Genética y Fisiología, Universidad de Málaga, Campus Teatinos, 29071 Málaga, Spain (P.H.C., J.R.-A., E.R.B.)

Living organisms are subjected to constantly changing environments that compromise survival. As sessile organisms, plants have optimized a myriad of strategies to adapt, avoid or tolerate stress conditions. A general type of mechanism associated to environmental responses is post-translational modification (PTM), which acts as a fast and reversible regulator of key proteins. Relevant PTMs includes small peptides such as ubiquitin and ubiquitin-like modifiers, namely the Small Ubiquitin-like Modifier (SUMO). SUMO may exert different effects depending on the target protein, either controlling its conformation, or even creating or blocking interacting interfaces that ultimately modulate its activity. SUMO attachment or sumoylation requires SUMO peptides to first be processed by SUMO proteases (ULP/SENP family), and then conjugated to a target's lysine via SUMO E1 activase and SUMO E2 conjugase, aided by SUMO E3 ligases. Deconjugation of the SUMO peptide can subsequently be carried out by the SUMO proteases. A great number of SUMO targets are involved in nuclear-related functions, and are part of essential cellular processes. This is particularly evident in plants since loss-of-function mutants of most SUMO components display embryonic lethality or pleiotropic defects. SUMO-conjugation levels vary in respect to growth conditions, tissues and developmental stages. It is therefore expected that highly dynamic SUMO conjugation and deconjugation machinery is at play. Unlike ubiquitin, SUMO conjugation machinery components are less abundant in the plant genome. SUMO proteases (ULPs) constitute a fairly large gene family and are the potential sources of specificity within the pathway by displaying different enzymatic activity, subcellular localization and expression levels. Based on a reverse genetics strategy, several sets of Arabidopsis ULP T-DNA insertion mutants have been characterized. Results place ULPs as fundamental regulators of both plant development and the response to environmental stress.

Work was supported by FCT/FEDER (refs. PTDC/BIA-PLA/3850/2012 and FCOMP-01-0124-FEDER-028459).

O3 Anti-bacterial potential of silver nanoparticles green synthesised using *Withania somnifera* aqueous leaf extract

*Gregory Marslin [1,], Selvakesavan R.K[1], Franklin G[1], Bruno Sarmento [2,3]**,** Alberto C.P. Dias [1]*

1 Centre for the Research and Technology of Agro-Environment and Biological Sciences (CITAB-UM), AgroBioPlant Group, Department of Biology, University of Minho, Portugal
2 INEB – Instituto de Engenharia Biomédica, University of Porto, Portugal
3 CICS, Department of Pharmaceutical Sciences, Instituto Superior de Ciências da Saúde-Norte, Gandra, Portugal

In the present study, we report on green synthesis of silver nanoparticles (AgNPs) using *Withania somnifera* aqueous leaf extracts as reducing agent and their characterization. The formation, size and shape of green synthesized AgNPs were confirmed by physical-chemical techniques such as UV-Visible spectroscopy, laser Doppler anemometry, Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), X-ray diffraction (XRD) and X-ray energy dispersive spectroscopy (EDX). The SEM and AFM images confirmed that the size of synthesised AgNPs ranged between 70-110 nm, mainly spherical and some in hexagonal shape. AgNPs exhibited significantly higher antibacterial activity (up to 200x) against human as well as plant pathogens (*Escherichia coli, Pseudomonas aeruginosa* and *Agrobacterium tumefaciens*) compared to AgNO3 solution and *W. somnifera* leaf extract. The cellular interaction study coupled with SEM analysis revealed the effective uptake of AgNPs by the bacteria. The compounds responsible for the nanoparticle formation were identified as phenolics and whitanolids present in the extract, as revealed by HPLC analysis.

O4 Antioxidant capacity of *Melia azedarach* under water deficit conditions

Azevedo C, Dias MC, Costa M, Pinto G and Santos C*

Department of Biology and CESAM – Centre for Environmental and Marine Studies, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal.
**Correspondig author: celeste.dias@ua.pt*

This work aims to evaluate the effects of water stress (WS) on the antioxidant capacity and on the photosynthetic apparatus of the medicinal plant *Melia azedarach* L. Two month old plants of *M. azedarach* were exposed to WS (plants at 20% of field capacity) during 20 days. After this period, plant performance was evaluated through the measurement of the water potential, plant growth, chlorophyll *a* fluorescence, gas exchange, pigment content, H_2O_2, proline, the activities of antioxidant enzymes and antioxidant metabolites, cell membrane permeability and lipid peroxidation. IRGA analysis demonstrated that WS induced stomatal closure, reduced the net CO_2 assimilation rate (*A*) and decreased the CO_2 availability in the intercellular spaces of mesophyll cells (*Ci*). However, WS did not affect the photosynthetic efficiency of PSII or plant growth (dry weight accumulation and plant height). WS increased cell membrane permeability and induced an up-regulation of the antioxidant enzymes, CAT, APX and Gr and also an over production of antioxidant metabolites. Despite these effects on photosynthetic apparatus and increased antioxidant capacity, plant growth was not affected, supporting that this species may be used in re/afforestation programs for drought prone habitats. Moreover, it would be interesting to analyse if the stress-induced increase of the antioxidant capacity may be used as a positive strategy to increase antioxidant properties of this species.

O5 Reflorestação de zonas degradadas pela seca com amargoseira: I. crescimento e fotossíntese

Maria da Costa [1,2], Glória Pinto[1], Conceição Santos[1]

[1]Departamento de Biologia, Universidade de Aveiro, 3810-193, Aveiro Portugal
[2]Ministério da Educação de Timor-Leste, Dili, Timor-Leste

Melia azedarach, ou amargoseira, é uma espécie lenhosa que produz madeira com interesse comercial, e apresenta propriedades medicinais utilizadas na tradição etnobotânica de Timor-Leste. Esta espécie existe em quase todas as regiões do país –litorais e montanhosas-. Contudo, Timor-Leste apresenta problemas crescentes de degradação de solos devido a períodos de seca, agricultura itinerante, desmatamento descontrolado, etc. A necessidade de valorizar recursos florestais, proteger práticas etnobotânicas e combater a desertificação justifica a introdução da amargoseira na arborização de solos degradados pela seca em Timor-Leste. Esta medida permitiria melhorar a qualidade de vida e auxiliaria a implementação do Programa Estratégico para o Desenvolvimento (PED) do Governo Leste-Timorense.
Para esta estratégia é necessário avaliar o grau de suscetibilidade da amargoseira a stresse hídrico. Para isso, usaram-se sementes e plantas com 2-3 meses de idade, a crescer em estufa. Lotes de sementes e de plantas foram expostos a condições normais de irrigação (controlos) e outros lotes de sementes e plantas foram expostos a stresse hídrico (20%). O stresse hídrico atrasou a germinação das sementes e afetou de forma diferente as raízes e a porção aérea (eg., maior peso das raízes). A sobrevivência das plantas não foi afetada. O stresse diminuiu a eficiência fotossintética, e reduziu o índice de abertura estomática, limitando as trocas gasosas. Diminuiu também o conteúdo de pigmentos, o que pode justificar uma menor eficácia na captação de luz pelos PSI/PSII. Houve uma tendência para aumentar o F0 enquanto Fv e Fv/Fm apresentaram uma tendência de decréscimo com o stresse. Sendo os cloroplastos um organelo muito sensível a stresse, analisou-se também a degradação membranar (EL). Os dados preliminares sugerem que a fotossíntese na amargoseira é afetada pelo stresse hídrico, embora não seja suficiente para comprometer a viabilidade das plantas.

O6 Dot-blot validation of novel DNA markers for detection and genotyping of *Ralstonia solanacearum* species complex

Albuquerque P[1], Caridade CMR[2,3], Marçal ARS[2,4], Tavares F[1,5]

1- CIBIO- Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, Vairão, Portugal
2- CICGE-Centro de Investigação em Ciências Geo-Espaciais, Faculdade de Ciências, Universidade do Porto, Porto, Portugal
3- ISEC-Instituto Superior de Engenharia de Coimbra, Coimbra, Portugal
4- CMUP-Centro de Matemática da Universidade do Porto, Faculdade de Ciências, Universidade do Porto, Porto, Portugal
5- FCUP- Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Edifício FC4, Via Panorâmica nº 36, Porto, Portugal

Ralstonia solanacearum (*Rs*) is a Gram-negative rod that causes systemic wilting disease in several crops, namely potato, tomato, banana and tobacco, causing important economic losses. In order to enforce large-scale monitoring and quarantine phytosanitary procedures there is the need to develop specific detection and typing methods for this pathogen, particularly due to the high genotypic diversity observed within the *Rs* species complex. In this regard, DNA-based methods present several advantages over culture-based methods of detection, mainly concerning their specificity, detection resolution and assay time.

In this work, novel *Rs* specific markers, able to detect and discriminate *Rs* strains belonging to different races and biovars, were selected and validated. *Rs*-specific protein domains were identified using the Pfam database and a follow-up BLAST analysis of the corresponding nucleotide sequences, as previously described [1]. The 17 novel putative *Rs*-specific DNA markers were assessed for their specificity and consistency across diverse *Rs* strains and non-target species using PCR and hybridization-based approaches as detailed by Albuquerque *et al.* [2]. The data obtained showed that while some markers were broad spectrum, i.e. present throughout the different tested *Rs* strains, the majority displayed strain-specific patterns. The simultaneous use of broad spectrum and strain-specific markers in high-throughput assays, such as inverted dot blots, provides discriminatory hybridization patterns useful for presumptive infrasubspecific discrimination of *Rs* isolates. Ultimately, this work might contribute to determine epidemiologic patterns which are important to implement suitable control measures and infer dissemination models.

References:

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O7 Contribution of the plant rhizosphere system to the phytoremediation of cadmium in estuarine areas

Marta Nunes da Silva[1], Ana P. Mucha[2], A. Cristina Rocha[1], Catarina Teixeira[2], Carlos R. Gomes[1], C. Marisa R. Almeida[2]

1 CIMAR/CIIMAR, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal.
2 CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal.

Some salt marsh plants have already shown to have potential for metal phytoremediation in estuarine areas [1], being important to study and test strategies to enhance that potential. The aim of this work was to evaluate how the rhizosphere of *Juncus maritimus* and *Phragmites australis* plants influenced Cd removal from sediments favoring environmental decontamination. For this study, plants of both species, collected together with the sediment involving their roots, were placed in vessels and maintained in greenhouses, exposed to natural environmental and light conditions. A nutritive saline solution was added to all vessels through an automated irrigation system (2 daily cycles of flood / draught) to mimic the tides and maintain plants at optimum nutritional conditions. After 2 weeks of acclimation, all vessels were spiked with a saline Cd solution, which was in contact with the plant rhizosphere for about 6h. Afterwards a solution containing an autochthonous enriched consortium of microorganisms resistant to Cd (prepared in the laboratory) was added to half of the vessels. Vessels were maintained for 2 months in the abovementioned conditions, being afterwards disassemble. For that, plants aboveground tissues were separated from belowground structures, which in turn were carefully separated from the sediment. Cadmium was determined as before [1]. Results suggest that both plants were able to accumulate considerable amounts of Cd, especially in their belowground tissues, without showing signs of toxicity. The addition of the microbial consortium seemed to promote Cd translocation to *P. australis* stems, increasing the phytoextraction potential of this plant. In addition, the phytostabilization potential of *J. maritimus* was also enhanced. Therefore, the addition of autochthonous microorganisms resistant to Cd seems to be a valuable strategy to potentiate the ability of these plants to phytoremediate this metal in saltmarshes, being useful for the recovery of moderately impacted estuaries.

Acknowledgments: To FCT, Portugal, for PTDC/MAR/099140/2008.

