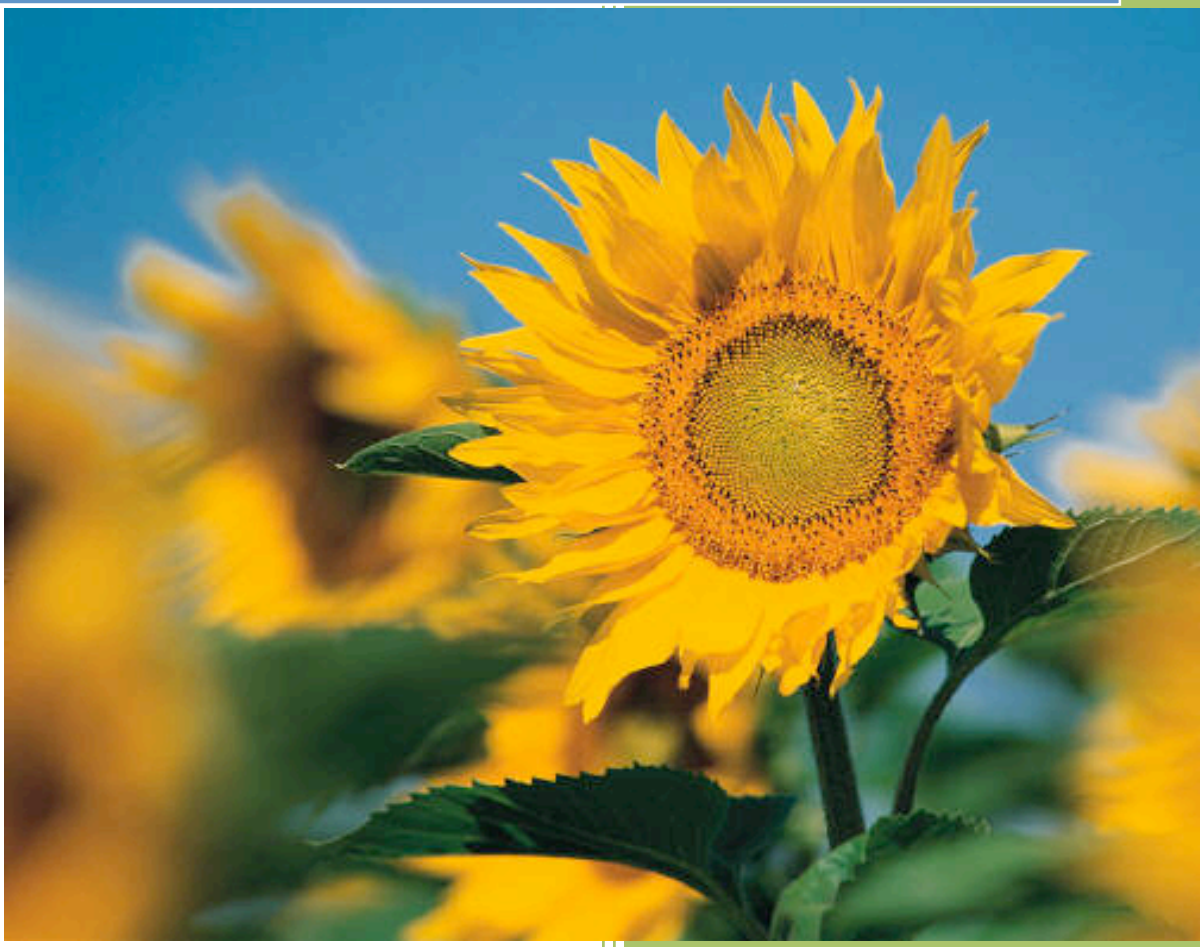
2012

3rd Annual MAP-BioPlant Workshop

Becoming a Plant: mechanisms and physiology of plastid acquisition

Joint Doctoral Program in Plant Biology



BOOK OF ABSTRACTS

3rd Annual MAP-BioPlant Workshop – Becoming a Plant: mechanisms and physiology of plastid acquisition

4–5 December
Auditorium of the Department of Environmental Sciences
University of Aveiro 3810-193, Portugal

Organization
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António José Calado – Department of Biology, UA

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Welcome

Welcome to the 3rd Annual Workshop of the Joint Doctoral Program in Plant Biology, the MAP-BioPlant!

The BioPlant gathers together professors and researchers of three Universities of the North of Portugal (UA, UM and UP). Similarly to the previous years, all researchers and professors of these institutions aim at, in an integrated and synergic way, contribute to a solid training of highly qualified scientists and to an investigation of excellence standards at national and international levels.
The third year became the consolidation of a huge effort developed by many professors, researchers and students, all bound by a strong conviction that research in plant sciences in Europe is gaining relevance as its impact at all its dimensions (social, economic, environmental, health,...) is being better recognized. But this recognition also claims a better connection between industry and academia, which can be achieved through this particular Program.
I quote the first MAP-BioPlant Director in 2009-2010: "We want a Doctoral Program of reference, offering an education of excellence. We want an evolutionist Doctoral Program, offering advanced education of growing quality, as it will itself empower the dynamics and internationalization of the science we do. We want a Doctoral Program that jumps over the walls of University, and comes across needs of companies and producers to which we have competences to respond". These were the main objectives during the MAP-BioPlant foundation and remain the main objectives today!
During this third year, BioPlant has an increased number of students working with companies, and the internationalization of students is maintained. The advanced courses offered are consolidated, and many more will be offered in the next years.
This National MAP-BioPlant Workshop shows some of the best research in plants that is being done in Portugal. I hope you enjoy it.
I also want to thank to all that supported the consolidation of this objective!

Aveiro, 4th December 2012

Conceição Santos
Director of the 3rd Edition of BioPlant

Programme

4 December 2012

09:00 – **Opening and welcome**
Welcoming committee

09:20 **– Presentation of invited speakers and main conference theme**

Becoming a Plant: mechanisms and physiology of plastid acquisition

Chairman: António José Calado, Univ. Aveiro / Bioplant scientific committee

09:30 – **Invited lecture 1**
The eukaryotic tree of life and the 'phylogenies' of plastid acquisition
Øjvind Moestrup, University of Copenhagen, Denmark

10:30 – **Coffee Break and poster affixation**

11:00 – **Invited lecture 2**
Ecological and physiological aspects of a versatile 'plant-animal' lifestyle
Per Juel Hansen, University of Copenhagen, Denmark

12:00 – **Invited lecture 3**
Stealing from thieves: chloroplast transfer from cryptophytes through *Mesodinium* to *Dinophysis*
Øjvind Moestrup, University of Copenhagen, Denmark

12:30 – **Lunch and Poster affixation**

Programme

4 December 2012

14:30 – **Session: Stress & Development**
Chairman: Conceição Santos, Univ. Aveiro / Bioplant scientific committee

14:30 – **Invited Lecture 4**
Vacuolar transport of the alkaloid vindoline is mediated by a proton antiport in the medicinal plant *Catharanthus roseus*
Mariana Sottomayor, Univ. Porto, Porto.

15:00 – **Kleptoplasty: plastid acquisition in sea slugs**
Sónia Cruz, Univ. Aveiro, Aveiro.

15:15 – **Responses during water stress and recovery in two *Eucalyptus* clones: physiological profiles**
Bárbara Correia, Univ. Aveiro, Aveiro.

15:30 – **A putative role for the cell wall in vacuolar sorting**
Marta A. L. Figueiredo, Univ. Porto, Porto.

15:45 – **Characterization of two new vacuolar sorting signals uncovers different vacuolar routes**
Cláudia Sofia Pereira, BioFig / Univ. Porto, Porto.

16:00 – **Coffee Break**

16:30 – **Oxidative stress modulation by sulforaphane in MG-63 cells**
Miguel Oliveira, Univ. Aveiro, Aveiro.

16:45 – **Antifungal activity and mechanism of action of phlorotannins in *Candida albicans* yeast: possible role in the respiratory chain and induced oxidative stress**
Graciliana L. L. Lopes, REQUIMTE / FFUP, Porto.

17:00 – **Influence of *Hypholoma fasciculare* in chestnut grove sustainability**
Francisca Rodrigues dos Reis, BioFig / Univ. Minho, Braga.

17:15 – **Sulforaphane-induced DNA damage and cytostaticity in osteosarcoma cells**
Helena Oliveira, Univ. Aveiro, Aveiro.

17:30 – **Posters**

Programme

5 December 2012

09:30 – **Session: The Plant Genome**
Chairman: Helena Oliveira, Univ. Aveiro / CESAM

09:30 – **Invited Lecture 5**
Applications of flow cytometry in plant population biology and ecology
Sílvia Castro and João Loureiro, Centro Ecol. Funcional, Dep. Ciências da Vida, Univ. Coimbra.

10:00 – **Invited Lecture 6**
Effects of phytocompounds on DNA damage and repair: lessons from the comet assay
Isabel Gaivão, UTAD, Vila Real.

10:40 – **Coffee Break**

11:00 – **Genotoxicity / antigenotoxicity activity of *Calendula officinalis* and *Lavandula angustifolia*.**
Ana Raquel C. P. dos Santos, UTAD, Vila Real.

11:15 – **Cadmium-induced cyto- and genotoxicity in lettuce**
Cristina Monteiro, Univ. Aveiro, Aveiro.

11:30 – **A genetic triangle: how three MYB proteins determine flower asymmetry in *Antirrhinum***
João Raimundo, Univ. Minho, Braga.

11:45 – **ABA pre-treatment reduced the negative impact of water stress in *Ulmus minor* Mill.**
Maria Celeste Dias, Univ. Aveiro, Aveiro.

12:00 – **New technologies and approaches for industry interest in plant research at UA**
Glória Pinto, Univ. Aveiro.

12:15 – **Award of best communication in the 3rd annual workshop MAP-BioPlant**

12:30 – **Lunch and Posters**

Programme

5 December 2012

14:30 – **Session: Presentations by MAP-BioPlant students**
Chairman: Paula Melo, Univ. Porto, Porto.

14:50 – **Cloning expression and localization of VvSIP1 and functional studies of the purified protein in artificial phosphatodylethanolamine liposomes**
Henrique L. S. Noronha, Univ. Minho.

15:10 – **The effect of high temperature on sugar transport in grape cells**
Henrique L. S. Noronha, Univ. Minho.

15:30 – **Characterization, micropropagation and conservation of selected *Pinus* genotypes**
Sandra Nunes, Univ. Aveiro/Klon Co., Biocant.

15:50 – **Genetic characterization of *Pinus* genotypes with high oleoresin production: molecular and functional studies**
Diana Sousa, Univ. Aveiro/Klon Co., Biocant.

16:10 – **Analysis of the bioactivity of flavonoids in different osteosarcoma lines**
Sónia Pinho, Univ. Aveiro, Aveiro.

16:50 – **Closing address**

16:50 – **Coffee Break and poster removal**

17:10 – **Session: oral presentation of projects by 2nd year students**
Bioplant scientific committee

17:40 – **(title not provided)**
Nilson Paraíso, Univ. Minho, Braga.