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O8 The Plant Specific Insert and Its Molecular Role in Protein Sorting

Peixoto B.[1,2], Pereira C.[1], Veríssimo P.[2], Pissarra J.[1]

[1]BioFIG – Centre for Biodiversity, Functional and Integrative Genomics, Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre, s/nº, 4169-007, Porto, Portugal.
[2]Molecular Biotechnology Laboratory, Center for Neuroscience and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal.

A particular characteristic of plant aspartic proteinases is the presence of an approximately 100 amino-acids long insertion, highly homologous to both saposins and saposin-like proteins and whose physiological function is currently unknown – the Plant Specific Insert (PSI). This PSI domain is characterized by a closely packed globular structure comprised by five amphipathic α-helices linked to each other by three disulfide bridges.This domains' importance in vacuolar trafficking has already been demonstrated in transient expression experiments using tobacco protoplasts expressing a PSI-lacking phytepsin. However, additionally to the PSI's involvement in protein sorting to the plant vacuole, this domain's properties in inducing vesicle leakage *in vitro* have been demonstrated, a result that suggests plant aspartic proteinases might be bifunctional molecules, acting both as membrane-destabilizing agents and proteinases.
Recently, a novel AP has been discovered in *Chlamydomonas reinhardtii*, its characterization has revealed a series of intriguing features, such as an 80 amino-acid long alanine-rich insertion in the PSI domain, as well as a chloroplastidial subcellular localization, both of which had never been reported for typical aspartic proteases, turning this novel proteinase, chlapsin, into a most promising model for studying the molecular mechanisms associated with the PSI's role in protein sorting. Recent work by our group, with the objective of further characterizing these domains, from a bioinformatics, biochemical and cellular perspectives have recently identified functional diversity associated with PSI-mediated protein sorting routes, which could prove useful from a biotechnological point of view.

O9 A highly efficient leaf protoplast expression system for the study of anticancer alkaloid metabolism in *Catharanthus roseus*

Patrícia Duarte[1], Diana Ribeiro[1,2], Inês Carqueijeiro[1,3] and Mariana Sottomayor[1,3,*]*

[1] IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Rua do Campo Alegre, 823, 4150-180 Porto, Portugal
[2] Departamento de Biologia, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[3] Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal

Catharanthus roseus leaves accumulate in low levels the anticancer terpenoid indole alkaloids (TIAs) vinblastine and vincristine, and intense research has uncovered a number of TIA biosynthetic steps and regulatory genes. However, there are still enormous gaps in the knowledge on the TIA biosynthetic pathway, its regulation and TIA transmembrane transport. At present, several *C. roseus* transcriptomic projects are unveiling a high number of candidate genes, urging the development of easy and efficient molecular tools for functional characterization. Leaves are the single *C. roseus* organ where the biosynthesis of the anticancer TIAs is completed, and therefore mesophyll cells are the ideal target for TIA gene functional analysis. Here, a highly efficient method for *C. roseus* mesophyll protoplast isolation and transformation is reported, enabling to reproducibly test TIA candidate genes in the precise cells where the biosynthesis and accumulation of the anticancer TIAs occur. As a proof of principle of the application of this molecular tool, the subcellular sorting determination of CroPrx1, a class III peroxidase involved in TIA biosynthesis, was investigated. Transient expression of different *CroPrx1-GFP* fusions revealed that CroPrx1 is sorted to the vacuole by a C-terminal peptide signal. After customized optimization of protoplast isolation, the transformation procedure seems to be easily adaptable to different species, suggesting that this is a plant-transferable tool that may be applied to non-model species for the functional characterization of the wealth of candidate genes emerging from next generation sequencing.

O10 Photosynthesis in grape berry tissues: an *in vivo* approach by imaging PAM fluorometry

Richard Breia[1,2], Sónia Vieira[3,4], Jorge Marques da Silva[3], Hernâni Gerós[1,2] and Ana Cunha[1,2]

[1] Centro de Investigação e de Tecnologias Agro-ambientais e Biológicas CITAB-UM, Portugal
[2] Grupo de Investigação em Biologia Vegetal Aplicada e Inovação Agroalimentar (AgroBioPlant), Departamento de Biologia, Escola de Ciências Universidade do Minho, Braga, Portugal
[3] Departamento de Biologia Vegetal e Centro de Biodiversidade, Genómica Integrativa e Funcional (BioFIG), Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal
[4] Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

Photosynthesis is not exclusive of green leaves. In fact, some sink tissues, like fruits, which are chlorophyllous during early developmental phases, could exhibit photosynthetic activity. To characterize the photosynthetic properties of the green grape berry, the pulse amplitude chlorophyll fluorescence imaging (imaging-PAM) was used. Also the effect of the saturation pulse (SP) intensity on photochemical parameters was tested. Results revealed a tissue-specific distribution pattern of photosynthesis and a strong effect of SP on maximum fluorescence (F_m) and in rapid light curves (RLC) parameters, such as F'_m and effective quantum yield (Φ_{II}). After RLC modeling, the exocarp showed the highest photosynthetic capacity and the lowest photoinhibition susceptibility and the mesocarp, revealed the lowest fluorescence signals and photochemical competences. Notably, the seed outer integument presented a high photochemical capacity, similar to the exocarp, although it is more prone to photoinhibition. Globally, all tissues saturated the PSII reaction centers at relatively low SP light intensities. Above 5000 µmol m^{-2} s^{-1}, F_m, F'_m and Φ_{II} were significantly affected, but inner tissues under light-adapted conditions were susceptible at lower saturating light (3200 µmol m^{-2} s^{-1}) indicating a photoinhibitory interaction between SP and actinic light intensities and repetitive exposure to saturating pulses (1). These results open the way to further studies concerning the involvement of tissue-specific photosynthesis in the highly compartmentalized production and accumulation of organic compounds during grape berry development.

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Acknowledgments: This work was supported by European Union Funds (FEDER/COMPETE-Operational Competitiveness Programme) and by national funds (FCT-Portuguese Foundation for Science and Technology) under the projects FCOMP-01-0124-FEDER-022692 and European COST action FA 1106 and the research project PTDC/AGR-ALI/100636/2008. RB was supported through a research grant fellowship (UMINHO/BI/177/2011) associated with the project PTDC/AGR-ALI/100636/2008.

O11 Copper transporters in grapevine

Viviana Martins[1,2], António Teixeira[1,2], Elias Bassil[3], Eduardo Blumwald[3], Hernâni Gerós[1,2]

[1]Centro de Investigação e de Tecnologias Agro-Ambientais e Biológicas (CITAB), Portugal; [2]Grupo de Investigação em Biologia Vegetal Aplicada e Investigação Agroalimentar – Agrobioplant, Departamento de Biologia, Escola Ciências, Universidade do Minho, Campus de Gualtar, Braga, 4710-057, Portugal; [3]Department of Plant Sciences, University of California, One Shields Ave, Davis, CA 95616, USA

Fungicides based on copper salts have been extensively used in viticulture since the late 1800s upon the development of the 'Bordeaux mixture', a broad spectrum fungicide that consists of copper sulfate and lime. Despite being an essential element for plant health, excess copper may be a cause of toxicity, and several mechanisms such as chelation and compartmentation of metal ions have evolved to avoid negative effects at cell level while insuring their appropriate delivery within the cell compartments. Previous studies have shown that the viability of grape cells decreases with the increase in copper concentration in a dose-dependent manner and that the sequestration of the metal ion in the vacuole may constitute an effective mechanism to avoid its inherent toxicity. Copper transporters (COPT/Ctr) operate at the plasma membrane level and in internal membranes, such as the tonoplast. These high-affinity transporters have been mostly characterized in yeast, humans and in some plant species. In the present study, eight putative *Vitis vinifera* Copper Transporters (*VvCTrs*) were identified and the expression of *VvCTr1* was investigated in grapevine tissues throughout development and ripening and in response to Bordeaux mixture. Furthermore, the localization of VvCTr1 was assessed in plant cells after transient transformation with fusion proteins of VvCtr1 with GFP and RFP. *VvCTr1* successfully restored the growth-defect of yeast mutants lacking Ctr transporters, validating its function as a copper transporter and giving insights on its contribution for copper mobilization within the cell.

Acknowledgments: This work is supported by European Union Funds (FEDER/COMPETE-Operational Competitiveness Programme) and by portuguese national funds (FCT-Portuguese Foundation for Science and Technology) under the project FCOMP-01-0124-FEDER-022692, the research project FCOMP-01-0124-FEDER-008760 (Refª. FCT PTDC/AGR-ALI/100636/2008), the project FCT/5955/27/5/2013/S - scientific cooperation Portugal-Tunisia, and the PhD grant no. SFRH/BD/64587/2009 to VM. This work is also supported by the European project INNOVINE (ref. 311775), by the networking activities within the european funded COST ACTION (FA1106 "QualityFruit" and FA1003 "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding").

O12 - Desenvolvimento Sustentável e Ecoinovação - ferramentas de combate à crise

Ecoinside

O13 Identification of heavy metal transporters in Solanaceae plants

A.M. Pessoa1,2, S. Pereira1,2 and J.Pissarra1,2

1University of Porto, Faculty of Sciences, Biology Department, Edifício FC4, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal;
2BioFIG - Center for Biodiversity, Functional & Integrative Genomics, Plant Molecular Biology & Biotechnology Lab, University of Porto, Portugal;

The Solanaceae include commonly edible plants such as Potato (Solanum tuberosum), Tomato (Solanum lycopersicum) and Eggplant (Solanum melongena). This plant taxon is considered to be one of the most economically important and the most valuable regarding vegetable crops. The two model plants tomato and potato had their genome recently sequenced and is now publicly available.

Metal transport and uptake of metals are essential for plant growth, development, nutrition and signal transduction. In addition, plant metal transports systems are a very important part of mechanisms for decrease the toxicity of certain metal ions.

Therefore, many metal transporters are known to transport toxic cations (such as heavy metals) along with cationic nutrients.

The ZIP family (ZRT, IRT-like proteins) plays prominent roles in metal ions uptake, transporting Zn, Fe and Mn from outside the cell into the cytoplasm. The CDF family (cation diffusion facilitators) transports a wide range of metal ions and is known to promote Zn compartmentalization by pumping the metal out of the cell or into the lumen of organelles. The NRAMPs (natural resistance-associated macrophage proteins) are known to participate into the transport and regulation of Fe. All of these three families of transporters are known to possess Cd transport ability.

A large number of heavy metal transporters have already been described for Thlaspi and Arabidopsis as well as for other plant species species, but only a limited number has been identified for Solanaceae. However, there are several inconsistencies in the heavy metal transporter sequences published and also many unannotated Solanaceae sequences in NCBI databases (unknown DNA clones, ESTs, GSSs, genome sequences) that may represent putative metal transporters.

Using these annotated sequences along with the sequence information from NCBI, and data mining and gene prediction techniques, several putative transporters from the ZIP, CDF and NRAMP family were identified and characterized in tomato and potato, as well as compared with other plant species, such as Thlaspi and Arabidopsis.

Our conserved primers design tool, PrimerIdent (http://primerident.up.pt), was then used for designing suitable primers that allowed not only to confirm the predicted transporter sequences, but to identify them in other Solanaceae plants as well.

We expect that these results will be a stepping stone in the understanding of metal homeostasis in Solanaceae plants.