Invited lectures and Oral Communications

4 December 2012

Invited Lecture #1

The eukaryotic tree of life and the 'phylogenies' of plastid acquisition

Øjvind Moestrup
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The tree of life has undergone major changes following the discovery that plastids in all groups of algae are endosymbionts. In the heterotrophic common ancestor of green algae and red algae (an amoeba or a flagellate), one or more blue-green algae were ingested and retained in the cell as endosymbionts, which continued to perform photosynthesis. Thus arose the first plastid or chloroplast. Most of the genes from the blue-green alga were transferred to the protozoan nucleus or lost and the two membranes limiting the plastids in this so-called primary endosymbiosis are of blue-green algal origin. However, the plastid-containing red algae and green algae were subsequently ingested by other heterotrophic protozoa, and this secondary endosymbiosis gave rise to plastids of many groups: cryptomonads, haptophytes, heterokonts, chlororachniophytes, many dinoflagellates, and euglenoid flagellates. It resulted in more complex conditions, the plastids being separated from the host by 3 or 4 membranes of different origins. The red algal nucleus was retained in cryptomonads, the green algal nucleus in chlorarachniophytes, in both cases as a so-called nucleomorph containing 3 chromosomes. In the other groups of algae, all traces of the red algal or the green algal nucleus were lost. Finally in certain other dinoflagellates, tertiary endosymbioses took place, when the dinoflagellate ingested a cryptomonad, a haptophyte or a heterokont (diatom). As a consequence of these complex events, indicators of phylogenetic affinity between algal groups are no longer found in the plastids, but in the structure of other parts of the cell (notably the flagellar apparatus) and in the DNA of the nucleus and perhaps the mitochondria, the origin of the latter being much less complex. Based on present evidence the eukaryotes are now grouped in 5 major assemblages ("Kingdoms"): the Opisthokonts (incl. terrestrial fungi and metazoans), the Amoebozoa (amoebae incl. myxomycetes), Archaeplastida (red and green algae and their descendents, the land plants), the Excavata (a number of protozoan groups and euglenoids), and the very large SAR group (S: stramenopiles/heterokonts, A: Alveolata, R: Rhizaria) which includes brown algae, diatoms, dinoflagellates, ciliates, sporozoa, foraminiferans and several other groups. Cells of most species of sporozoan (or apicomplexan) parasites contain a reduced plastid (of green algal or red algal origin) which has taken over other functions than photosynthesis and no longer contains chlorophyll.

Invited Lecture #2

Ecological and physiological aspects of a versatile 'plant-animal' lifestyle

Per Juel Hansen
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The classic portrayal of plankton is dominated by phytoplanktonic primary producers ("plants") and zooplanktonic secondary producers ("animals"). In reality, many if not most plankton traditionally labelled as phytoplankton or microzooplankton should be identified as mixotrophs, contributing to both primary and secondary production. Mixotrophic protists (i.e. single-celled eukaryotes that perform photosynthesis and graze on particles) do not represent a minor component of the plankton, as some form of inferior representatives of the past evolution of protists; they represent a major component of the extant protist plankton. This talk will give an overview of how mixotrophy are organized in different protists and then put special emphasis the dinoflagellates, because of their versatility. Mixotrophic species with permanent chloroplasts generally display a growth response towards irradiance like an ordinary autotrophic alga. However, some species cannot grow in the light on a standard inorganic nutrient medium, because they require the ingestion of prey for sustained growth. This includes species with various types of chloroplast origin. Only a few species have been shown to be able to grow in the dark if supplied prey. About half of the studied species are primarily phototrophic species, and food uptake marginally increases their growth rates at low irradiances. In the remaining species, food uptake increases to a large degree their growth rate when light is limiting and in some cases even when irradiance is not limiting growth. Some of these species grow relatively fast at high irradiances without food, while other species only grow slowly or cannot even maintain themselves at high irradiances without food. Dinoflagellates, which form symbioses with endo- and ectosymbionts are a very heterogeneous group, which have been studied only sporadically. Some species are clearly primarily phototrophs, while others rely heavily on food uptake for growth.

Invited Lecture #3

Stealing from thieves: chloroplast transfer from cryptophytes through *Mesodinium* to *Dinophysis*

Øjvind Moestrup
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moestrup@bio.ku.dk

Mesodinium is a genus of ciliates comprising both purely heterotrophic (protozoan) species, species that ingest cryptomonads and retain them in the cell as endosymbionts for a time before digesting them (kleptoplastids), and species which harbour photosynthetic cryptophytes as permanent symbionts. The latter comprises the famous *Mesodinium rubrum*, a red ciliate known to occur in huge numbers in the marine plankton in many parts of the world. Because of the red colour of the cryptophyte endosymbionts, cells of *Mesodinium rubrum* may stain the water red, thus forming so-called red tides (reported as far back as by Darwin in the Beagle). *Mesodinium rubrum* usually contains many cryptophyte cells within the cell, and the nuclei of the all endosymbionts are generally removed and fused to form a common nucleus. The plastids are nearly intact and retain the nucleomorph, the nucleus of the red algal ancestor of the cryptophyte plastid. *Mesodinium rubrum* can now be maintained in laboratory culture but needs a constant, small supply of cryptophytes which are ingested.
Dinophysis is a genus of marine dinoflagellates, known particularly because of the ability of many species to form DSTs, diarrhetic shellfish toxins. The toxins accumulate in shellfish which filter water containing *Dinophysis*, but do not poison these. However, humans and others may be poisoned when they eat the shellfish. In 2006 Korean researchers made a breakthrough in research on harmful algae, when they reported that *Dinophysis* could be maintained in culture by feeding with *Mesodinium rubrum*. This opened up for detailed studies of the toxic potential of *Dinophysis*. *Dinophysis* ingested whole *Mesodinium* cells, and molecular studies indicate that the plastids of *Mesodinium* were retained as plastids in *Dinophysis*. If this scenario is supported by additional evidence it represents a truly exceptional series of events, the plastid serving in turn in no less than 4 different hosts. It is a transformed blue-green alga which served first in a red alga, then in a cryptophyte which ate the red alga, then in *Mesodinium* which ate the cryptomonad and finally in *Dinophysis*, which ate *Mesodinium*. While the first three steps are generally accepted, the last step is presently debated as major structural differences are found between the plastids of *Mesodinium* and *Dinophysis*.

Invited Lecture#4

Vacuolar transport of the alkaloid vindoline is mediated by a proton antiport in the medicinal plant *Catharanthus roseus*

Inês Carqueijeiro[1,2], Henrique Noronha[3,4], Patrícia Duarte[1], Hernâni Gerós[3,4], Mariana Sottomayor[1,2*]
1 IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto
2 Departamento de Biologia, Faculdade de Ciências da Universidade do Porto
3 Centro de Investigação e de Tecnologias Agro-Ambientais e Biológicas (CITAB)
4 Departamento de Biologia, Universidade do Minho, Campus de Gualtar
** Corresponding author e-mail: msottoma@ibmc.up.pt*

Catharanthus roseus accumulates in the leaves the dimeric terpenoid indole alkaloids (TIAs) vinblastine and vincristine, which are valuable agents used in cancer chemotherapy. These TIAs are produced in very low levels in the leaves of the plant, from the monomeric precursors vindoline and catharanthine, and much is known about their biosynthesis. However, TIA membrane transport mechanisms are basically uncharacterized, in spite of their importance to understand TIA metabolic fluxes and to develop strategies aiming to increase TIA levels. Here, we have characterized the transport of vindoline across the tonoplast of mesophyll cells. Tonoplast vesicles isolated from leaves showed strong H^+-ATPase activity insensitive to azide and vanadate, indicating the absence of contamination with plasma membrane or mitochondrial membranes. It was shown that the tonoplast transmembrane pH gradient resulting from V-H^+-ATPase activity was dissipated by $CaCl_2$ and $CuCl_2$, indicating the involvement of Ca^{2+}/H^+ and Cu^{2+}/H^+ antiport systems. Vindoline incubated in the presence of ATP-energized tonoplast vesicles also induced the dissipation of the trans-tonoplast pH gradient, and the initial velocities of proton uptake followed a Michaelis-Menten kinetics, suggesting the involvement of mediated transport. Moreover, the observed pH gradient dissipation was confirmedly due to vindoline/H^+ exchange and not to inhibition of proton pumping or interference with membrane integrity. Finally, this vindoline/H^+ antiport activity was shown to be specific, since no significant activity was observed in the presence of other alkaloids. Overall, our data strongly indicates that vindoline is accumulated in the vacuole of *C. roseus* mesophyll cells by a specific proton antiport system.

Oral Communication #1

Kleptoplasty: plastid acquisition in sea slugs

Sónia Cruz
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A range of sacoglossan sea slugs from the superfamily Plakobranchoidea have developed the capacity of acquiring phototrophic-mediated carbon by sequestering plastids into tubule cells of their digestive diverticula, a mechanism often named kleptoplasty or kleptoplastidy. A general introduction to the mechanisms and physiology of plastid acquisition in sacoglossan sea slugs will be presented along with a brief description of the photobehaviour and photoprotection.
Acknowledgements: Work developed at the University of Aveiro using the local species *Elysia viridis*.

Oral Communication #2

Responses during water stress and recovery in two *Eucalyptus* clones: Physiological profiles

Correia B, Pintó-Marijuan M, Neves L, Dias MC, Brossa R, Araujo C, Costa A, Chaves MM, Santos C and Pinto G
Laboratory of Biotechnology and Cytomics, University Aveiro

In Portugal, about 700,000 ha have been established with *Eucalyptus globulus* clones selected for their high growth rates, high pulp yield and environmental adaptability. However, productivity in *E. globulus* plantations has encountered serious limitations, mostly because of water availability. Drought is a major abiotic stress negatively affecting plant growth and development that causes an array of physiological, biochemical and molecular responses in plants.
A number of studies reporting on plant responses to drought stress is currently available, but only a few reports provided evidence about the plants' capacity of recovering and the underlying processes. Moreover, ecophysiological studies have reported that different genotypes differ in their capacity to cope with drought. Considering that the capacity to survive and recover rapidly after rewatering should be the most important consideration in plant productivity, the aim of this study was to characterize the water perception and gather physiological features about the recovery capacities in different genotypes. It is expected that this approach will allow an early selection of suitable clonal collections for sustainable plantations in a Mediterranean climate.
For this propose, two *E. globulus* clones (AL-18 and AL-126) were subjected to a three-week water stress period, followed by one week recovery. Each genotype was analysed and a physiological profile was obtained for each one. Growth, water status, lipid peroxidation, photosynthetic responses, gas exchange and ABA concentration were assessed during maximum stress day and also after one day and one week of recovery. The main results of this work led has to conclude that: i) the chosen genotypes were highly tolerant to the conditions tested; ii) the selected clones presented a similar response in most of the tested parameters (except for MDA, pigments, fluorescence parameters and ABA); iii) clone AL-126 was the most resilient to drought, maintaining higher growth rates under stress and after rewatering.
Acknowledgments: This study was supported by the Portuguese Foundation for Science and Technology (FCT) through the fellowship of Maria Celeste Dias (SFRH/BPD/41700/2007).

Oral Communication #3

A Putative Role for the Cell Wall in Vacuolar Sorting

Marta Figueiredo, Cláudia Pereira, Susana Pereira and José Pissarra
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

In the past few years, the plant cell wall has been drawing more and more attention from the scientific community, particularly regarding the protein trafficking to and from this compartment. Although the transport of soluble proteins to the extracellular matrix is commonly accepted to occur by bulk flow, new data has emerged regarding the existence of signals and intermediates to control these pathways. In fact, experiments by our group have pointed towards some form of regulation of protein transport by the cell wall. Using cardosins A and B as experimental models, we have shown that the absence of cell wall in *Nicotiana tabacum* protoplasts expressing cardosins, which are typically vacuolar in this system, have originated partial protein secretion. In addition, the importance of cardosins' vacuolar sorting determinants (VSD) in this regulation was explored and particularly important observations were collected when comparing the divergent results obtained with the Plant Specific Inserts (PSI) and with the C-terminal peptides. This study enhances the dynamic and active role of the cell wall in the protein sorting events and is the starting point of several studies exploring its participation on the regulation of intracellular sorting.

Oral Communication #4

Characterization of two new vacuolar sorting signals uncovers different vacuolar routes

Cláudia Pereira[1, 2], Susana Pereira[1], Béatrice Satiat-Jeunemaitre[2] and José Pissarra[1]
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Several vacuolar sorting determinants (VSDs) have been identified for protein transport to the vacuoles in plant cells. Due to the variety in plant models, cell types and experimental approaches used to decipher vacuolar targeting processes, it is however not clear whether the well-known three groups of VSDs exhaust all the vacuolar targeting mechanisms in plants, nor if they reflect certain protein types or families. We used the aspartic proteinase cardosin A to study vacuolar trafficking in plants, as this protein is processed along the vacuolar pathway and accumulates in different types of vacuoles. Our results demonstrate that either the PSI or the C-terminal domains were necessary and sufficient to direct proteins to the vacuole, confirming that they are indeed VDSs. Further analyses using blockage experiments of the secretory pathway revealed that these two VSDs mediate two different trafficking pathways. Furthermore, the presence or the absence of a glycosylation site on the PSI domain appears as a key structure in determining the route to be taken by the PSI-driven targeting.

Oral Communication #5

Oxidative stress modulation by sulforaphane in MG-63 cells

Miguel Oliveira, Maria Costa, Tiago Pedrosa, Helena Oliveira, Conceição Santos
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Osteosarcoma (OS) is the major primary bone cancer in infants and young adults. Classical OS chemotherapy is mostly ineffective and highly toxic to normal cells. Sulforaphane (SFN), a phytochemical present in e.g. broccoli and Brussels sprouts, shows antiproliferative and proapoptotic activities in a number of cancer systems, both in vitro and in vivo. SFN has a dicothomous role in the protection against oxidative stress. It may transiently increase the formation of reactive oxygen species (ROS) but also increase the cells long-term antioxidant capacity. Considering these effects in other cancer systems, we decided to investigate SFN effects in the osteosarcoma MG-63 cell line. For this, cells were exposed to SFN up to 20 uM for a maximum period of 48h. SFN decreased cell viability (Trypan Blue) and increased intracellular ROS formation. Moreover, the total antioxidant activity was negatively affected by SFN. Different enzymes involved in ROS handling were studied. Increasing SFN resulted in decreased gene expression and enzyme activity for enzymes involved in ROS handling and glutathione recycling, viz. glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST). Both GPx and catalase (CAT) are responsible for hydrogen peroxide degradation and like GPx, also CAT was less expressed upon SFN exposure. This suggests that in MG-63 cells, SFN inhibits hydrogen peroxide degradation, which may constitute the main mechanism for ROS build-up in these cells.
Acknowledgements:This study was supported by the Portuguese Foundation for Science and Technology (FCT) trough the post-doctoral fellowships of J .M.P. Ferreira de Oliveira (SFRH/BPD/74868/2010) and Helena Oliveira (SFRH/BPD/48853/2008).

Oral Communication #6

Antifungal activity and mechanism of action of phlorotannins in *Candida albicans* yeast: possible role in the respiratory chain and induced oxidative stress

Graciliana Lopes [a], Eugénia Pinto[b], Paula B. Andrade [a], Teresa Mouga [c] and Patrícia Valentão [a]
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The widespread use of antifungal agents over the past few decades resulted in the increasing expression of resistance by fungal organisms. *Candida albicans* is among the most common fungal agents, frequently responsible for a variety of infections. Hence, considering the emerging multidrug resistance, substantial attention has been focused on natural products with antifungal properties.
While the medicinal properties of herbs have been recognized since ancient times, there has been a resurgence of interest in the antimicrobial properties of marine organisms. Among them, marine algae are particularly attractive, not only for the abundance of substances with industrial interest, but also for the diversity on secondary metabolites. Phlorotannins, a class of compounds specific from brown algae, are particularly interesting.

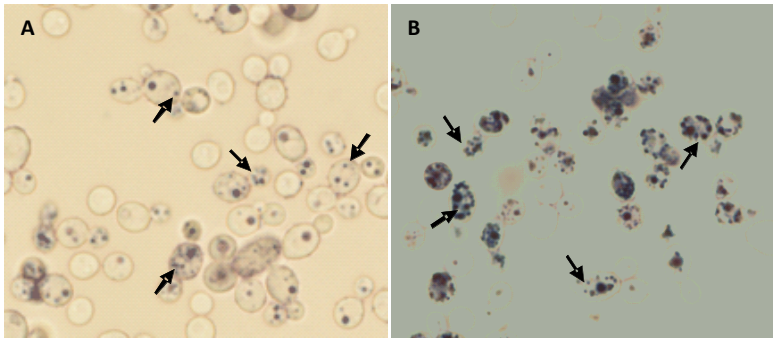

Figure 1: Effect of *C. nodicaulis* phlorotannins extract on the conversion of the yellow salt thiazolyl blue tetrazolium bromide (MTT) to formazan (515nm), by the action of the mitochondrial dehydrogenases of *C. albicans* ATCC 10231. Control (**A**), *C. nodicaulis* extract (**B**). Arrows show the formazan salt produced by *C. albicans* mitochondria.

Phlorotannins from *Cystoseira nodicaulis* (Withering) M. Roberts, *Cystoseira usneoides* (Linnaeus) M. Roberts and *Fucus spiralis* Linnaeus showed fungistatic activity over several *Candida* strains. *C. albicans* ATCC 10231 was used for the study of the mechanism of antifungal action of these compounds. The results showed that they act by affecting the dimorphic transition in *C. albicans* yeast, by affecting the activity of mitochondrial reductase enzymes (**Fig. 1**) and by altering the respiratory chain events, which can be related to oxidative stress. While phlorotannins doesn't seem to affect the level of chitin on the cell wall, they have a slight effect on the ergosterol levels of the cell membrane. Among the studied algae species, *C. nodicaulis* was the most promising one, presenting low minimum inhibitory concentration.
Phlorotannins can be promising antifungal agents, since their mechanisms of action are plural and different from those of the antifungals commonly used in therapy. Moreover, the use of these natural compounds as antifungals can be an alternative to circumvent the increasing fungal resistance.

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Oral Communication #7

Influence of *Hypholoma fasciculare* in chestnut grove sustainability

Francisca Reis, Eric Pereira, Rui Tavares, Paula Baptista, Teresa Lino-Neto
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The chestnut tree (*Castanea sativa* Miller) has an enormous economic importance at national level, mainly due to the value of the fruit (chestnut) and the high quality of the wood. The presence of the fungus *Hypholoma fasciculare*, which presents an expressive antagonist action against other soil-borne fungi present in chestnut groves, was recently detected. In order to assess the consequences arising from the presence of *H. fasciculare* on soil microbial diversity, the fungal community of soils from three chestnut groves presenting different levels of *H. fasciculare* was compared. After DNA extraction from 10 soil cores from each orchard soil, the ITS regions were amplified using the adequate primers for fungal sequences and 454 pyrosequencing was used for the assessment of soil metagenomes. Results will be presented taking into account the diversity of fungal trophic groups that could be important for chestnut sustainability, mainly the ectomycorrhizal fungi. These results will contribute to the identification of soil microbial species most affected by the presence of H. fasciculare, making it possible to assess the action of this fungus on beneficial mycorrhizal fungi to the chestnut tree.

Oral Communication #8

Sulforaphane induced DNA damage and cytostaticity in osteosarcoma cells

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Osteosarcoma is an aggressive bone malignancy, with high incidence in children and adolescents. This type of cancer is associated with low survival rates since it is highly refractory to current therapeutics. Therefore, it is essential to find alternative compounds with tolerable side effects that are able to increase the responsiveness of osteosarcoma cells. Sulforaphane (SFN) is an isothyocianate with important role as antiproliferative and antitumoral agent *in vitro* and *in vivo* and has been considered a promising chemotherapeutic compound. In this work we evaluate the potential of SFN as an antiproliferative and genotoxic agent in osteosarcoma cells. The osteosarcoma cell line MG-63 was exposed *in vitro* to increasing doses of SFN (0-20 uM) up to 48 h. Cells were analyzed for cell cycle dynamics, genotoxicity (clastogenicity, DNA breaks) and apoptosis. The results showed that SFN affected cell cycle dynamics with an arrest at G2 phase and increased cell genomic instability by increasing DNA strand breaks, clastogenicity and nuclear and mitotic abnormalities. The nuclear division index (NDI) decreased with SFN doses, and negatively correlated with the raises of nucleoplasmic bridges (NPBs), micronuclei (MN), cell cycle blockage at G2/M phase transition. Overall, the NPBs, MN, and comet data positively correlated with the loss of viability and increase of apoptosis. Together, these data point to genotoxic damages as important events in SFN-induced cytotoxicity in MG-63 cells, with still unknown consequences in therapy.
Acknowledgments:This study was supported by the Portuguese Foundation for Science and Technology (FCT) trough the post-doctoral fellowships of Helena Oliveira (SFRH/BPD/48853/2008) and J.M.P. Ferreira de Oliveira (SFRH/BPD/74868/2010).

5 December 2012

Invited Lecture #5

Applications of flow cytometry in plant population biology and ecology

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Flow cytometry (FCM) is a high-throughput technique that simultaneously measures and analyses multiple parameters of individual particles. Over the last decade, the applications and use of FCM in plant ecology have increased dramatically. The unsurpassed speed and reliability on estimating differences in nuclear DNA content by FCM allowed large-scale surveys at the landscape, population, individual and tissue levels, with the majority of the studies being focused in spatial distribution and evolutionary significance of genome duplication (polyploidy). Also, representative sampling opened the possibility to gain novel insights into the extent of intra- and inter-population ploidy variation, niche differentiation, and ecological preferences of particular cytotypes. In combination with molecular and phenotypic approaches, FCM promises qualitative advances in our understanding of genome multiplication and the population biology of vascular plants. In the present talk we will briefly explore the importance of this technique in the areas of plant population biology and ecology, namely by presenting a case study on the breeding barriers between cytotypes of *Aster amellus* complex.