Posters

P1 Polyol transport and metabolism adjustments in *Vitis vinifera* in response to water stress

Artur Conde[1,2], Ana Regalado[3], Diana Rodrigues[2], M. Manuela Chaves[3,4] and Hernâni Gerós[1,2]

[1] *Centro de Investigação e de Tecnologias Agro-ambientais e Biológicas CITAB-UM, Portugal*
[2] *Grupo de Investigação em Biologia Vegetal Aplicada e Inovação Agroalimentar (AgroBioPlant), Departamento de Biologia, Escola de Ciências Universidade do Minho, Braga, Portugal*
[3] *Instituto de Tecnologia Química e Biológica, Apartado 127, 2781-901 Oeiras, Portugal*
[4] *Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal*

Polyols, or sugar alcohols, are widely distributed compounds among plants and frequently act as osmoprotectants. In *Olea europaea*, mannitol is an important osmoprotectant under drought and salinity, where mannitol transport and intracellular oxidation are well coordinated (1). GC-TOF-MS analysis revealed that several polyols are present in grapevine (*Vitis vinifera*) mature leaves and berry mesocarp in significant concentrations. Several polyols accumulate during water deficit in the final maturation stages of berry development. A significant inhibition (50%) of sorbitol oxidation to fructose via sorbitol dehydrogenases (VvSDHs) in mesocarp is clear in the full mature stage of grape berries under drought. Mannitol dehydrogenase (VvMTD) activity is also severely repressed (4-fold) in the mature and full mature stages under water deficit conditions. These data are well in line with our previous observations regarding polyol transport by VvPLT1. The combination of metabolomic, molecular biology and traditional biochemical approaches provided new insights on the role of polyols in grapevine defense against drought stress. Additionally, increased concentrations of polyols in grape berries may constitute a novel biomarker for drought-tolerant grapevine cultivars and a bioindicator of plant health/adaptation under water deficit.

(1) Conde et al. (2011) Plant Cell Physiol 52(10):1766-75

Acknowledgments: This work was supported by European Union Funds (FEDER/COMPETE-Operational Competitiveness Programme) and by national funds (FCT-Portuguese Foundation for Science and Technology) under the projects FCOMP-01-0124-FEDER-022692 and European COST action FA 1106 and the research projects PTDC/AGR-ALI/100636/2008 and PTDC/AGR-AAM/099154/2008. AC was supported by the PhD grant no. SFRH/BD/47699/2008.

P2 Reporting On Endemic Endangered Plant Species Using Predictive Models

Rita S. Silva[1]*, Paulo Alves*[2]*, Antoine Guisan*[3]*, João Honrado*[1,2]*, Angela Lomba*[2,3,4]

[1] *Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal*
[2] *Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO), Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal*
[3] *Laboratoire de Biologie de la Conservation (LBC), Département d'Ecologie et d'Evolution (DEE), Université de Lausanne, Bâtiment de Biologie, CH-1015 Lausanne, Switzerland*
[4] *Alterra Wageningen University and Research Centre, NL-6708 Wageningen, The Netherlands*

Current patterns of species distributions are largely determined by changes in climate and land uses, with its impacts on biodiversity and associated ecosystem services being widely acknowledged. Rare species are a growing concern in the context of biodiversity conservation worldwide, since these species are assumed to undergo higher risk of extinction. Rare species are characterized by restricted geographic ranges, habitat specialization and/or small population sizes. Even though datasets on rare species distribution usually have few observations, limited spatial accuracy and lack of valid absences, the development of predictive models that relate species occurrences to environmental descriptors have been proved to be particularly useful to realistically capture a large part of these species ecological niche. *Veronica micrantha* Hoffmanns. & Link is an endangered plant species, endemic of the Iberian Peninsula and protected under the EU Habitats Directive. Under Article 17 of this Directive, EU Member States are obliged to undertake monitoring, assessment and reporting of conservation status for all habitats and species of Community interest. Thereby, this study aimed to assess the usefulness of predictive models as assessment tools to effectively fulfil the reporting obligations, and as tools to identify environmental conditions within which populations can be maintained. The potential distribution of *Veronica micrantha* was estimated based on two different approaches: minimum convex polygon and species distribution modelling. The former resulted in an overestimation of the species distribution, whereas the latter gave a more conservative, presumably more realistic, assessment of the distribution of the species while also allowing a better understanding of the ecology and dynamics of the species. Therefore, the proposed approach constitutes a step forward in the study of the distribution patterns of endemic endangered plant species, as well as in the use of these more informative projections to improve the reporting and monitoring of these at-risk species.

A. Lomba is supported by the Portuguese Science and Technology Foundation (FCT) through Post Doc Grant SFRH/BPD/ 80747/2011. This study was partially funded by FEDER through "Programa Operacional Factores de Competitividade – COMPETE" and by the Portuguese Government through "FCT – Fundação para a Ciência e a Tecnologia", in the form of project grant "EcoSensing: indicators, methods, and protocols for reporting and monitoring the condition of biodiversity and ecosystems in rural landscapes" (PTDC/AGR-AAM/104819/2008).

P3 Plant species assemblages along a gradient of forest naturalness in dairy-farmlands of the Northwestern Portugal

Ana Sofia Vaz[1]*, João Honrado*[1,2] *and Angela Lomba*[1,3,4]

[1]*Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Edifício FC4, Rua Campo Alegre s/n, 4169-007 Porto, Portugal*
[2]*Faculdade de Ciências (Departamento de Biologia), Universidade do Porto, Edifício FC4, Rua Campo Alegre s/n, 4169-007 Porto, Portugal*
[3]*Alterra Wageningen University and Research Centre, NL-6708 Wageningen, The Netherlands*
[4]*Départment d'Ecologie et d'Evolution (DEE), Université de Lausanne, Bâtiment de Biologie, CH-1015 Lausanne, Switzerland*

Knowledge of plant assemblages is essential to comprehend ecological processes, and to enhance efficient management and conservation measures across forests worldwide.

A total of 50 stands were surveyed along a gradient of forest naturalness in dairy-farmlands in Portugal, and classified considering their dominant tree: *Eucalyptus globulus*, *Pinus pinaster*, and mixed stands with both species. Vascular plants were recorded within $10m^2$, $100m^2$ and $1000m^2$ squares centred in the stand centroid, and within the whole stand. Species richness, assemblages and species-area relationships were evaluated across forest types. For the whole community, plant assemblages were assessed calculating co-occurrence indices. For the tree community, assemblages were explored within $100m^2$ squares, where trees with ≥1.30m height were mapped and analysed through point pattern analyses.*Pinus* stands exhibited smallest areas and highest species richness. For all forest types, species richness increased with area within each stand. Segregation patterns prevailed in plant communities regardless of the scale and forest type. Still, random patterns were found in mixed and *Eucalyptus* stands. Tree assemblages revealed trends for regular patterns in *Pinus* stands, and aggregation patterns in the other forests. Overall, results converge with previous research evaluating the impacts of forest naturalness on plant diversity. *Pinus* stands showed independent relations between area, community species richness and assemblages, possibly due to high environmental heterogeneity and low disturbance levels related to forest management. Tree assemblages appeared to reflect shifts in the management intensity of each forest, with close-to-nature regularities in *Pinus*, and fine-scale aggregations in mixed and *Eucalyptus* stands. This suggests that structural changes within stands could determine plant diversity and interactions, and ecosystem processes and services.

Results highlight forest naturalness and management as key drivers of plant diversity and assemblages, so extensively managed semi-natural stands must be promoted for sustainable provisions of ecosystem services from planted forests in intensive farmlands.

Acknowledgements: A. Lomba is supported by the Portuguese Science and Technology Foundation (FCT) through Post Doc Grant SFRH/BPD/ 80747/2011. This study was partially funded by FEDER through "Programa Operacional Factores de Competitividade – COMPETE" and by the Portuguese Government through "FCT – Fundação para a Ciência e a Tecnologia", in the form of project grant "EcoSensing: indicators, methods, and protocols for reporting and monitoring the condition of biodiversity and ecosystems in rural landscapes" (PTDC/AGR-AAM/104819/2008).

P4 Lipoxygenase in *Vitis Vinifera* culture cells and grape berry

António Teixeira[1,2], Diogo Araújo[2], Henrique Noronha[1,2], José Eiras Dias[3], Hernâni Gerós[1,2]

[1]Centro de Investigação e de Tecnologias Agro-Ambientais e Biológicas CITAB-UM, Portugal
[2]Grupo de Investigação em Biologia Vegetal Aplicada e Inovação Agroalimentar (AgroBioPlant), Departamento de Biologia, Escola de Ciências, Universidade do Minho, Braga, Portugal
[3]Instituto Nacional de Investigação Agrária INIA, Quinta da Almoinha, 2565-191 Dois Portos, Portugal

Vitis vinifera is a major crop worldwide of extreme socioeconomic importance. Environmental factors like heat, drought, soil salinity or pathogen infections can affect berry composition and thus compromising quality. Lipoxygenases (LOXs) are a group of dioxygenases that catalyse oxygenation of polyunsaturated fatty acids (PUFAs) and lipids, and initiate the formation of biologically active compounds known as oxylipins. LOXs can play several roles in plant metabolism and the products of its reaction are signaling molecules in wounding and pathogen attack, inducers of cell death, inhibitors of mycotoxin synthesis, and signaling molecules in several other stresses like water deficit. Thus, its study may provide information on how grapevine responds to environment. In grape genome (*Vitis vinifera* L.) LOX family consists of 18 individual members. While the expression of *VvLOXC* and *-D* is evenly distributed between seeds, pulp and skin, and *VvLOXO* is mostly expressed in the seed, *VvLOXA* is the most abundant isoform in berry skins in all developmental stages. In the present study LOX activity was measured in grape cells (Cabernet Sauvignon Berry) subjected to salt, osmotic and heat stress, and to treatments with stress related hormones (salicylic acid - SA and abscisic acid - ABA). LOX activity was also measured in grapes (green pea, *veraison* and mature stages) from the cv. Alvarinho, sampled in two different Portuguese regions: Demarcated Region of Vinho Verde (Estação Vitivinícola Amândio Galhano, EVAG) and Estremadura Region (Instituto Nacional de Investigação Agrária, INIA). Results showed that except for salt treatment, which decreased enzyme activity by 25%, all the treatments caused a significant increase of LOX activity, in particular ABA and SA. The hormone ABA is likely to stimulate LOX activity at the transcriptional level because the increased enzyme activity in grape cells correlated with an increase of *VvLOXA* transcripts measured by qPCR. Concerning measurements in intact berries, results showed that LOX activity picked at the veraison stage in both cultivars and was higher in INIA than in the north.

Acknowledgments: This work is supported by European Union Funds (FEDER/COMPETE-Operational Competitiveness Programme), European COST action FA 1106, and by national funds (FCT-Portuguese Foundation for Science and Technology) under the project FCOMP-01-0124-FEDER-022692 and the research project PTDC/AGR-ALI/100636/2008.

P5 Photomixotrophic grape berry cell suspensions: a suitable cell model for the study of abiotic stresses in fruit photosynthesis

Richard Breia[1,2], Fábio Ramalho[2], João Serôdio[3], Hernâni Gerós[1,2] and Ana Cunha[1,2]

1 Centro de Investigação e de Tecnologias Agro-ambientais e Biológicas CITAB-UM, Portugal
2 Grupo de Investigação em Biologia Vegetal Aplicada e Inovação Agroalimentar (AgroBioPlant), Departamento de Biologia, Escola de Ciências Universidade do Minho, Braga, Portugal
3 Departamento de Biologia e CESAM – Centro de Estudos do Ambiente e do Mar, Universidade de Aveiro, Campus de Santiago, Aveiro, Portugal (richardgoncalves@bio.uminho.pt)

Fruit are essentially sink organs, so fruit photosynthesis has been seldom overlooked. However, recent findings proved that some grape berry tissues exhibit high photochemical activity, which may contribute to fruit and seed development (1). In the present study, two lines of photomixotrophic suspension cells derived from CSB (Cabernet Sauvignon Berry) cells and exocarps of grape berries from cv. “Alvarinho” were used to determine the effect of cooper and water stress in cell growth, chlorophyll content and maximum photochemical quantum efficiency (F_v/F_m). While copper-based fungicides have been widely used against several grapevine (*Vitis vinifera* L.) diseases since the late 1800s, although its intensive application has raised phytotoxicity concerns, water deficit is probably the most important environmental stress factor in agriculture, responsible for major reductions in plant productivity. Several studies have shown the negative impact of these stresses in leaf photosynthesis in many species, including grapevine, but little information is still available concerning fruit photosynthesis. In the present study, water deficit conditions (Ψw = -0.4 MPa and -0.8 MPa) caused a reduction in the fresh weight and an increase in dry weight seven days after sub-culture of both cell lines. Chlorophyll content was reduced in CSB-derived photomixotrophic cell cultures, but maximal photochemical potential (F_v/F_m) decreased in both cell lines. When 40 or 80 μM $CuSO_4$ were added to the culture media, cell growth, chlorophyll content and F_v/F_m values were reduced in a dose-dependent manner in the CSB-derived photomixotrophic culture cells, but none of these parameters were affected in exocarp-derived culture cells. Results are discussed in terms of putative differential effects of copper and drought over the photochemical activity of grape berry mesocarp and exocarp tissues.