Invited Lecture #6

Effects of phytocompounds on DNA damage and repair: lessons from the comet assay

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Abstract not available

Oral Communication #9

Genotoxicity / antigenotoxicity activity of *Calendula officinalis* and *Lavandula angustifolia*

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The use of plants for therapeutic purposes is prior to the development of science. Every nation has its own set of medicinal herbs, plants usually common in the territory where they live, whose applications are passed down through generations. The *Calendula officinalis* is commonly used as an ornamental and medicinal plant with multiple applications. The *Lavandula angustifolia* (lavender) is considered an aromatic plant, used in perfumery and folk medicine.
In vitro culture is an important tool to get a large number of plants in a short period of time. So, it is very important to evaluate if the characteristics of the plants obtained in vitro are similar to the plants in the field.
Somatic mutation and recombination tests (SMART) in *Drosophila melanogaster*, have been developed in recent decades, they show high sensitivity, specificity and accuracy. In particular the w/w^+ test, has been widely used in the analysis of a large number of substances with different mechanisms of action, showing an extensive ability to detect genotoxic effects. In this study we used the SMART w/w^+ test, using OregonK flies. We made experiments with different concentrations of the plants to determine whether these induce genotoxicity and with different concentrations of the plants together with paraquat (that induce reactive oxygen species) to observe if there is less damage caused by the agent.
We used a chi-square ($\chi 2$) with a dual decision multiplication factor of two (m = 2) for statistical analysis. The formulation of two cases into four categories allows to evaluate the effect of treatment; positive, weakly positive, negative and inconclusive.
The results obtained for the plants ex vitro and in vitro indicate that both plants have some genotoxicity. Concerning about the effect antigenotoxic the results are inconclusive or negative at Lavandula, while in Calendula seems to exist a decrease in the genotoxicity on the treatment with plant and paraquat simultaneously.

Oral communication #10

Cadmium-induced cyto- and genotoxicity in lettuce

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Cadmium (Cd) contamination of soil and water due to anthropogenic activities may lead to its bioaccumulation in humans through the food chain, causing several health problems like liver, renal and bone injuries.
To understand the extent of Cd-induced cyto- and genotoxicity in plants, lettuce (*Lactuca sativa* L.), an important crop which accumulates Cd and is an ISO species, was exposed to Cd.
L. sativa was cultured in Cd solutions ranging from 1 to 50 µM, for 28 days. In roots and leaves, Cd cyto- and genotoxicity was assessed using multiple endpoints.
Data indicate that roots accumulated more Cd than leaves. Moreover, oxidative stress occurred in roots and leaves after Cd exposure. As H_2O_2 increased some antioxidant enzymes were stimulated to scavenge the reactive oxygen species (ROS). Overall, the total antioxidant activity was decreased in leaves and increased in roots with Cd concentration. In both organs, Cd induced lipid and protein oxidation with consequent increase of membrane permeability. Genotoxicity was observed by increasing the DNA damage and micronuclei formation in both organs. In response to DNA damage, root cells showed a cell cycle blockage trend at G_2 checkpoint at 1 µM, while those exposed to higher Cd concentrations presented S-phase delay.
These cyto- and genotoxicity biomarkers contributed to an overall comprehension of Cd effects in lettuce, depending on the plant organs (roots and leaves). The high accumulation of Cd and large biomass, compared to other species, allows lettuce to be considered in phytoremediation strategies.
Acknowledgments: This study was supported by the Portuguese Foundation for Science and Technology (FCT) through the fellowships of Cristina Monteiro (SFRH/BD/48204/2008), Helena Oliveira (SFRH/BPD/48853/2008) and Maria Celeste Dias (SFRH/BPD/41700/2007).

Oral Communication #11

A Genetic Triangle: How three MYB proteins determine flower asymmetry in *Antirrhinum*

João Raimundo
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The establishment of domains with different transcriptional activities in meristems is essential for many developmental processes. The asymmetry of the *Antirrhinum majus* flower is established by transcription factors with an asymmetric pattern of activity in the floral meristem. To better understand how this asymmetrical pattern is established, we have studied the molecular basis of the antagonism between the dorsal MYB protein RADIALIS (RAD) and the ventral MYB transcription factor DIVARICATA (DIV). We show that RAD and DIV compete for binding with another class of MYB proteins, termed RIPs (RAD-Interacting Proteins). RIP1 and DIV interact and form a protein complex that binds to the DIV DNA consensus region suggesting that that the RIPs may act as a co-regulator of DIV transcriptional activity. In the presence of RAD, the interaction between RIP1 and DIV-DNA complex is disrupted and the RIPs are sequestered in the cytoplasm, thus, preventing or reducing the formation of RIP-DIV heterodimers in the nuclei. Taken together, our results suggest that the establishment of the asymmetric pattern of gene activity in the *Antirrhinum* flower, responsible for the repression of ventral identity in the dorsal part of the flower, is most likely generated by the antagonism that RAD has over DIV activity through a subcellular competition for the RIP proteins.

Oral Communication #12

ABA pre-treatment reduced the negative impact of water stress in *Ulmus minor* Mill.

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Drought is one of the main abiotic stress factors that affect plant growth and development reducing crop yield. The effects of drought are expected to increase with environmental climate change and growing water scarcity. Abscisic acid (ABA) is an important regulator in many aspects of plant growth and development, and is pivotal for stress resistance. This work aims to determine if ABA exogenous application can alleviate the negative effects of drought on the photosynthetic performance of *Ulmus minor*. One month old plants were sprayed with 50 or 100 µM of ABA during 25 days. After this period plants were exposed to water deficit by withholding water for 6 days. Water stress (WS) induced an impairment of photosynthesis and caused oxidative stress. However, these effects were diminished in plants previously treated with ABA. The lower concentration of ABA studied (WS conditions) induced higher protection against water stress. This work demonstrated that ABA pre-treatment alleviated drought stress effects and reduced oxidative damage.
Acknowledgments: This study was supported by the Portuguese Foundation for Science and Technology (FCT) through the fellow of Maria Celeste Dias (SFRH/BPD/41700/2007).

Oral Communication #13

New Technologies and Approaches for Industry Interest in Plant Research at UA

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Plants are essential to human life, providing the majority of the food we consume, feed for our livestock, and valuable non-food products. Plant sciences therefore face important challenges at the European and global scale due to the increasing demands from a burgeoning world population. Competition for land, water and energy will increase the need to adapt to and mitigate the effects of climate change and adopting new techniques is crucial.
Plant science is needed to appreciate, understand, and thereby conserve biodiversity in a rational way. Improved knowledge of plants in the environment will help develop management strategies for both conservation and production that will have a far-reaching impact on the environment and Portuguese economy.
One of the University of Aveiro mission is to strengthen communication among faculty and with stakeholders and continually identify strategic opportunities in plant research and outreach. During this presentation several examples of plant research highlighting the interaction between the Department of Biology and industry will be presented. New technologies are key strategies to industry interest in plant-based interest. Examples where in vitro culture is applied, selection for more tolerant plants, supporting selection policies concerning plant production and pathology and the technology used during plant research in Biology department will be presented.

Posters

Poster #1

Hyaluronidase inhibition and antioxidant capacity of phlorotannins from Fucales collected in the Portuguese west coast: possible candidates for the development of an anti-aging formula

Tatiana Mendo, Graciliana Lopes, Paula B. Andrade, Carla Sousa and Patrícia Valentão
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Reactive oxygen species (ROS) formed during aerobic life, such as superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($HO^{\bullet}$) and hydrogen peroxide (H_2O_2), are recognized for being associated not only to initiation, but also to promotion and progression of multiple diseases, disorders and aging. Skin is particularly vulnerable to ROS, since it is exposed to oxidative stress from both endogenous and exogenous sources.
The effect of phlorotannins against ROS is related not only to the prevention of skin aging, but also to the reduction of inflammatory states, allergy and migration of cancer cells. These compounds can effectively contribute to the recovery of skin homeostasis and consequently prevent the downstream events that physically damage dermal matrix structure. They are not only potent ROS scavengers, but have also demonstrated a huge capacity to inhibit HAase and to minimize the oxidative stress through a synergy created by the elimination of ROS and enhancement of the antioxidant defence capacity. The non-toxic nature of these compounds should be valued, as they show an unparalleled low toxicity when compared with other natural antioxidants.
Phlorotannins from *Cystoseira tamariscifolia* (Hudson) Papenfuss, *Cystoseira usneoides* (Linnaeus) M. Roberts and *Fucus spiralis* Linnaeus (**Fig. 1**) showed potent antioxidant activity against several ROS, and potent hyaluronidase inhibitory capacity, *F. spiralis* being the most promising species.

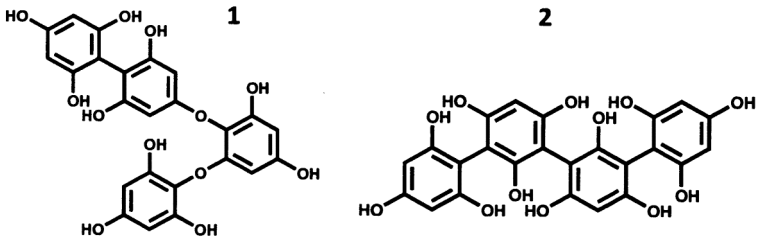

Figure 1. Phlorotannins isomers ($[M+H]^+$ at *m/z* 499) of fucodiphloroethol (1) and tetrafucol (2) tentatively identified in *F. spiralis* and *C. usneoides*.

This feature, along with the potent anti-aging activities, is the hallmark of phlorotannins that enables effective protection against the loss of elasticity of aged skin. For these reasons, the use of natural anti-aging products derived from marine sources is gaining prominence and attracting researchers' attention.

Acknowledgements: G. Lopes is grateful to FCT, FSE and POPH for the grant (SFRH/BD/61565/2009).

Poster #2
Phytochemical compounds and biological activities of *Euphrasia rostkoviana* Hayne leaves

Alvarina R. Teixeira[1], Silvia R. Chañi[1,2], Paula B. Andrade[1], Patrícia Valentão[1], Luís R. Silva[1]
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Euphrasia rostkoviana Hayne, synonym of *E. oficinallis* L. (Scrophulariaceae), commonly known as "eyebright" or "eyewort", is a wild plant growing in the temperate Himalayas [1]. Eyebright is stated to possess anticatarrhal, astringent and anti-inflammatory properties. Traditionally it has been used for nasal catarrh, sinusitis and specifically for conjunctivitis when applied locally as an eye lotion [2].
This study was conducted in order to assess the metabolite profile and biological activities of hydroethanolic extract and infusion obtained from *E. rostkoviana* leaves. Phenolic compounds and organic acids were analysed by HPLC-DAD and HPLC-UV, respectively. Biological activities (antioxidant, anticholinesterases and antibacterial) were also assessed.
Regarding the phenolic and organic acids composition, some qualitative and quantitative differences were noticed between the two analysed extracts, infusion being the richest one. Both extracts presented hydroxycinnamic acids and flavonoids, the latter predominating. Citric was the main organic acid in both hydroethanolic extract and infusion.
A concentration-dependent antioxidant effect was observed, infusion being the most active extract against all of the tested reactive species, which can be partly explained by its higher amounts of phenolics and organic acids. In addition, hydroethanolic extract and infusion showed good inhibitory effect on acetylcholinesterase and butyrylcholinesterase.
Both extracts displayed potent activity against Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Enterococcus faecalis* and *Micrococcus luteus*) and Gram-negative bacteria (*Salmonella typhimurium*, *Proteus mirabilis*, *Escherichia coli* and *Pseudomonas aeruginosa*).