1 - Breia et al. 2013. Mapping Grape Berry Photosynthesis by Chlorophyll Fluorescence Imaging: The Effect of Saturating Pulse Intensity in Different Tissues. *Photochemistry and Photobiology*, 89: 579–585.

Acknowledgments: This work was supported by European Union Funds (FEDER/COMPETE-Operational Competitiveness Programme) and by national funds (FCT-Portuguese Foundation for Science and Technology) under the projects FCOMP-01-0124-FEDER-022692 and European COST action FA 1106 and the research project PTDC/AGR-ALI/100636/2008. RB was supported through a research grant fellowship (UMINHO/BI/177/2011) associated with the project PTDC/AGR-ALI/100636/2008.

P6 Unravelling the molecular network regulating zinc content in plants: from Arabidopsis to rice

Joana G. Guedes[1], Diego Almeida[2], Mark G.M. Aarts[3], Nelson J. M. Saibo[2], Ana G.L. Assunção[1]

[1]Plant Evolution Group, CIBIO/InBIO, Universidade do Porto, R. Padre Armando Quintas, 4485-661 Vairão, Portugal
[2]Genomics of Plant Stress Laboratory, ITQB, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal
[3]Laboratory of Genetics, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

As a plant micronutrient, zinc is of major interest in agronomy and for human and cattle nutrition. Zinc deficient soils are widespread all over the world and the risk of inadequate diet and zinc malnutrition is estimated to affect one third of the global human population, i.e. around 2 billion people. Developing countries, where people depend on cereal-rich diets for sustenance, are the most affected. Improvement of crop efficiency in zinc deficient environments, and bio-fortification to alleviate human nutrition are plant-based solutions as source of micronutrients. An incomplete understanding of zinc homeostasis mechanisms in plants is however a limitation. Recently the first transcription factors regulating zinc homeostasis in Arabidopsis were identified (Assunção et al. 2010). The AtbZIP19 and AtbZIP23 were shown to be essential for the adaptation to zinc deficiency. Transcript profiling revealed only a small set of genes, to be induced in wild-type plants in response to zinc deficiency, but not in the *bzip19bzip23* Arabidopsis double mutant line. A suggested conservation of the zinc deficiency response regulatory network within the plant kingdom makes it very attractive to explore the molecular basis of such network in rice (*Oriza sativa* L.). Rice is one of the most important crops worldwide and it is the staple food for more than half of the world's population. Additionally it is a very relevant crop in Portugal. Transferring the knowledge from Arabidopsis to rice is of major importance. We present our preliminary results in the identification and analysis of the bZIP19/bZIP23 rice orthologs.

P7 Níveis taxonómicos Superiores (e.g. género e família) como Indicadores de Riqueza Específica de Briófitas

Cristiana Alves[1], Helena Hespanhol[2], Cristiana Vieira[2] & Rubim Almeida da Silva[1,2]

[1]Faculdade de Ciências da Universidade do Porto
[2]Centro de Investigação em Biodiversidade e Recursos Genéticos, CIBIO-UP

Devido ao rápido declínio da biodiversidade que se tem vindo a acentuar nas últimas décadas, é necessária uma monitorização dos habitats para avaliar o estado dos ecossistemas.
Por isso, uma das soluções que tem vindo a ser utilizada para descrever padrões de diversidade é o uso de níveis taxonómicos superiores como indicadores do número de espécies. Este método é vantajoso, uma vez que, a identificação até ao género ou família é mais fácil e menos demorada do que a identificação de espécies. O potencial de utilização desta metodologia para prever padrões de riqueza específica para diferentes grupos taxonómicos tem sido bastante explorado porém, ainda não foi alvo de investigação no caso do grupo das briófitas. O objectivo deste estudo foi analisar se níveis taxonómicos superiores, como o género ou família, podem ser utilizados como indicadores fiáveis da riqueza específica de briófitas. Os dados foram recolhidos em áreas pertencentes à rede NATURA 2000, no Norte e Centro de Portugal, em dois tipos de habitats: saxícolas e fluviais. Nos habitats saxícolas foram encontradas 129 espécies que pertencem a 73 géneros de briófitas, enquanto que nos habitats fluviais foram encontradas 175 espécies de briófitas distribuídas por 99 géneros. Para os dados dos habitats saxícolas foi usada a correlação de Pearson e para os dados dos habitats fluviais foi usada correlação de Spearman. Nos dois tipos de habitats estudados, a correlação entre a riqueza específica e o número de géneros e famílias é positiva e significativa ($p < 0.01$) e o coeficiente de correlação é alto ($r > 0.8$). Este estudo sugere que níveis taxonómicos superiores, podem ser bons indicadores de riqueza específica de briófitas e, como tal, pode ser considerado um método rápido, eficiente e de baixo custo que pode ser implementado em futuros estudos da diversidade de briófitas.

P8 A Trait-Based Approach to Assess Dune Vegetation Responses to Changes in Coastal Dynamics

José António Macedo[1,2], João Tereso[1], Paulo Alves[1], Renato Henriques[3,4], Francisco Ignacio Pugnaire[5], João Honrado[1,2]

[1] Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Edif. FC4, Rua Campo Alegre s/n, 4169-007 Porto, Portugal
[2] Faculdade de Ciências da Universidade do Porto, Edif. FC4, Rua Campo Alegre s/n, 4169-007 Porto, Portugal
[3] Centro de Geologia da Universidade do Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal
[4] Departamento de Ciências da Terra, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[5] Estación Experimental de Zonas Áridas, Consejo Superior de Investigaciones Científicas, Ctra. de Sacramento s/n, La Cañada de San Urbano, Almería, Spain

Research on plant traits has become a successful way to understand species coexistence patterns as a result of ecological filtering processes. Stability, an important property of biotic communities, is promoted by species' traits mediating their responses to changes in environmental stress and/or disturbance events. Also, theory predicts the restriction of traits by assembly rules and thereby a limit to the similarity of coexisting species associated to the partition of space and resources. Therefore, non-random patterns of trait (dis)similarity are a common feature of biotic communities. Moreover, trait-based analyses are essential to understand the relationship between environmental gradients and species sorting, which can then support powerful ecological assessment frameworks.Coastal erosion is a prevailing process in much of the Portuguese coastline, particularly when dunes are the dominant geomorphologic elements. Aligned with the theoretical framework described above, this research addresses patterns of plant traits in coastal foredunes, which are dynamic systems subject to severe stress and frequent disturbance. Specifically, we compared prevailing plant traits between dune systems under erosive vs. meta-stable conditions. We analyzed the variability of distinct functional metrics (e.g. trait variance and dissimilarity, functional diversity indices), which revealed non-random associative patterns related to the distinctive conditions of coastal dynamics. Understanding patterns of plant trait distribution and variation in dune vegetation may help to anticipate functional modifications driven by environmental changes on dune ecosystems.

Acknowledgements: José António Macedo is supported by a PhD scholarship (Reference SFRH/BD/48610/2008) from FCT, the Portuguese Foundation for Science and Technology.

P9 Padrões de Coocorrência de Espécies da Vegetação Dunar no Estudo dos Processos de Dinâmica Costeira e na Monitorização Ecológica

José António Macedo[1,2], Ana Sofia Vaz[1], Ângela Lomba[1], Paulo Alves[1], Renato Henriques [3,4], Francisco Ignacio Pugnaire [5], João Honrado [1,2]

[1] *Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Edif. FC4, Rua Campo Alegre s/n, 4169-007 Porto, Portugal*
[2] *Faculdade de Ciências da Universidade do Porto, Edif. FC4, Rua Campo Alegre s/n, 4169-007 Porto, Portugal*
[3] *Centro de Geologia da Universidade do Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal*
[4] *Departamento de Ciências da Terra, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal*
[5] *Estación Experimental de Zonas Áridas, Consejo Superior de Investigaciones Científicas, Ctra. de Sacramento s/n, La Cañada de San Urbano, Almería, Espanha*

As interações entre as espécies, e destas com as condições do respetivo meio em que se inserem, constituem aspetos centrais nos estudos ecológicos. Nas zonas costeiras, a distribuição das espécies vegetais ao longo dos principais gradientes ambientais apresenta padrões característicos, relacionados com a ação dos diversos fatores ambientais, sob a forma de gradientes direcionais, e das suas interações. Por outro lado, os modelos de organização das comunidades vegetais preveem que a coexistência entre as espécies é função do balanço de interações bióticas como a competição e facilitação, cujo predomínio é determinado pelas condições ambientais. Os diversos fatores de stresse e de perturbação assumem assim um papel crucial na regulação dos padrões de ocorrência que dependem deste sistema de interações. Os sistemas dunares costeiros apresentam condições de adversidade e instabilidade ambientais acrescidas para as espécies vegetais que neles ocorrem, sendo expectável uma prevalência dos fenómenos de facilitação sobre os de competição, em sintonia com a teoria ecológica. No entanto, nestes ambientes extremos, alterações importantes nas condições e nos recursos disponíveis (e.g. relacionadas com alterações na dinâmica costeira) podem modificar o balanço entre interações positivas e negativas. Deste modo, a deteção de padrões de coocorrência ou de exclusão mútua entre espécies poderá apresentar um assinalável carácter diagnóstico para detectar precocemente alterações ecológicas em curso.
Considerando este quadro teórico geral, neste trabalho foram analisados os padrões de variação destes processos interativos na vegetação de dunas frontais (tipicamente dominada por Ammophila arenaria), comparando situações de dinâmica transgressiva (erosiva) com situações meta-estáveis. A análise de coocorrência entre espécies, utilizando diferentes métricas indicadoras de tendências de agregação versus segregação, revelou padrões de associação não-aleatórios, relacionados com as distintas condições de dinâmica costeira. Esta avaliação dos padrões espaciais permite uma identificação mais robusta dos efeitos isolados ou combinados dos fatores abióticos e bióticos. O conhecimento detalhado das regras de estruturação destas comunidades constitui assim uma importante ferramenta na deteção precoce de variações nos processos de dinâmica costeira, bem como na monitorização ecológica dos habitats dunares.

Agradecimentos: José António Macedo é financiado pela FCT – Fundação para a Ciência e a Tecnologia, através da Bolsa de Doutoramento com a Referência SFRH/BD/48610/2008.