References:
[1] Porchezhian, E., Ansari, S.H., Shreedharan, N.K. (2000). Antihyperglycemic activity of *Euphrasia officinale* leaves. *Fitoterapia*, *71*(5): 522-526; [2] Barnes, J., Anderson, L. A., & Phillipson, J. D. (2007). Herbal medicines. Pharmaceutical press, London , 256-257.

Poster #3
Physiological response of cork oak (*Quercus suber* L.) to salt stress

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Salt stress is considered a major abiotic stress since approximately 20% of the cultivated area and 50% of the irrigated lands worldwide are affected by salinity. Excessive amounts of NaCl in the soil lead to molecular damages in the plant, limits growing and development and, in severe situations, results in plant loss. In order to avoid or mitigate these effects plants have developed mechanisms that regulate NaCl accumulation or exclusion. Cork oak (*Q. suber*) occupies about 23% of the total Portuguese forest area, being the most abundant native species and having a crucial ecological, social and economical role. Portugal is the world's biggest producer of cork and its derivates. Hence, a better knowledgement of the physiological and metabolic change that occur during salt exposure are a priority to improve salt stress tolerance in this species. This study aims to evaluate the physiological response of cork oak to salt stress**.** Two months old plants were watered with 300 mM NaCl during 6 days. Leaf samples were collected before watered with NaCl (day 0) and after 1 and 6 days of the beginning of the experiment. The results obtained indicated that salt stress leads to a decrease of *Q. suber* physiological performance and lipid damage (oxidative stress). Proline didn't show to be a sensitive salt stress biomarker in *Q. suber*. Further physiological studies should be performed in order to have an in deep/complete knowledge of the performance of *Q. suber* under salt stress.

Poster #4
In the pursuit of novel anti-depressant drugs

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Monoamine oxidase A (MAO-A) inhibitors are used to treat depression, a psychiatric disorder that affects 150 million people worldwide. However, due to the side effects of some of the classic drugs, the search for safer and more efficient inhibitors is urgent. Since medicinal plants are known as an inexhaustible source of new bioactive metabolites, the hydromethanolic extracts of *Jacaranda caroba* (Vell.) A. DC (Bignoniaceae), *Cochlospermum angolensis* Welw. (Bixaceae), *Grindelia robusta* Nutt. (Asteraceae) and *Gelsemium sempervirens* L. (Loganiaceae) were studied both from chemical and biological points of view. Among the four species, *J. caroba* and *C. angolensis* were the most active anti-MAO-A, displaying IC_{50} values of 23 and 42 µg/mL, respectively (Fig. 1). The HPLC-DAD-ESI/MS^n analysis revealed the presence of four dicaffeoyl acid derivatives and nine flavonoids (quercetin, kaempferol and isorhamnetin derivatives) in *J. caroba* extract, isorhamnetin-3-*O*-rhmanoside-7,4´-di-*O*-glucoside and quercetin-3-*O*-(2-pentosyl)hexoside being the major compounds. On the other hand, methyl ellagic acid pentoside isomer is the main metabolite of *C. angolensis* extract, followed by other methyl ellagic acid and ellagic acid derivatives. These results highlight the value of these two species as sources of antidepressant compounds.

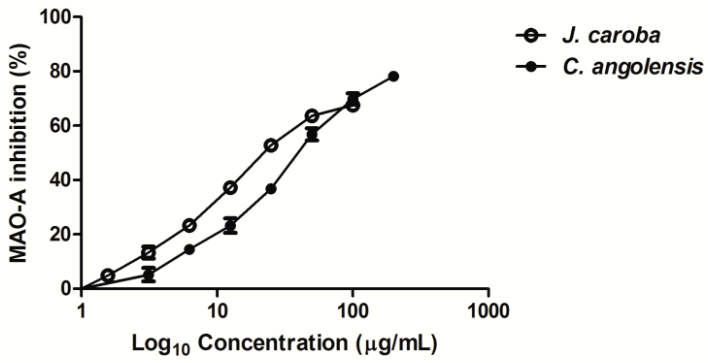

Figure 1: MAO-A inhibitory activity of the hydromethanolic extracts of *J. caroba* and *C. angolensis*.

Acknowledgements: Clara Grosso thanks FCT for the Post-Doc fellowship (SFRH/BPD/63922/2009).

Poster #5
Plant Specific Insert (PSI) Domains as Modulators of Protein Sorting and Maturation

Bruno Peixoto[1], Marta Figueiredo[1], Susana Pereira[1], Cláudia Pereira[1,2], and José Pissarra[1].
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Typical plant aspartic proteinases (APs) are characterized by the presence of an approximately 100 amino-acid long insertion termed the "Plant Specific Insert". Despite the high degree of homology observed between different plant APs, PSI domains show remarkably higher plasticity both in terms of their cDNA and amino acidic sequences. Structurally, however, these domains demonstrate a highly conserved topology, identical to that of mammalian saposin C and other saposin-like proteins (SAPLIPs). Cardosins' A and B PSI domains have so far been identified as vacuolar sorting determinants, but recent findings by our group hints at a larger role for this domain, which might be responsible for controlling tissue-specific vacuolar routes and protein maturation.

Poster #6
Further insights into the chemical profile and bioactivities of *Ficus carica* L. fruits

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Fig tree (*Ficus carica* L.; Moraceae) is one of the first plants cultivated by humans as a seasonal food, its fruits being important constituents of the Mediterranean diet. In this work, a targeted metabolite analysis was performed on the fruits (peels and pulps) of three dark varieties of *F. carica*. Phenolics and organic acids profiles were determined by HPLC-DAD and HPLC-UV, respectively. All fruits presented a similar phenolic profile composed, by 3- and 5-*O*-caffeoylquinic acids, ferulic acid, quercetin-3-*O*-rutinoside, psoralen and bergapten. Citric, malic, shikimic and fumaric acids were the organic acids found. Additionally, fatty acids, sterols and triterpenes were also determined by GC-MS (**Fig. 1**).

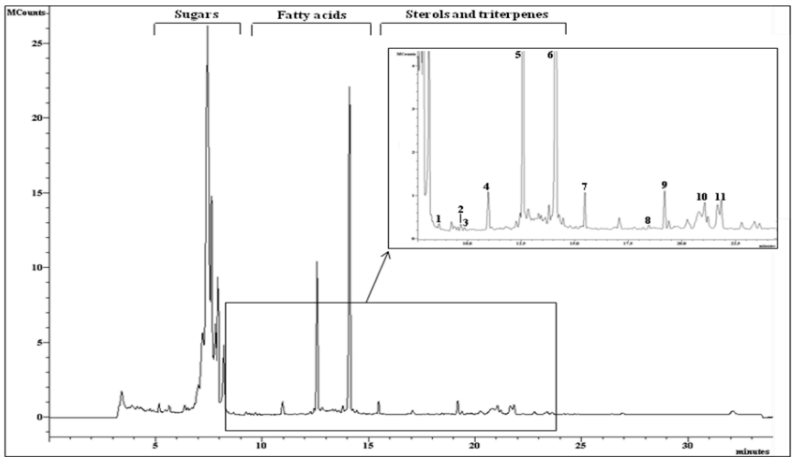

Figure 1. GC-MS chromatogram of *F. carica* peels. (1) Palmitic acid; (2) oleic acid; (3) stearic acid; (4) arachidonic acid; (5) palmitic acid derivative; (6) stearic acid derivative; (7) cholesterol derivative; (8) stigmasterol; (9) β-sitosterol; (10) β-amyrin and (11) lupeol acetate.

The lyophilized aqueous extracts of peels and pulps showed a dose-dependent response against reactive nitrogen species. The same effect was observed in the α-glucosidase inhibitory assay. Furthermore, acetylcholinesterase and butyrylcholinesterase inhibitory capacities were evaluated, but no activity was found. The results suggest that this fruit is a good source of bioactive metabolites, and may contribute to the prevention of diseases in which homeostasis is impared by oxidative features.

Acknowledgments: Andreia P. Oliveira is indebted to FCT for the grant (SFRH/BD/47620/2008).

Poster #7

Phenolic profile of three different varieties of *Colocasia esculenta* (L.) Shott from Azores

Ana Margarida Silva[a], Rui F. Gonçalves[a], Artur M. S. Silva[b], Patrícia Valentão[a], João B. Silva[c], Delfim Santos[d] and Paula B. Andrade [a]

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[b] *Department of Chemistry & QOPNA, University of Aveiro, Campo Universitário de Santiago, 3810-193 Aveiro, Portugal*
[c] *Unidade de Investigação Geobiotec, Departamento de Geociências, Universidade de Aveiro, Campo Universitário de Santiago, 3810-193 Aveiro, Portugal*
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Colocasia esculenta (L.) Shott is an annual herbaceous plant belonging to the Araceae family, commonly known in Portugal as "Inhame dos Açores". The cultivation of this species is mainly due to its tuber, taro, an essential food for millions of people. In addition to its nutritional value, taro is known for its medicinal properties and has been traditionally used in the treatment of several diseases. The welfares exerted by this plant can be related to the presence of phenolic compounds, which are known for their health benefits. Therefore, seeking the valorization of taro crop in the Azores archipelago, this work aimed to characterize, quantify and compare the phenolic composition of the different varieties and culture conditions of taro from Azores.

Forty one phenolic metabolites (eleven hydroxycinnamic acid derivatives and thirty glycosylated flavonoids) were identified by High Performance Liquid Chromatography-Diode Array Detection-Electrospray Ionization/Mass Spectrometry (HPLC-DAD-ESI/MS^n) by our group in the leaves of two *C. esculenta* varieties.[1] Further phenolics quantification was achieved by an accurate and sensitive validated HPLC-DAD method. Apigenin and luteolin derivatives were the metabolites in higher amounts in the three varieties. Qualitative and quantitative differences observed lead us to conclude that this HPLC-DAD method can be an important tool in the discrimination of the different varieties of this species. The established phenolic profile is an added value for the authenticity and quality control of *C. esculenta*.

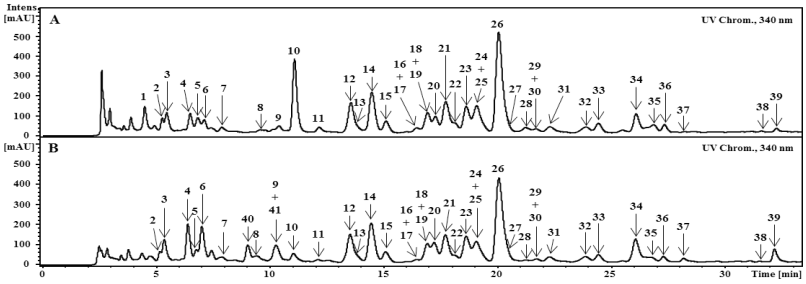

Figure 1. HPLC-DAD (340 nm) phenolic profile of aqueous extract from leaves of *C. esculenta*. (**A**) "giant white" and (**B**) "red" varieties. (**1-6**) caffeic acid derivatives; (**7**) sinapoyl hexoside; (**8**) apigenin-6-*C*-pentoside-8-*C*-hexoside-7-*O*-hexoside; (**9**) luteolin-6,8-di-*C*-hexoside; (**10**) caffeic acid; (**11**) luteolin-6-*C*-(6-*O*-hexosyl)hexoside; (**12**) luteolin-6-*C*-hexoside-8-*C*-pentoside; (**13**) apigenin-6,8-di-*C*-hexoside; (**14**) luteolin-6-*C*-hexoside-8-*C*-pentoside; (**15**) luteolin-6-*C*-pentoside-8-*C*-hexoside; (**16**) apigenin-6-*C*-(6-*O*-hexosyl)hexoside; (**17**) apigenin-6-*C*-pentoside-8-*C*-hexoside; (**18**) apigenin-6-*C*-pentoside-8-*C*-hexoside; (**19**) luteolin-6-*C*-(3-*O*-hexosyl)hexoside-8-*C*-pentoside; (**20**) *p*-coumaric acid; (**21**) apigenin-6-*C*-pentoside-8-*C*-hexoside; (**22**) luteolin-6-*C*-pentoside-8-*C*-hexoside; (**23**) luteolin-8-*C*-hexoside; (**24**) apigenin-6-*C*-hexoside-8-*C*-pentoside; (**25**) chrysoeriol-6-*C*-hexoside-8-*C*-pentoside; (**26**) luteolin-6-*C*-hexoside; (**27**) luteolin-6-*C*-(2-*O*-pentosyl)hexoside; (**28**) apigenin-6-*C*-pentoside-8-*C*-(2-*O*-hexosyl)hexoside; (**29**) diosmetin-6-*C*-hexoside-8-*C*-pentoside; (**30**) apigenin-6-*C*-(2-*O*-hexosyl)hexoside-8-*C*-pentoside; (**31**) apigenin-8-*C*-hexoside; (**32**) apigenin-8-*C*-(2-*O*-pentosyl)hexoside; (**33**) apigenin-6-*C*-hexoside-8-*C*-pentoside; (**34**) apigenin-6-*C*-hexoside; (**35**) chrysoeriol-8-*C*-hexoside; (**36**) chrysoeriol-6-*C*-hexoside; (**37**) luteolin-7-*O*-rhamnosyl(1→2)hexoside; (**38**) chrysoeriol-7-*O*-hexoside; (**39**) chrysoeriol-7-*O*-rhamnosyl(1→6)hexoside; (**40**, **41**) dihydrocaffeoylquinic acid derivatives.

References: [1]Ferreres, F.; Gonçalves, R. F.; Gil-Izquierdo, A.; Valentão, P.; Silva, A. M. S.; Silva, J. B.; Santos, D.; Andrade, P. B. Further knowledge on the phenolic profile of *Colocasia esculenta* (L.) Shott. . *J. Agric. Food Chem*. **2012**, *60* (28), 7005–7015.

Poster #8
Vacuolar transport of the alkaloid vindoline is mediated by a proton antiport in the medicinal plant *Catharanthus roseus*

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Catharanthus roseus accumulates in the leaves the dimeric terpenoid indole alkaloids (TIAs) vinblastine and vincristine, which are valuable agents used in cancer chemotherapy. These TIAs are produced in very low levels in the leaves of the plant, from the monomeric precursors vindoline and catharanthine, and much is known about their biosynthesis. However, TIA membrane transport mechanisms are basically uncharacterized, in spite of their importance to understand TIA metabolic fluxes and to develop strategies aiming to increase TIA levels. Here, we have characterized the transport of vindoline across the tonoplast of mesophyll cells. Tonoplast vesicles isolated from leaves showed strong H^+-ATPase activity insensitive to azide and vanadate, indicating the absence of contamination with plasma membrane or mitochondrial membranes. It was shown that the tonoplast transmembrane pH gradient resulting from V-H^+-ATPase activity was dissipated by $CaCl_2$ and $CuCl_2$, indicating the involvement of Ca^{2+}/H^+ and Cu^{2+}/H^+ antiport systems. Vindoline incubated in the presence of ATP-energized tonoplast vesicles also induced the dissipation of the trans-tonoplast pH gradient, and the initial velocities of proton uptake followed a Michaelis-Menten kinetics, suggesting the involvement of mediated transport. Moreover, the observed pH gradient dissipation was confirmedly due to vindoline/H^+ exchange and not to inhibition of proton pumping or interference with membrane integrity. Finally, this vindoline/H^+ antiport activity was shown to be specific, since no significant activity was observed in the presence of other alkaloids. Overall, our data strongly indicates that vindoline is accumulated in the vacuole of *C. roseus* mesophyll cells by a specific proton antiport system.

Poster #9
Molecular Markers Associated to Aluminium Tolerance in *Secale cereale*

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Soil acidity associated with aluminium (Al) toxicity is a major factor in limiting crop production, mainly due to the inhibition of the plants root elongation. The Al is then considered as a problem for crop production on acid soils.
Different crop species have distinct responses to the stress induced by Al toxicity. Between cereals, rye has proven to be the most tolerant species. As such, the *Secale* genus has become a current use in breeding programs, in relation to other crops with economic and agricultural importance.
In the present study, five wild species and seven cultivated ryes were assessed at 5ppm of Al concentration. Tolerance and sensibility to Al toxicity were observed in plants with different genetic backgrounds. From the results obtained it was observed that some ryes showed a great variability (*S. ancestrale* and Dankowskie Zlote), while others showed small variability, as *S. Montanum* and Riodeva, predominantly sensitive, and JNK and Portuguese regional populations, principally tolerant.
Tolerant and sensitive plants from each rye were selected and analyzed using RAPDs (Random Amplified Polymorphic DNA) markers. Ten primers were used to detect the markers associated to Al tolerance. A total of 18 markers, possibly related to tolerance mechanisms were found. The markers obtained in this work presented a high polymorphism rate of 72,19% where 109 of 151 amplified fragments were polymorphic.
However, further studies with more primers and molecular markers, like ISSRs (Inter Simple Sequence Repeat) are required for association analysis.

Poster #10
Application of *ISSRs* to detect the genotoxic effects of aluminium in *Plantago almogravensis* plantlets

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Plantago almogravensis Franco (Plantaginaceae) is an endemic and rare species from the Portuguese Southwest coast. This species is at risk of global extinction and is legally protected under the European Habitats Directive 92/43/EEC and by the Portuguese law. Recently, *P. almogravensis* Franco has been described as aluminum hyperaccumulator. Aluminum (Al) is the third most abundant metallic element in the Earth's crust and its toxicity, associated with acidic soils, is one of the majors limiting factors for plant development. For these areas the research is focused on hyperaccumulator plants, in the study of mechanisms of tolerance/resistance to Al, genes isolation and metals transporters. With our study we intended to evaluate the genotoxicity of *P. almogravensis* in stress conditions (in presence of Al), using molecular markers (*ISSRs – Inter Single Sequence Repeats*).
To understand if Al induces DNA damage, the same genotypes of *P. almogravensis* were exposed for 21 days to a 400 µM of Al concentration. Ten ISSR primers were used to detect DNA damage in roots and leaves of the same plantlets at 7 and 21 days. Polymorphisms became evident as the presence and/or absence of DNA fragments in treated samples compared with the untreated one. At 21 days, a high number of both missing bands and new amplified fragments were observed. From a total of 179 ISSR fragments, 118 were polymorphic.
The polymorphism ratios between control and treated plants, at 7 and 21 days of culture, were 12.3% and 14.9% in the leaves, and 18.6% and 31.6% in the roots, respectively.
DNA polymorphisms detected by ISSR analysis can be used as an investigation tool for environmental toxicology and as a useful biomarker assay for the detection of genotoxic effects of Al on plants.

Poster #11
Effect of TiO_2 NPs on *Lactuca sativa* seed germination and seedling root growth

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The application areas of nanoparticles (NPs) extended in the past few years. Engineered NPs (ENPs) production increased and, in consequence, its release to the environment. Several studies revealed that NPs are toxic to different species, including algae, bacteria, aquatic organisms and humans. In the past few years, more studies have been done in order to comprehend the impact of NPs exposure in plants species, however much is still to be done. In order to study the effects of TiO_2 in the germination of *Lactuca sativa*, seeds were germinated in TiO_2 NPs suspensions. The seeds were exposed to 5; 10; 50; 100 and 150 mg/L TiO_2 suspensions during five days. Germination rate was determined and four days old seedlings were used to evaluate root growth and cell cycle profile. Exposure to TiO_2 NPs did not lead to a significant decrease in the germination rate, nevertheless was detected a decreasing trend. Exposed seedlings presented an increment in the root length, which was significantly higher in seedlings exposed to 5 mg/L TiO_2 NPs. Using FCM, root cell cycle profile was studied. NP exposure altered the cell cycle dynamics (significantly different in seedlings exposed to 150 mg/L): was observed a trend of decreasing cells in the G_0/G_1 phases and to increasing of cells in the S phase. So, results point to a blockage of the S phase at the expense of decreases in G_0/G_1 phases.

Acknowledgments: FCT supported Sonia Silva (SFRH/BPD/74299/2010) and Helena Oliveira (SFRH/BPD/48853/2008) grants.

Poster #12
Micropropagation of selected *Pinus* genotypes

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Clonal propagation of forest species has significant advantages for the mass propagation of selected genotypes and is used in breeding strategies to produce improved plant stocks more rapidly than conventional seed orchard procedures. Micropropagation is an effective way of capturing genetic gain with the potential to provide very high multiplication rates of selected tree genotypes, resulting in short-term silviculture gains. Moreover, it overcomes problems of irregular seed cone production, long life cycles and recalcitrance of conventional vegetative propagation methods.
Slash Pine (*Pinus elliottii* var *elliottii*), Caribbean Pine (*Pinus caribaea* var *hondurensis*) and the resulting hybrid (*Pinus elliottii* var *elliottii* x *Pinus caribaea* var *hondurensis*) were introduced in Brazil in reforest programs due to their great economic value in wood and resin industry. It is known that the hybrid has a high growth ratio, which makes it more suitable for production of wood and resin than its parents. Several studies on micropropagation of Pinus have been reported in the literature. These studies include axillary shoots proliferation and induction of direct and indirect organogenesis. However, information about micropropagation protocols for these particular species is still scarce. The aim of this project is to develop efficient protocols for the micropropagation of *Pinus elliottii var elliottii*, *Pinus caribaea var hondurensis* and the hybrid, *Pinus elliottii var elliottii x Pinus caribaea var hondurensis,* in order to integrate *in vitro* culture technology in ongoing breeding programs.
Acknowledgments: This study was supported by the FCT through the grants of MCDias (SFRH/BPD/41700/2007) and J .M.P. Miguel Oliveira (SFRH/BPD/74868/2010)

Poster #13
An overview of transgenics' applications in human health

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The advances in recombinant DNA technology, since the 1970 decade, were at the basis of the development of biotechnological tools that became essential to human health research and services. Transgenic products are already present on a global scale. We update here, some of the most relevant scientific advances in the last decades, of transgenic development focused on human health. For example, we highlight in this scientific review the development of transgenic crops with desirable traits, such as resistance to pathogens and improved nutritional characteristics. We also discuss the recent efforts to develop transgenic organisms to combat diseases and/or as potential tools of vaccines, among other potentialities.