P10 Class III peroxidases peroxidase catalytic intermediates CoI and CoII are reduced by arabinogalactan proteins

Sara Bettencourt[1,2] and Mariana Sottomayor[1,2]

[1]IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Rua do Campo Alegre, 823, 4150-180 Porto, Portugal
[2]Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre s/n 4169-007 Porto, Portugal

Class III peroxidases (Prxs) are typical plant enzymes that have been implicated in key processes determining the architecture and defence properties of the plant cell wall, mainly through H_2O_2 dependent oxidation activities leading to the cross-linking of cell wall components. Previously, we have obtained indirect evidence that arabinogalactan proteins (AGPs) are an *in vivo* substrates of Prx, and that Prxs are involved on the appearance /disappearance of several specific AGP glycosidic epitopes implicated in important aspects of plant development and plant cell physiology. In this work, the capacity of purified AGPs from the leaves of the medicinal plant *Catharanthus roseus* to reduce the catalytic intermediates of class III peroxidases was investigated. *C. roseus* peroxidase 1 (CroPrx1), the main Prx present in the leaves of the plant, was purified and its oxidation by H_2O_2 and consequent interaction with AGPs showed that these proteoglycans are suitable electron donors for the oxidizing intermediates of CroPrx1 - compound I (CoI) and compound II (CoII). Similarly, AGPs were also capable of reducing horseradish peroxidase-type II CoI and CoII, indicating that the capacity to oxidize AGPs is a general feature of Prxs. The K_m for AGP oxidation by CoII was lower for CroPrx1 than for HrPrxII, indicating a higher affinity of CroPrx1 for AGPs coming from the same species/organ. Moreover, immunofluorescence co-labelling of leaf sections with a monoclonal antibody recognizing a specific AGP glycosidic epitope and an anti-Prx antibody clearly showed co-localization with a punctuated distribution in plasma membrane and tonoplast of mesophyll cells from *C. roseus*. This data strongly indicates that AGPs are in vitro and possibly in vivo substrates of class III peroxidases, with potential implication for the developmental and cell physiology functions attributed to AGPs.

Acknowledgments: Project funding by i) FEDER funds through the Operational Competitiveness Programme – COMPETE and by National Funds through FCT – Fundação para a Ciência e a Tecnologia under the project FCOMP-01-0124-FEDER-022718 (PEst-C/SAU/LA0002/2011), ii) a Scientific Mecenate Grant from Grupo Jerónimo Martins, and iii) the FCT scholarship SFRH/BD/48283/2008

P11 Impact analysis of riverscapes fragmentation on the conservation of bryophyte communities

Ana Paula Portela[1], Cristiana Vieira[2], Helena Hespanhol[2], Bruno Marcos[2], João Honrado[1,2] & Rubim Almeida da Silva[1,2]

[1] Faculdade de Ciências da Universidade do Porto
[2] Centro de Investigação em Biodiversidade e Recursos Genéticos, CIBIO-UP

The objective was to conduct a regional assessment of impacts caused by fragmentation elements (dams, small hydropower schemes, wind farms, roads and railway) on the riverscapes, identifying simple or cumulative impact zones and potential zones of ecological protection, of fluvial bryophyte communities.We used a compilation of databases including information on bryophytic communities (2000-2013), from 257 watercourses in the North of Portugal. Floristic composition was classified (TWINSPAN, CAP) and typified (SIMPER, CAP) and ecological parameters of sites statistically explored (DCA and CCA, CANOCO; SPSS) to obtain the regional communities types and establish species-environment relations. Spatial information analyses were performed in GIS program (ArcMap 10.1). We spatialized bryophytic communities in the watersheds, established buffers of impact per fragmentation element and analysed the loss of potential occurrence of communities.We obtained 8 types of bryophytic communities characterized by 1 to 6 core taxa and found indicator pseudo-species (TWINSPAN) concordant with many of the taxa responsible for the cohesion of the groups.Communities were spatialized according to their fluvial Strahler order, summer precipitation, annual average temperature, local slope, altitude and hillshade; areas of occurrence were calculated for each community. The impacts are mainly due to dams and small hydropower schemes and the areas of loss and potential preservation of each community type were calculated and presented in a detailed map that allows regional and local conservation planning. Bryophytic communities are spatially restricted in the context of Portuguese, Iberian and European biodiversity, where communities show high species richness and diversity and many taxa of Atlantic distribution. Especially in mountain areas, these communities are vulnerable because of fragmentation caused by small hydropower schemes, dams and wind farms implementation.These results allowed us to analyse the impact of energy and communication elements on the assessment of cumulative impacts and identify areas of cumulative impact and conservation.

P12 Effect of different factors on the callus induction of *Pinus elliottii* and *Pinus elliottii x Pinus caribaea*

A. Almeida[1], G. Pinto[1], C. Dias[1], A. Costa[1], V. Pereira[2], L. Marum[2], S. Correia[2], C. Santos[1]*

*[1]Department of Biology,& CESAM – Centre for Environmental and Marine Studies , University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal. E-mail: *gpinto@ua.pt*
[2]KLÓN, Innovative Technologies from Cloning, Biocant Park, Núcleo 4, Lote 4A, 3060-197 Cantanhede, Portugal

Pinus elliottii and the hybrid *Pinus elliottii x Pinus caribaea* have significant commercial importance. Therefore it urges the need to develop a battery of in vitro culture protocols for micropropagation and for general research (eg, physiology, molecular biology, etc.) of these conifers. The main objective of this study was to analyze the effect of various endogenous and exogenous factors on the induction of: a) callus tissue (important in fundamental studies of physiology, maintenance of germplasm, morphogenesis, etc.); b) embryogenic callus (important for plant regeneration by somatic embryogenesis, cryopreservation studies, etc). For that, mature zygotic embryos (MZE) and cotyledons of *Pinus elliottii* and *Pinus elliottii x Pinus caribaea* were exposed to different growth regulators (2,4-D, NAA and TDZ). First callus was visible after 15 days. The most efficient growth regulators conditions in inducing callus were: 2,4-D with 85% induction of calli in MZE; 2,4-D and NAA in cotyledons, both leading to 55% induction of calli. MZE were subjected to different growth regulators (2,4-D+24-epiBr; 2,4-D+BAP), carbon sources (sucrose and maltose) and stress factors, in particular heat (80 °C), cold (-15 °C), salicylic acid, proline, H_2O_2 and putrescine. The most efficient combination of growth regulators was 2,4-D+BAP with approximately 30% rate of calli induction. The more efficient stress conditions were cold and putrescine. These results support the possible induction of callus in these two conifers, and supports preliminary data towards the optimization of effective protocols for SE.

P13 Relação entre padrões ambientais locais e a distribuição de espécies de líquenes dominantes no Vale do Côa (NE Portugal)

Cláudia Oliveira[1], Joana Marques[1,2], Rubim Almeida[1,2]

[1] Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Edifício FC4, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal.
[2] CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

O Vale do Côa (Vila Nova de Foz Côa, Nordeste de Portugal) tornou-se conhecido em finais dos anos 90 após a descoberta da maior e mais importante coleção de arte rupestre ao ar livre do Paleolítico Superior, atualmente classificada como património mundial pela UNESCO.Desde então têm sido realizados diversos estudos de caracterização das dinâmicas de meteorização dos afloramentos rochosos e superfícies gravadas, integrando dados ambientais, geo-físico-químicos e biológicos, que visam o desenvolvimento de ferramentas de gestão e conservação da arte rupestre.Os líquenes dominam as comunidades que colonizam as superfícies gravadas e desempenham um papel importante no processo de deterioração dessas superfícies. No entanto, pouco se sabe sobre os padrões de variação dos fatores ambientais à escala local e sobre a forma como estes atuam sobre a composição destas comunidades. Estudos anteriores indicam que *Lecanora pseudistera* é a espécie dominante nas superfícies orientadas a noroeste, *Aspicilia hoffmaniana* a sudeste e *Caloplaca irrubescens*, igualmente abundante, parece ser indiferente à exposição. Com este estudo pretende-se avaliar de que forma os padrões de variação de fatores ambientais à escala local afetam a sua abundância e distribuição destas três espécies na área do Parque Arqueológico do Vale do Côa. Foram recolhidos dados de temperatura e humidade relativa de dois anos consecutivos recorrendo a três estações meteorológicas e onze sensores distribuídos pela área de estudo a altitudes que variam entre os 136 e os 332 metros, orientados a norte (3), sul (6), este (1) e oeste (1). Será discutida a relação entre os padrões de distribuição destas espécies e os padrões de variação local dos fatores ambientais registados, e respetivas implicações para a conservação da arte rupestre no Vale do Côa.

P14 Effect of Cr III in *Lactuca sativa* L. physiology

Raíssa CS Faria[1,2], Willian GD Bedo[1,2],C Dias[1], G Pinto[1], C Santos[1]

[1]Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal
[2] FHO|Uniararas - Fundação Hermínio Ometto, 055 19) 3543-1400, Brasil

Cr(III) is an essential nutrient to humans, being ingested through different food sources (eg., crops, nuts, meat and eggs). Despite their essentiality to humans Cr (independently of its valence) is currently considered to be a toxic element to plants. Most studies on Cr phytotoxicity are based on the Cr(VI) valence, while Cr(III) effects remain less studied. One of the most well studied crops for Cr(VI) toxicity is L*actuca sativa*, a worldwide consumed crop, and an excellent model in ecotoxicology. Lettuce plants were grown hydroponically on Hoaglands (1/3 strengh) for 15 days and exposed to Cr (III) (20, 50, 150 and 200 mg/L). All independent experiments show a hormesis effect (up to 20 mg/L), with plants showing an increase of lengh and growth. This preliminary result led us to raise the hypothesis of Cr(III) being a beneficial nutrient to this crop. For that we analyzed some physiological parameters namely photosynthesis. Curiously, all Cr doses decreased A (net photosynthetic rate), and led to a stomatal closure. These Cr(III) doses did not significantly affect the Ci (internal CO2 availability). Also, considering that plant growth is a result of cell division and extension, the impact of these doses on mitotic index (MI) and mitosis catastrophe (micronuclei) in the root apex is under discussion. These data support that Cr(III) hampers some photosynthetic parameters, and the pathways involved in the growth stimulation at lower doses deserve further studies. The growth stimulation at lower doses also support that, contrarily to Cr(VI), Cr(III) has potential to be considered a beneficial nutrient when at very low doses, despite further studies are ongoing.

P15 Chemical profile and anti-allergic screening of *Fucus spiralis* Linnaeus and *Padina pavonica* (Linnaeus) Thivy

Mariana Barbosa, Joana Costa, Tiago Pereira, Patrícia Valentão, Paula B. Andrade, Brígida R. Pinho

REQUIMTE/Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, R. Jorge Viterbo Ferreira n.º 228, 4050-313 Porto, Portugal.

Presently, the research on marine products has shown that seaweeds are a rich source of a wide variety of natural compounds belonging to different metabolic pathways, and with numerous interesting properties. In fact, more than 15000 primary and secondary metabolites have already been reported in macroalgae and different applications are assigned to them [1]. From a human health point of view, both primary and secondary metabolites exhibit numerous biological activities, like antioxidant, antitumor and anti-inflammatory [1].
Macroalgae can be classified into three classes based on their pigmentation: green algae (Chlorophyta), brown algae (Phaeophyta) and red algae (Rhodophyta). There is a wide diversity of brown algae that are important members of many marine communities with significant economic importance [2]. In this work, ethanolic extracts of two brown seaweeds species [*Fucus spiralis* Linnaeus and *Padina pavonica* (Linnaeus) Thivy], collected in different places of the west coast of Portugal were chemically characterized and their anti-allergic properties studied. Eleven compounds were identified by GC–MS (Table 1), *F. spiralis* presenting a great variety of compounds. The anti-allergic screening was assessed by inhibition of calcium ionophore stimulated RBL-2H3 cells degranulation by non-toxic concentrations of the two ethanolic extracts. Preliminary data point *P. pavonica* as the most promising species, because it reduced 51.5 ± 8.56 % (mean ± SEM) the cells degranulation at 12.5 mg/mL, while *F. spiralis* reduced the degranulation in 40.0 ± 4.59%, for the same concentration.
In conclusion, in spite of *F. spiralis* to be more chemically rich than *P. pavonica*, the last one showed better anti-allergic activity, which could be related, at least partially, with the presence of α-linoleic acid (C18:3) and of phloroglucinol.

Table 1 - Metabolic composition of *F. spiralis* and *P. pavonica* samples (mg/100 g dry algae)[a]

Compounds	RI_{calc}[b]	RI_{lit}[c]	*F. spiralis*	*P. pavonica*
Proline	1286	1299 [3]	39.68 (9.68)	13.48 (2.78)
Phloroglucinol	1631	1647 [4]	< LOQ	21.02 (0.06)
C14:0	1837	1843 [4]	6.98 (0.27)	-
Mannitol	1896	1912 [4]	127.38 (3.48)	38.32 (1.58)
C16:0	2043	2040 [4]	60.60 (2.06)	12.16 (3.38)
C18:3	2225	2218 [4]	-	186.02 (2.20)
C18:1	2238	2248 [4]	2169.06 (166.76)	146.92 (4.88)
C18:0	2253	2234 [5]	20.84 (2.14)	14.04 (2.42)
C20:4	2389	2373 [4]	46.56 (2.90)	-
C20:5	2398	2380 [4]	21.70 (1.14)	-
Fucosterol	3361	3330 [5]	31.76 (0.94)	16.48 (0.56)

[a] Values are expressed as mean (SD) of three determinations; LOQ – limit of quantification. [b] RI_{calc}: Retention index obtained in the experiment. [c] RI_{lit}: Retention index described in literature.

Acknowledgements: **This work was supported by Fundação para a Ciência e a Tecnologia (FCT): (PEst-C/EQB/LA0006/2011). B. R. Pinho is indebted to FCT for the grant (SFRH/BD/63852/2009).**

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P16 Carga orgânica adicionada a FitoETAR por colmos de *Phragmites australis* mantidos nos leitos durante o outono/inverno

Luis Manuel Oliveira[1], António Mário Almeida[2] e Isabel A-P. Mina[1, 3]

[1] *Departamento de Biologia, Escola de Ciências da Universidade do Minho (DB-ECUM)*
[2] *Centro de Física, Escola de Ciências da Universidade do Minho (CF-ECUM)*
[3] *Centro de Investigação em Tecnologia Agro-Ambiental e Ciências Biológicas (CITAB)*

As Estações de Tratamento de Águas Residuais (ETAR) têm por objetivo reduzir a carga orgânica e aumentar a qualidade sanitária das águas afluentes.

As FitoETARs utilizam leitos de plantas para o tratamento secundário de águas residuais sendo sobretudo adequadas para o tratamento de águas residuais domésticas de pequenas comunidades. É já considerável o número de FitoETARs existentes em Portugal e a maioria utiliza leitos plantados com *Phragmites australis* (caniço). Entre setembro e fevereiro, esta Poaceae cosmopolita seca, e os seus colmos são, em muitas FitoETARs, mantidos nos leitos. Neste período do ano, frio e geralmente caracterizado por fortes aguaceiros, os colmos permanecem imersos durante largos períodos.Para estimar a carga orgânica adicionada a leitos de FitoETARs pelo encharcamento causado pelas chuvas invernais, realizaram-se em laboratório ensaios em microcosmos.Os ensaios preliminares até agora realizados evidenciam que, colmos de caniços imersos em água da torneira durante uma semana, conduzem a um considerável teor de carga orgânica na “água da chuva experimental”. Ao longo do tempo, a carga orgânica da “água da chuva” estimada pela Carência Bioquímica de Oxigénio (CBO_5) diminui, enquanto que o pH e a tensão superficial aumenta, mostrando tendência para estabilizar ao fim de algumas semanas.

P17 Halotolerant Plants for Phytoremediation in Saline Environments

Jesus[a], J., Gonçalves[b], Ana T., Mina[c], Isabel, Borges[b,d], Maria-Teresa,

a – FEUP – CIGAR - Centro de Investigação em Geo-Ambiente e Recursos, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, s/n 4200-465 Porto, Portugal
b - FCUP – Departamento de Biologia, Faculdade de Ciências da Universidade do Porto,
Rua Campo Alegre s/n, 4169-007 Porto, Portugal.
c – UM - Departamento de Biologia - Universidade do Minho, Campus de Gualtar 4710-057 Braga Portugal
d - CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal.

Halotolerant and halophytic plants have been the subject of intense research due to their capacity to withstand high salinity which opens up new opportunities for phytoremediation in extreme conditions. This study focus on *Typha latifolia* and *Arundo donax* ability to tolerate high salinity and on methods to successfully propagate and acclimatize these species aiming at gathering practical information for *in situ* remediation applications like saline effluents treatment.

Typha latifolia was collected and propagated with intact rhizomes. It revealed adequate adaptation to the tested substrate, expanded clay, after 2 weeks. However, survival rate was dependent on sampling season, with higher values in autumn (75 to 90% survival) and lower values in late spring (11%), probably due to a lower carbohydrate concentration in rhizomes. Furthermore, it was able to withstand salinity values of up to 2.4% with 94% survival rate, despite some growth inhibition. *Arundo donax* was collected and propagated through secondary shoots. The best results were obtained in the spring. Expanded clay utilization increased plant growth and survival. With proper acclimation, survival rate was high (=100%) up to 3.5% salinity level. Furthermore, *Typha latifolia* was also able to withstand and treat highly saline wastewater from a recirculating aquaculture farm. However, it remains unclear if these plants species are able to uptake dissolved salts. Although expanded clay itself is able to adsorb significant amounts of salts some salinity reduction cannot be explained by substrate adsorption alone.

Further studies, therefore, will be aimed at clarifying the potential use of these plants for remediation of saline environments.

P18 Monogalactosyl diacylglycerols from the edible brown algae *Fucus spiralis* Linnaeus: Anti-inflammatory potential on RAW 264.7 macrophage cell line

G. Lopes[1], G. Daletos[2], P. Proksch[2], P. B. Andrade[1] and P. Valentão[1]

[1]REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, University of Porto, R. de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[2]Intitute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine University, Universitätstrasse 1, 40225 Düsseldorf, Germany

As the majority of marine organisms, seaweeds have developed physical and chemical defence mechanisms in order to survive in a competitive environment, with extreme atmospheric conditions. These defence strategies resulted in the production of a significant diversity of secondary metabolites, which have attracted scientists' attention in the past few years.
The edible brown seaweed *Fucus spiralis* Linnaeus is widely distributed over the Portuguese west coast. This species is rich in secondary metabolites, among which fatty acids constitute a very special group. Fatty acids are one of the most representative classes of compounds, both in diversity and contents, and are associated with diverse biological activities. *F. spiralis* has a diverse lipid composition, dominated by mono and polyunsaturated long chain fatty acids, oleic acid being the major one [1]. A purified fraction containing two monogalactosyl diacylglycerols (MGDGs) and a monoglyceride (**Figure 1**) were isolated from the non-polar fraction of the ethyl acetate extract of this seaweed by using reversed phase silica chromatography. Their structure was ascertained by 1H NMR and MS spectroscopy. The anti-inflammatory activity of the compounds was assessed on RAW 264.7 macrophage cell line.

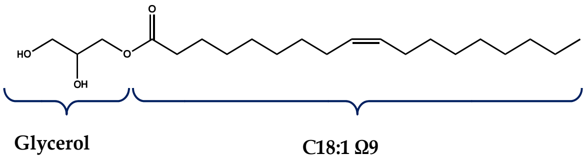

Figure 1. Monoglyceride isolated from the non-polar fraction of the ethyl acetate extract of the brown algae *F. spiralis.*

The MGDGs fraction and the monoglyceride displayed anti-inflammatory activity with IC_{50} values of 85.67 and 65.96 µg/mL, respectively. These results highlight the potential of these compounds as anti-inflammatory agents.

Acknowledgements:The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT) through grant no. PEst-C/EQB/LA0006/2011. G. Lopes (SFRH/BD/61565/2009) is indebted to FCT, FSE and POPH for the grant. [1] Food Chemistry, 2013, *138*, 1819-1828.

P19 Neuroprotective effect of steroidal alkaloids from *Lycopersicon esculentum* Mill. on glutamate-induced toxicity in SH-SY5Y cells

M. Taveira, M. Maciejewska, M. Costa, I. Costa, P. Valentão, P. B. Andrade

REQUIMTE/Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, nº. 228, 4050-313 Porto, Portugal

Lycopersicon esculentum Mill. (tomato plant) is one of the most important crop in the world, with economic and health impact. Nevertheless, there are several pests that can destroy its cultures, such as *Spodoptera littoralis* (Bois.). The losses caused by this pest justified the chemical characterization and the evaluation of the biological potential having in mind its possible use as source of bioactive compounds, taking profit from the infestation. The metabolic profile of several *S. littoralis* materials (larvae, pupae, adults and excrements) and the host plant material (leaves) were characterized in terms of phenolics, alkaloids, amino acids, fatty acids and sterols. These metabolites were determined by HPLC-DAD and GC-MS techniques. Phenolics were identified only in *L. esculentum* leaves, being absent in *S. littoralis* materials. Steroidal alkaloids were identified in all matrices, especially in leaves and *S. littoralis* excrements. Amino acids and fatty acids seem to be bioaccumulated by *S. littoralis* larvae.
The antioxidant capacity of these matrices was checked in chemical microassays against several reactive species (Figure 1). The ethanolic extract from tomato leaves was the most active antioxidant, followed by that of *S. littoralis* excrements.

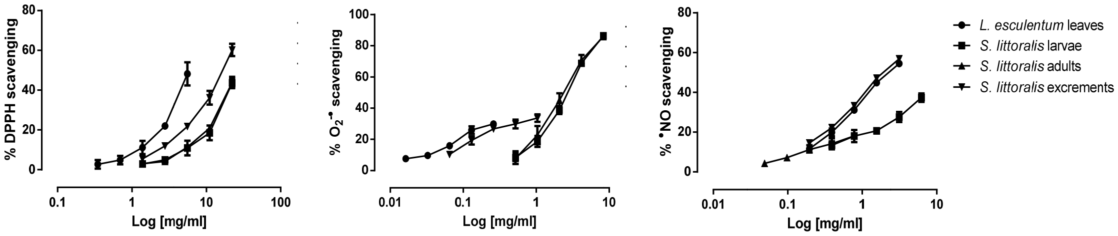

Figure 1. Effect of ethanolic extracts against DPPH$^\bullet$, $O_2^{\bullet-}$ and $^\bullet$NO. Values show mean ± SE from three experiments performed in triplicate.

The acetyl and butyrylcholinesterase inhibitory capacity was also evaluated using a colorimetric microassay. As verified for the antioxidant potential, the tomato leaves and *S. littoralis* excrements were the most active matrices. Their activity can be associated to the content in steroidal alkaloids. By evaluating the biological fate of these compounds in the *S. littoralis*/*L. esculentum* system we can discover the strategies used by the plant to overcome the pest, and also those developed by the insect to feed and survive on the plant. Based on the obtained information, a practical application would be in the breeding of crop plants more resistant to the attack by the insect.

Acknowledgements: The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT) for Grant No. PEst-C/EQB/LA0006/2011. M. Taveira (SFRH/BD/62662/2009) is indebted to FCT for the Grant.