Poster #14

Antioxidant enzymes in response to metalaxyl stress in *Solanum nigrum* L. cell suspension cultures

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Phytoremediation is a bioremediation process that uses plants and their associated microbes to clean up toxic elements such heavy metals and persistent organic compounds from contaminated areas (soils, groundwater and sediments) [1]. Metalaxyl is a systemic fungicide with a protective and curative action used to control diseases caused by fungi of the order Peronosporales and therefore widely used in agriculture. This fungicide is stable to a wide range of pH, light and temperature conditions and consequently it has a tendency to accumulate in soil and groundwater representing a serial concern not only to the environment but also to public health [2]. Plant tissue cultures of *Solanum nigrum* L. have been used as a model system applied in phytoremediation research [3,4]. Following previous results [5,6], the aim of the present study was to evaluate the ROS homeostasis and the antioxidant enzymes response of *S. nigrum* cell suspensions cultures against the stress induced by metalaxyl. Green and friable calli were used to establish cell suspensions in Murashige & Skoog medium (1962) supplemented with 2 mg/L 2,4-D and 0.5 mg/L BA, at pH 5.7. Cells were propagated on a rotary shaker (120 rpm), in the dark, at 25ºC. To evaluate the effect of metalaxyl on the antioxidant enzymes, cells were transferred to identical MS medium supplemented with 0 µM; 71.60 µM and 143.20 µM of metalaxyl and cell samples were collected at 312 h (13 days). Exposure of cell suspensions to metalaxyl resulted in the increase of TBARS, hydrogen peroxide and proline levels observed along the cultural cycle with higher incidence at the highest concentration of the fungicide.
Superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX; EC 1.11.1.11) activities were increased, particularly at the higher concentration of metalaxyl tested (143.20 mM)), however, non-denaturing PAGE showed that metalaxyl did not change any enzyme isoform patterns in which seven SOD isoenzymes, two CAT isoenzymes and nine APX isoenzymes were detected. Globally, the data showed that the presence of the fungicide in the culture medium affected all the antioxidant enzymes evaluated, on a concentration-dependent manner. In conclusion, the enhanced activity of these enzymes suggested that the enzymatic antioxidant system is involved in the defense response that enables *S. nigrum* cells to cope with metalaxyl-induced stress and in ROS homeostasis. In addition, the results provided useful information for the understanding of the specific physiological role of each antioxidant enzyme in cell suspension cultures of *S. nigrum* in response to metalaxyl exposure.

References: [1] Pilon-Smits E. (2005). *Annu. Rev. Plant Biol*. 56: 15-39; [2] Sukul, P., Spiteller, M. (2000). *Rev. Environ. Contam. Toxicol*. 164: 1-26.; [3] Rezek, J. *et al*. (2007). *Chemosphere*. 88: 1221-1227.; [4] Kucerová, *et al*. (2000). *Plant and Soil*. 225: 109-116.; [5] Sousa A *et al.* (2011a). *IJUP' 11 - 4th Meeting of Young Researchers of U.Porto*. Porto. Portugal.; [6] Sousa A *et al.* (2011b). *IJUP' 11 - 4th Meeting of Young Researchers of U.Porto*. Porto. Portugal.

Poster #15
Molecular biology of grape berry phenolic maturation

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When grape berries ripen through maturation they change in both size and composition. The most striking change in berry development occurs at veraison, as the grapes enter into the ripening phase. One of the most important changes is the alteration in colouration of red and black skinned varieties as they ripen, being the skin colour one of the most important qualities used as the basis for selection in breeding programs.
The project PTDC/ AGR-PRO/ 12020264/ 2010 with the title "Molecular Biology of grape berry phenolic maturation", inserted in the main scientific area of Agricultural Science and Forestry – Agricultural Production has as principal contractor the University of Trás-os-Montes and Alto Douro (UTAD). An international partner University, Universidad Politécnica de Madrid, will also give its contribution to developing this Project. Portuguese teams involved belong to the University of Trás-os-Montes and Alto Douro (UTAD) and to the University of Porto (UP) and are integrated in two Associated Laboratories (Instituto de Biotecnologia e Bioengenharia – IBB and Rede de Química e Tecnologia– REQUIMTE) and two Research Centres (Centro de Química – CQVR/UTAD; Centro de Investigação e Tecnologias Agro-Ambientais e Biológicas – CITAB/UTAD).
Thereby, this project aims a better understanding of the genetics behind grape berry colour accumulation, by studying different grape skin colour mutants (related red, light-red and white grape cultivars). It also aims the elucidation of the mechanisms which determine the phenolic quality of grape berries, by doing an effective combination of modern molecular biology, genomics and metabolomic approaches and traditional physiological, chemical and agronomical technologies. Several tasks will be pursued, namely molecular approaches to study the genes involved in the anthocyanin biosynthesis since grape colour results from their synthesis and accumulation.

Poster #16
Effects of water stress on the physiological performance of *Melia azedarach*

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Melia azedarach is one of the most used plants in traditional medicine. A number of biological activities have already been described for crude extracts. Among the various environmental factors that can affect plant performance, the water stress (WS) is probably the one that has more impact. WS affects photosynthesis, biomass production reducing plant growth and productivity. Timor is a country with an unstable climate due to different latitudes that causes rainfall and drought cycles. A consequence of this climate is deforestation that leads to soil erosion and floods. This work aims to analyse the physiological performance of one species found in Timor, Melia azedarach, under WS conditions for future use of this species in reforestation programs in this island. Plants of *M. azedarach* were exposed to WS (plants at 20% of field capacity) during 20 days. After this period plant performance were evaluated: the relative water content, water potential and the effective quantum yield of PSII were significantly reduced by WS; pigment content and biomass were not affected by WS. Moreover, WS did not affect cell cycle of *M. azedarach* leaves. These preliminary results indicated that WS induce a decrease of photosynthetic efficiency and affect the water relations of *M. azedarach*. However, others physiological parameters should be performed in order to have an in deep/complete knowledge of the performance of *M. azedarach* under WS conditions.

Poster #17

Another perspective for the medicinal use of *Glandora diffusa* (Lag.) D. C. Thomas

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Glandora is a genus that includes 6 species, *Glandora diffusa* (Lag.) D. C. Thomas being one of them. Commonly known in Portugal as "sargacinha" or "erva-das-sete-sangrias", *G. diffusa* is traditionally consumed as an infusion for diuretic, depurative and anti-hypertensive purposes. Additionally, its pollen is found in some honeys. In the present work, the phenolic profile of *G. diffusa* aqueous and ethanolic extracts were assessed by HPLC-DAD-ESI/MSn. Additionally, sugars, fatty acids, sterols and triterpenes composition of ethanolic extracts of *G. diffusa* purchased in the local market, from three different medicinal plants distributors, was determined by GC–MS.

Polymers of caffeic acid are the most abundant phenolic compounds, represented mainly by rosmarinic and salvianolic acids (Fig. 1) [1]. The ethanolic extracts are rich in sugars, fatty acids, sterols and triterpenes, quantitative differences being found between the different *G. diffusa* suppliers.

The chemical richness of this matrix justified the study of its biological activities. Thus, *G. diffusa* aqueous and ethanolic extracts showed a potent ability to inhibit *α*-glucosidase (Fig. 2), in addition to good antiradical activity. Nevertheless, scarce acetylcholinesterase inhibitory capacity was observed.

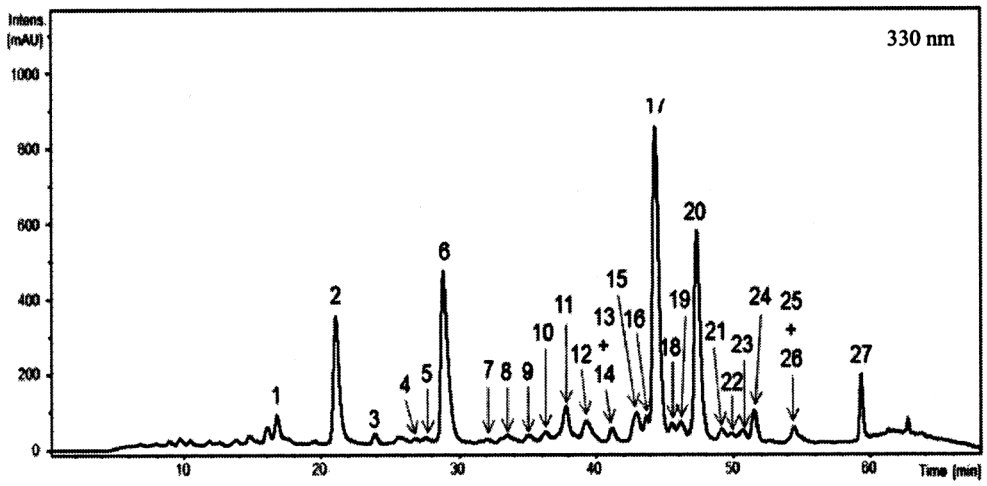

Figure 1. HPLC-UV phenolic profile of aqueous extract from *G. diffusa* aerial parts. (1) Caffeic acid; (2) Salvianolic acid H; (3) *p*-Coumaric acid; (4) Quercetin-3-*O*-(2,6-di-rhamnosyl)galactoside; (5) Quercetin-3-*O*-(2,6-di-rhamnosyl)glucoside; (6) Salvianolic acid E isomer; (7) Salvianolic acid I; (8) Kaempferol-3-*O*-(2,6-di-rhamnosyl)hexoside; (9) Kaempferol-3-*O*-(2-rhamnosyl)galactoside; (10) Kaempferol-3-*O*-(2-rhamnosyl)glucoside; (11) unknown; (12) Quercetin-3-*O*-(6-rhamnosyl)glucoside; (13) Salvianolic acid A isomer; (14) unknown; (15) Lithospermic acid isomer; (16) Salvianolic acid. E; (17) Rosmarinic acid; (18) unknown; (19) unknown; (20) Salvianolic acid B; (21) Kaempferol-3-*O*-(6-rhamnosyl)hexoside; (22) unknown; (23) Isorhamnetin-3-*O*-(6-rhamnosyl)hexoside; (24) unknown; (25) Methyl rosmarinic acid; (26) Salvianolic acid E isomer; (27) Salvianolic acid C isomer.

Our results point to the potential interest in adding *G. diffusa* aqueous and ethanolic extracts to both food supplements and pharmaceutical formulations, since it can suppress hyperglycemia and increase human antioxidant protection.

References: [1] Ferreres F., et al. *Food Chem.*, 2013, 136, 1390-1398.

Poster #18

HPLC-DAD-ESI/MS^n screening of bioactive compounds in *Lycopersicon esculentum* Mill. leaves

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High-performance liquid chromatography-diode array detection-electrospray ionization multi-stage mass spectrometry (HPLC-DAD-ESI/MS^n) is considered to be a very valuable tool for the characterization of compounds found in trace amounts in natural matrices. *Lycopersicon esculentum* Mill. leaves, usually considered as a by-product of tomato production, present several bioactive compounds, namely phenolics and alkaloids.