P20 *Grindelia robusta* Nutt. : Characterization of the phenolic profile and evaluation of its inhibitory potential against brain enzymes and oxidative species

C. Grosso[a], C. Azevedo[a], F. Ferreres[b], A. Gil-Izquierdo[b], P. Valentão[a], P. B. Andrade[a]

[a] *REQUIMTE/Laboratory of Pharmacognosy, Chemistry Department, Faculty of Pharmacy, University of Porto, Portugal*
[b]*Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), P.O. Box 164, 30100 Campus University Espinardo, Murcia, Spain*

Oxidative stress is considered to be one of the causes of the development of neuropsychiatric disorders, such as Alzheimer's disease (AD) and depression. Moreover, inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) ameliorate the symptoms of AD, while monoamine oxidase A (MAO-A) inhibitors are used to treat the symptoms of depression. These last also prevent the production of H_2O_2, a reactive oxygen species (ROS).
This study reports the biological potential of two extracts (aqueous and methanol/water) from *Grindelia robusta* Nutt. as ROS ($O_2^{\bullet -}$ and H_2O_2) and reactive nitrogen species [RNS, (${}^{\bullet}NO$)] scavengers and as AChE, BuChE and MAO-A inhibitors. The phenolic profile was characterized by HPLC-DAD-ESI-MS^n, both extracts being dominated by the pairs diosmetin-7-*O*-glucosyl-3´-*O*-pentoside+apigenin-7-*O*-glucosyl-4´-*O*-pentoside and apigenin-7-*O*-glucoside+diosmetin-7-*O*-glucoside (Fig. 1). Concerning the antioxidant activity, the extracts displayed better $O_2^{\bullet -}$ scavenging capacity than ascorbic acid, but they were weaker than the reference compound against the other oxidative species. The ability of the extracts to inhibit cholinesterases was weak, but the aqueous extract proved to be a good MAO-A inhibitor, which can be attributed to its higher content of hidroxycinnamic acids in comparison with the hydromethanolic one.

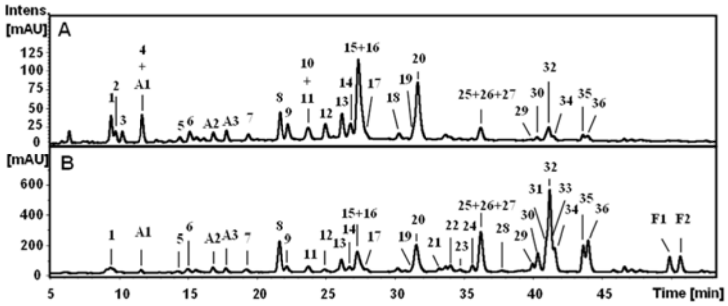

Figure 1 - HPLC-UV chromatogram (340 nm) of the aqueous (A) and hydromethanolic (B) extracts of *Grindelia robusta*. 1 – 5-caffeoylquinic acid; 2 – 3-feruloylquinic acid; 3 – 4-caffeoylquinic acid; 4 – quercetin-3-*O*-hexosyl-7-*O*-glucoside; 5 – 5-feruloylquinic acid; 6 – 4-feruloylquinic acid; 7 – 3,5-di-feruloylquinic acid; 8 – 3,5-di-caffeoylquinic acid; 9 – 3,4-di-caffeoylquinic acid; 10 – quercetin-7-*O*-glucoside; 11 – quercetin-3-*O*-hexoside; 12 – luteolin-7-*O*-glucosyl-3´/4´-*O*-pentoside; 13 – 4,5-di-caffeoylquinic acid; 14 – luteolin-7-*O*-glucoside; 15 – diosmetin-7-*O*-glucosyl-3´-*O*-pentoside; 16 – apigenin-7-*O*-glucosyl-4´-*O*-pentoside; 17 – 3-methyl-quercetin glucoside; 18 – 3-methyl-quercetin glucoside isomer; 19 – apigenin-7-*O*-glucoside; 20 – diosmetin-7-*O*-glucoside; 21 – quercetin; 22 – luteolin-3´/4´-*O*-pentoside; 23 – 3-methyl-quercetin pentoside; 24 – luteolin; 25 – diosmetin-3´-*O*-pentoside; 26 – apigenin-4´-*O*-pentoside; 27 – 3-methyl-quercetin; 28 – dimethyl-quercetin pentoside; 29 – apigenin; 30 – diosmetin; 31 – quercetagetin-3,6,3´/4'-trimethylether; 32 – quercetin-3,3´-dimethylether; 33 – quercetin-3,7- dimethylether; 34 – kaempferol-3-methylether; 35 – quercetagetin-3,6,7,3´/4´-tetramethylether; 36 – 6-hydroxy-kaempferol-3,6,7/4´-trimethylether; A1 – hydroxycinnamic acid 1; A2 – hydroxycinnamic acid 2; A3 – hydroxycinnamic acid 3; F1 – flavonoid 1; F2 – flavonoid 2.

Acknowledgements: The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT) for grant no. PEst-C/EQB/LA0006/2011, to "Operação NORTE-07-0124-FEDER-000069", to CYTED Programme (Ref. 112RT0460) CORNUCOPIA Thematic Network and project AGL2011-23690 (CICYT). Clara Grosso thanks FCT for the Post-Doc fellowship (SFRH/BPD/63922/2009).

P21 Enzymes inhibition by *Piper betle* L. leaves

Andreia P. Oliveira[1], Joana Ferreira[1], Sofia Riboira[1], Federico Ferreres[2], Angel Gil-Izquierdo[2], Patrícia Valentão[1], Paula B. Andrade[1]

[1]REQUIMTE/Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, nº 228, 4050-313 Porto, Portugal
[2]Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), P.O. Box 164, 30100 Campus University Espinardo, Murcia, Spain

Piper betle L., a member of Piperaceae, is a widely distributed plant in the tropical and subtropical regions of the world, recognized as a traditional herbal remedy for many diseases. The leaves act as breath-freshener and are used in the Indian system of medicine and health for their medicinal properties, such as anti-inflammatory, anti-allergic, hepatoprotective, antioxidant, antifungic, antihelmintic, xanthine oxidase inhibitor and chemopreventive [1, 2, 3]. In this work, aqueous lyophilized and ethanolic extracts of *P. betle* were analyzed regarding their chemical composition and biological potential. Amino acids, fatty acids, sterols and triterpenes were determined by GC-MS.Both extracts showed a good potential against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). In a general way, the aqueous extract revealed to be more active against BuChE (IC_{50} = 0.601 mg/mL) while the ethanolic one was more effective against AChE (IC_{50} = 0.264 mg/mL) (Fig. 1A and 1B). Furthermore, the α-glucosidase inhibitory activity was also evaluated, being ethanolic extract the most interesting (IC_{50} = 0.069 mg/mL) (Fig. 1C).

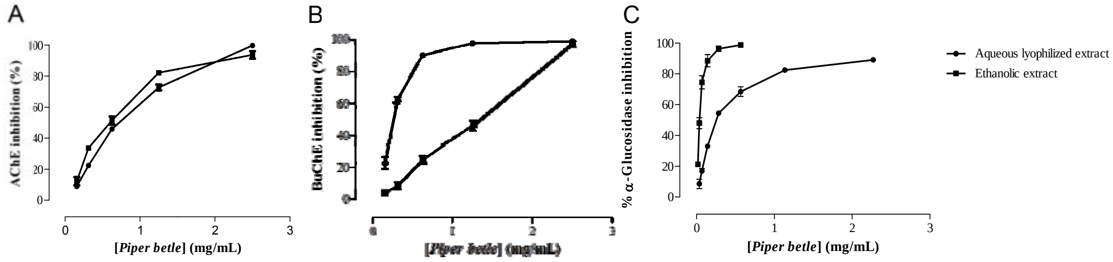

Fig.1. Enzymatic inhibitory activity of aqueous lyophilized and ethanolic extracts of *P. betle* leaves: (A) AChE, (B) BuChE and (C) α-glucosidase. Values show mean + SE from three experiments, performed in triplicate.

The results suggest that *P. betle* leaves could be useful to the prevention and treatment of Alzheimer´s disease and diabetes, due to their effects against cholinesterases and α-glucosidase, respectively.

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Acknowledgments: The authors are grateful to Fundação para a Ciência e a Tecnologia for grant no. PEst-C/EQB/LA0006/2011.

P22 Glandora diffusa (Lag.) D. C. Thomas: metabolic profile in authenticity control and the key of bioactivity

F. Fernandes[1], P. Almeida[2], F. Ferreres[3], A. Gil-Izquierdo[3], P. Valentão[2], P. B. Andrade

[1] Interdisciplinary Centre for Marine and Environmental Research (CIIMAR/CIMAR), Rua dos Bragas nº 289, 4050-123 Porto, Portugal.
[2] REQUIMTE/Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, nº 228, 4050-313 Porto, Portugal
[3] Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), P.O. Box 164, 30100 Campus University Espinardo, Murcia, Spain.

Glandora genus belongs to Boraginaceae family, in which *Glandora diffusa* (Lag.) D. C. Thomas is included. Usually known as scrambling-gromwell or shrubby-gromwell, the consumption of this species has been associated with diuretic, depurative, antihypertensive [1] and, more recently, antidiabetic and antioxidant properties [2].This work aimed the use of the chemical profile as a tool for authenticity control, and the assessment of some biological activities of *G. diffusa*, purchased in the local market, from three different medicinal plants distributors.
Amino acids, fatty acids, sterols and triterpenes were determined by GC-MS. The phenolic profile was characterized by HPLC-DAD. The studied samples revealed a similar qualitative composition. Nevertheless, different quantitative profiles were observed.The three commercial samples showed a potent dose-dependent response against free radicals and a moderate inhibitory effect on the enzymes acetylcholinesterase and butyrylcholinesterase. Additionally, extracts were cytotoxic to both human colorectal adenocarcinoma cell line (Caco-2) and human gastric adenocarcinoma cell line (AGS). Sample B revealed to the best anticarcinogenic properties (Fig. 1).

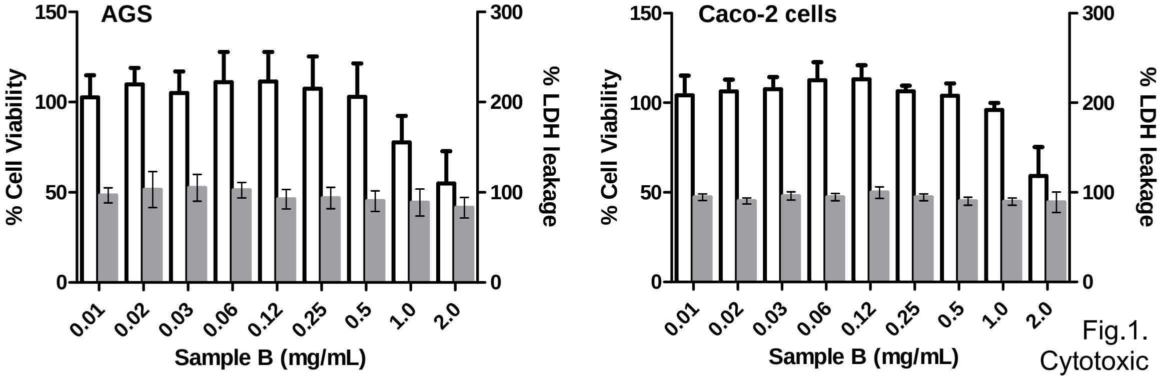

Fig.1. Cytotoxic effect of an extract from *G. diffusa* (sample B) on AGS and Caco-2 cells. Results are presented as mean ± SEM of 4 independent experiments performed in triplicate.

The phenolic compounds qualitative profile of the three samples revealed to be similar to the one previously reported [2], confirming their authenticity. Additionally, the diversity of described compounds, together with its biologic potential, suggests *G. diffusa* as a good source of bioactive compounds, and points its consumption as a probable surplus to human's health.

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Acknowledgments: The authors are grateful to Fundação para a Ciência e a Tecnologia for grant no. PEst-C/EQB/LA0006/2011 and to "Operação NORTE-07-0124-FEDER-000069".

P23 New insights on *SIZ1* function in the *Arabidopsis thaliana* stress response

Authors: ***Freitas S****, Castro PH, Couto D, Ruiz-Albert J, Tavares RM, Bejarano ER, Azevedo H*

Affiliation: Center for Biodiversity, Functional & Integrative Genomics (BioFIG), Plant Functional Biology Center, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal (P.H.C., D.C., S.F., R.M.T., H.A.)
Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Dept. Biología Celular, Genética y Fisiología, Universidad de Málaga, Campus Teatinos, 29071 Málaga, Spain (P.H.C., J.R.-A., E.R.B.)

Rapid and reversible posttranslational modifications (PTMs) are essential for the functioning of a living organism, particularly in response to a constantly changing environment. One class of PTMs subject to increasing focus of research employs small modifying peptides like the Small Ubiquitin-related Modifier (SUMO). Modification by SUMO may produce different effects on a target protein, such as conformational control, and creation or blocking of interacting interfaces. SUMO is not only involved in many aspects of plant growth and development, but also acts in the response to abiotic stress, including extreme temperatures, drought, salinity, and nutrient availability stresses. SUMO-conjugates accumulate quickly upon several stimuli and gradually disappear in a recovery phase. SUMO regulates numerous nucleus-associated mechanisms, namely by modulating transcription and chromatin remodeling factors for a rapid transcriptional reprogramming. Recent research efforts have been employed on determining plant's sumoylation targets, particularly in what concerns the plant response to stress. We have compiled this information and performed a gene ontology analysis, highlighting that SUMO-conjugates include chloroplast-targeted proteins. Moreover, our microarray analyses of Arabidopsis T-DNA insertion mutants in the sumoylation pathway show a de-regulation of chloroplast-associated genes. Taking these observations into consideration, we performed a functional characterization of the major Arabidopsis SUMO E3 ligase *siz1* mutant. Indeed, the *siz1* mutant displayed altered pigment content, de-regulated reactive oxygen species homeostasis and changes in chloroplast ultrastructure. Overall results establish a new role for sumoylation in the control of chloroplast functioning.

Work was supported by FCT/FEDER (refs. PTDC/BIA-PLA/3850/2012 and FCOMP-01-0124-FEDER-028459).

P24 Distribution of arabinogalactan proteins and pectins in *Quercus suber* female flower

M.I.Amorim, [a, b] C.Sousa,[a] M.Costa,[a,b] and S.Coimbra,[a,b]

[a] Department of Biology, Faculty of Science, University of Porto, Portugal.
[b] Center for Biodiversity, Functional & Integrative Genomics – BioFIG, Porto, Portugal

Coark oak (*Quercus suber*) is a dominant Fagacea tree species in the forests of Southern Iberian Peninsula. This is a monoecious tree species with a long progamic phase that provide a comprehensive system for comparative studies in development and sexual reproduction [1]. Arabinogalactan proteins (AGPs) and pectins belong to a superfamily of highly glycosylated hydroxyproline-rich glycoproteins cell components found in the entire plant kingdom, in almost all plant organs and cell types from root to flowers. At the subcellular level, AGPs can be found in the cell wall, in the apoplast or anchored to the plasma membrane via a GPI anchor attached to the C-terminal domain of the AGP backbone. Pectins, together with other polymers, are plant cell wall components that give physical strength to the plant body and provide a barrier against the outside environment. In reproductive tissues, the expression of AGPs is associated with the sporophyte–gametophyte transition, as our own previous work has shown, a specific AGP expression pattern during plant gametogenesis in *Arabidopsis thaliana* [2] and in *Trithuria submersa* [3]. A set of monoclonal antibodies (mAbs) directed against the carbohydrate moiety of cell wall polysaccharides were used for the immunolocalization of AGPs and pectins, such as: JIM 8, JIM13, JIM17 and LM7. The labeling obtained with anti-AGP antibodies in t female flowers show a dynamic distribution of those sugar epitopes in reproductive tissues. Moreover, these labeling may function as a molecular marker for the tissue specific expression pattern in pistil tissues. The changes in AGP or pectins specific mAbs distribution reveal that the expression of these is spatially regulated in cork oak female flower.

The authors are grateful for the financial support through FCT for the Project PTDC/AGR-GPL/118508/2010

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Lista de Participantes / *List of Participants*

Alberto Pessoa	Protein Trafficking and Development/BioFIG/FCUP – pessoa.am@fc.up.pt
Ana Assunção	PlantEvol/ CIBIO-UP – agla@cibio.up.pt
Ana Clara Grosso	Farmacognosia/ REQUIMTE/ FFUP – claragrosso@hotmail.com
Ana Cláudia Oliveira	CIBIO - up200906079@fc.up.pt
Ana Cunha	CITAB, DB - UM – accunha@bio.uminho.pt
Ana Luísa Silva	Bioactive Natural Products /IBMC analfgsilva@hotmail.com
Ana Paula Portela	CIBIO/UP - up200902686@fc.up.pt
Ana Raquel Almeida	Biotecnologia e Citómica/UA, almeidaraquel@ua.pt
Ana Rita Silva	BIOCON/Biodiversity and Conservation Ecology/ CIBIO rita.silva@fc.up.pt
Ana Sofia Vaz	Biodiversity and Conservation Ecology/ CIBIO sofia.linovaz@gmail.com
Andreia Patrícia Oliveira	Farmacognosia/ REQUIMTE/ FFUP andreiapsoliveira@gmail.com
António Teixeira	Departamento de Biologia /CITAB/UM antonio.teixeira@bio.uminho.pt
Artur Conde	CITAB, DB-UM, arturconde@bio.uminho.pt
Brígida Pinho	Farmacognosia/ REQUIMTE/ FFUP - brigidarpinho@gmail.com
Bruno Peixoto	Protein Trafficking and Development/BioFIG/FCUP - bpeixoto@msn.com
Cândida Learmonth	cmmc.learmonth@netcabo.pt
Carla Patrícia Azevedo	Biotecnologia e Citómica/Biologia do stress/UA - carlapsazevedo@ua.pt
Carolina Azevedo	Farmacognosia/ REQUIMTE/ FFUP carolina.azevedo@outlook.com
Conceição Santos	Biotechnology and cytomics/CESAM/UA, csantos@ua.pt
Cristiana Maia Alves	CIBIO/UP - up200901530@fc.up.pt
Cristiano Soares	BioFIG/FCUP - fsoares.cris@gmail.com
Cristina Marisa Almeida	CIIMAR – calmeida@ciimar.up.pt
Fernanda Fidalgo	Plant Molecular Biology and Biotechnology/ Plant Stress/ BioFIG/ FCUP – ffidalgo@fc.up.pt
Francisca Oliveira	BioFIG/FCUP - francisca.n.oliveira@gmail.com
Francisco Lima	Bioactive Natural Products/IBMC – francisco.lima278@gmail.com
Francisco Peixoto	CITAB/UTAD - fpeixoto@utad.pt
Graciliana Lopes	Farmacognosia/ REQUIMTE/ FFUP - gracilianalps@gmail.com

Hélder Gomes	Universidade de Aveiro – helder.alexandre@ua.pt
Henrique Noronha	CITAB, DB-UM, aluno Bioplant - henriquenoronha@bio.uminho.pt
Herlânder Azevedo	BioFIG/UM – hazevedo@bio.uminho.pt
Hernâni Gerós	CITAB, DB-UM – geros@bio.uminho.pt
Inês Carqueijeiro	Bioactive Natural Products/IBMC – Ines.Carqueijeiro@ibmc.up.pt
Isabel Amorim	BioFIG/FCUP - mpamorim@fc.up.pt
Isabel Mina	CITAB/UM - icapmina@bio.uminho.pt
Joana Almeida	FCUP - joanaalexandragar@gmail.com
Joana Gonçalves	Empresa ecoinside – joanagoncalves@ecoinside.pt
Joana Ferreira	Farmacognosia/ REQUIMTE/ FFUP – joanaguerraferreira@gmail.com
João Jesus	CIGAR /FEUP - joaojesuscta@hotmail.com
Jorge Teixeira	BioFIG /FCUP – agteixei@fc.up.pt
José António Macedo	Biodiversity and Conservation Ecology/CIBIO - jos.med.mac@gmail.com
José Feijó	IGC/Gulbenkian - jfeijo@igc.gulbenkian.pt
José Pissarra	BioFIG/FCUP - jpissarr@fc.up.pt
José Tiago Moreira	BioFIG/UM - josetmoreira@gmail.com
Juliana Oliveira	Secretariado Bioplant – juoliveira@fc.up.pt
Lorena Romero	Bioactive Natural Products/IBMC – lorena.almagro@um.es
Luís Carlos Andrade	luis.andrade@live.com.pt
Luís Manuel Oliveira	CITAB/UM - luismco12@gmail.com
Manuela Costa	BioFIG/UM – manuela.costa@bio.uminho.pt
Marcos André Monteiro	Farmacognosia/ REQUIMTE/ FFUP - taveira.marcos@gmail.com
Maria da Costa	Biotechnology and Cytomics/UA/aluna Bioplant – costamaria@ua.pt
Fátima Fernandes	CIIMAR/CIMAR - mfgfernandes@gmail.com
Maria Teresa Borges	Biodiversidade Costeira (LBC)- CIIMAR /FCUP - mtborges@fc.up.pt
Maria Teresa Braga	Plant Stress/ FCUP – up200806802@fc.up.pt
Maria Teresa Lino Neto	BioFIG/UM – tlneto@bio.uminho.pt
Mariana Nunes Barbosa	FFUP - mariana.nunes.barbosa@gmail.com
Mariana Sottomayor	FCUP/IBMC/Diretora Bioplant – msottoma@ibmc.up.pt
Marslin Gregory	CITAB/DB-UM/aluno Bioplant – marslingregory@gmail.com
Miguel Ângelo Faria	Plant Evolution Group/CIBIO - mfaria@cibio.up.pt
Patrícia Duarte	Bioactive Natural Products/IBMC – pduarte@ibmc.up.pt
Paula Melo	BioFIG / FCUP /CC Bioplant – pmmelo@fc.up.pt
Pedro Humberto Castro	UM/BioFIG – pedro1berto@bio.uminho.pt

Pedro Albuquerque	MDE - Microbial Diversity and Evolution/ CIBIO - palbuquerque@cibio.up.pt
Pedro Miguel Almeida	Farmacognosia/ REQUIMTE/ FFUP – mfgfernandes@gmail.com
Raissa Cristina Faria	Fisiologia vegetal/CESAM/UA - ra_issa_cristina@hotmail.com
Richard Maykel Breia	CITAB, DB-UM – richardgoncalves@bio.uminho.pt
Rui Tavares	UM/CC Bioplant – tavares@bio.uminho.pt
Sara Bettencourt Ramos	Bioactive Natural Products/IBMC – Sara.Bettencourt@ibmc.up.pt
Sara Freitas	BioFIG/UM - siofreitas@bio.uminho.pt
Sílvia Coimbra	BioFIG/FCUP – scoimbra@fc.up.pt
Simão Pedro Neves	BioFIG/FCUP - sbrancon@gmail.com
Sofia Barroso Riboira	Farmacognosia/ REQUIMTE/ FFUP – sofiariboira92@gmail.com
Valérie Pantano	FCUP – valeriepantano@gmail.com
Vanessa Vieira	Protein Trafficking and Development/BioFIG/FCUP – vanessa.aad@gmail.com
Viviana Martins	CITAB, DB-UM, aluna Bioplant – vmartins@bio.uminho.pt
Willian Giusebepe Bedo	Fisiologia vegetal/CESAM/UA - will_bedo@hotmail.com