Using HPLC-DAD-ESI-MS^n we were able to characterize 15 phenolic compounds in *L. esculentum* leaves, nine of them reported for the first time (**Fig. 1**). Some differences were found between leaves of "cherry" and "bull's heart" varieties. α-Tomatine was the main glycoalkaloid.

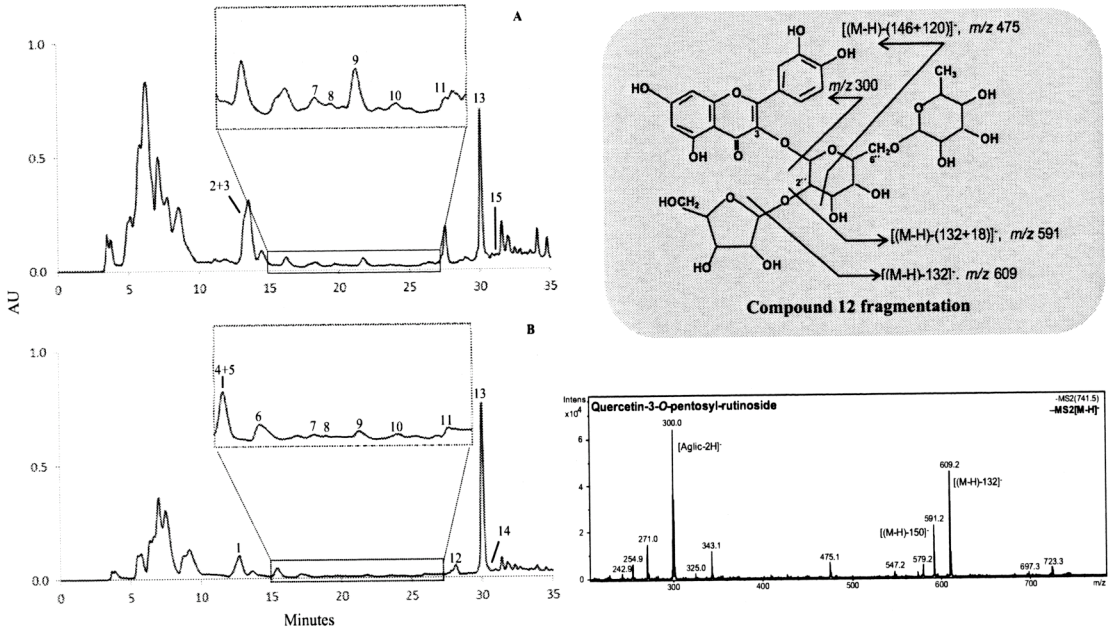

Figure 1. HPLC chromatogram (330 nm) of phenolic compounds in *L. esculentum* leaves and quercetin-3-*O*-pentosyl-rutinoside fragmentation. (A) "cherry" and (B) ''bull's heart'' varieties. Peaks: (1) 3-*O*-caffeoylquinic acid; (2) caffeoyl-hexoside acid; (3) *p*-coumaroyl-hexoside acid; (4) feruloyl-hexoside acid; (5) sinapoyl-hexoside acid isomer; (6) 5-*O*-caffeoylquinic acid; (7) *p*-coumaroylquinic acid; (8) sinapoyl-hexoside acid; (9) 5-*O*-feruloylquinic acid; (10) *p*-coumaroylquinic acid isomer; (11) feruloylquinic acid isomer; (12) quercetin-3-*O*-pentosyl-rutinoside; (13) quercetin-3-*O*-rutinoside; (14) sinapoyl derivative; (15) kaempferol-3-*O*-rutinoside.

The antioxidant capacity of the hydroalcoholic extract was evaluated against DPPH, superoxide and nitric oxide radicals, revealing promising results. Thus, tomato leaves extracts can be regarded as an alternative to synthetic preservatives in the food industry and also potentially used as food supplement or source of bioactive compounds. As so, a profitable use can be given to this discarded material.

Acknowledgments: M. Taveira is indebted to FCT for the grant (SFRH/BD/62662/2009).

Poster #19

Gene expression of different genes related with anthocyanin biosynthesis in blueberry

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Blueberry (*Vaccinium* spp) belongs to Ericaceae family, Vaccinoideae subfamily and *Vaccinium* genus. In last years, highbush blueberry production and consumption is increasing in the world, including Portugal. This fruit has a significant value because the exotic flavour, its economic value and their richness in nutrients, namely vitamin C, several minerals and anthocyanins. These nutrients were implicated in a wide range of health benefits. They are considered as a "longevity source", that can be explained especially due to the high anthocyanins content being, consequently, an excellent source of antioxidants.
The main objective was to identify and determine gene expression differences between genes related with anthocyanins biosynthesis in different maturation stages of blueberry fruit.
RNA extraction from 'Bluecrop' blueberry cultivar fruits in three different maturation stages was done using the RNaqueos *kit* (Ambion) and cDNA synthesis performed with High Capacity cDNA Reverse Transcription kit (Applied Biosystems). We selected two reference genes (Actin and GAPDH) and five genes related with anthocyanin biosynthesis (CHS, ANS, ANR, F3'5'H and UFGT) for amplification to confirm the gene expression.
The two reference genes and the five anthocyanins related genes amplified according to expected size. The two selected reference genes presented a constant expression level and did not differ across the maturation stages, as expected. The results allow to identify differences in the ANS, ANR and F3'5'H gene expression between the maturation stages.
The next step is to perform a quantitative real-time Q-RT-PCR to understand and analyze the anthocyanins biosynthesis gene expression profiling in highbush blueberry.

Poster #20

Nanoparticles Interactions with Plants

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Nanoparticles (NP) are considered to be the building blocks for nanotechnology, and are referred to particles with at least one dimension <100 nm. Due to their antimicrobial, catalytic and light emitting properties there are an increasing number of applications being developed in various fields, such as electronic, biomedical, pharmaceutical, cosmetic, energy, environmental, catalysis, and materials science. As nanotechnology is an innovative scientific growing area, more information is needed concerning the impacts of NPs in the environment and, particularly, in animals/humans health and in plants performance. Furthermore NPs when compared to their individual components may present also different toxicity profiles. The effects of NPs have been described in a wide variety of organisms, such as microorganisms, protozoa, invertebrates, and vertebrates. However, interactions of NPs with plants have been poorly studied, remaining unclear the general consequences of NPs exposure for plant cells. This lack of knowledge leads to a defective understanding of how NPs are transferred and accumulate in the various food chain levels. On this study we will describe some of the most relevant studies on NPs toxicity in plants.
Acknowledgments: This work was supported by COMPET program/FCT (Fundação para a Ciência e Tecnologia) with the project PTDC/AAC_AMB/113649/2009. FCT also funded the fellows of Helena Oliveira (SFRH/BPD/48853/2008) and Miguel Oliveira (SFRH/BPD/74868/2010).

Poster #21
***In vitro* DNA repair assay of human osteoblasts exposed to cadmium**

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Cadmium (Cd) is a known carcinogenic compound that may cause DNA damage in particular oxidative damage. In order to prevent the occurrence of mutations, DNA damage can be removed by repair enzymes. DNA repair capacity has been associated with the susceptibility to develop cancer. Moreover, has been shown that Cd interferes with different DNA repair systems (e.g. base excision repair (BER)). Thus, the evaluation of DNA repair capacity when cells are exposed to Cd is of great interest.
DNA BER assay consists on the incubation of protein extracts from cells exposed to Cd with substrates of cells treated with the specific damaging agent methyl viologen which induces 8-oxoguanine lesions. The repair enzymes present in extracts will convert the oxidative damage in breaks increasing the degree of tail DNA.
An osteoblast like cell line MG-63, was exposed to different Cd concentrations (20, 50 and 65 µM) for 48h. The slides were analyzed by visual scoring of 100 comets per duplicate gel, on a scale of 0-400 arbitrary units. As preliminary results, 50 µM of Cd showed the highest enhancement of DNA repair among the other Cd concentrations. However, further studies are needed to verify the DNA repair capacity when MG-63 cells are exposed to Cd.
Acknowledgments: This study was supported by Fundação para a Ciência e Tecnologia (FCT) through the fellows of Verónica Bastos (SFRH/BD/81792/2011), Cristina Monteiro (SFRH/BD/48204/2008) and Helena Oliveira (SFRH/BPD/48853/2008).

Poster #22
GMO: from its scientific concept to its environmental and economic impact

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Genetically modified organisms (GMOs), or transgenics, are organisms in which a trangene has been integrated into its genome. The development of robust protocols for genetic modification of plant/crops and its incorporation in agro-industry practices has become a powerful tool, and an example of agro-industry and biotechnology combination.
This assignment is based on a bibliographical research where several cases have been reported with respect to the implications of GMOs's release into the environment. These concerns relate to reductions of biodiversity, non-target interactions (NOT), variability/instability of the genome, pest resistance and economics. It is however, highlighted that the environmental and economic impacts of GMO remain a complex and controversial issue among scientists.
The aim of this presentation is to discuss the potential risks of the transgenic's release upon the environment, based on a systematic review of the most relevant scientific publications of the last decade.

Poster #23
Short-term climate change mitigation strategies for Mediterranean vineyards

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In Portugal the wine-grape sector has a crucial economic, social and cultural relevance, especially in Alto Douro Wine Region, which is the oldest viticultural legal region in the world. This region produces the world famous Port Wine and other remarkably good table wines. In recent decades the grapevine/wine sector has been modernized, to ensure a positive effect on the improvement of the productivity and wine quality. However, in the main viticultural areas, plants are often subjected to periods of severe drought associated with strong light and high temperature. Consequently, the vineyard experiences irreparable damage. As stress mitigation practices, some farms have invested in irrigation. However, given the high natural limitations in water resources is crucial to develop mitigation alternatives, not only in economic terms, but also in terms of grape quality and environmental sustainability. Among these mitigation alternatives, there has been a major effort undertaken by the scientific community to study the effect of inorganic substances in the improvement of light microclimate and water relations of leaves. The aim of this experiment was to evaluate the role of Kaolin (K) (protector agent of leaves during periods of strong light and heat) in plant protection against oxidative stress. *Vitis vinifera* plants with 2 years old were separated in 4 groups: a) plants watered at field capacity (FC) (control, C); b) C plants treated with 3% kaolin; c) plants watered at 1/3 of FC (water stress, WS); d) WS plants treated with 3% kaolin. After 3 weeks of treatment, proline content, MDA, Catalase and APX activity were determinate. Proline did not shown to be a sensitive stress biomarker in *V. vinifera*. Preliminary results indicated that kaolin application under WS conditions rise the levels of lipid peroxidation (MDA), but, also increased the antioxidant response (e.g. Catalase increase). Other parameters related to the antioxidant response (e.g. SOD activity and H_2O_2 content) should be performed in order to have a better knowledgement of the role of kaolin on the antioxidant system of *V. vinifera* under WS conditions.

Poster #24
Environmental Implications associated with Transgenics – an Overview

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Throughout the years, the transgenic technology has been developed in various fields. Its impact at different levels such as environment and human health has been largely discussed and is far from being consensual, making it an extremely controversial topic. Both scientists and the general public argue that there may not be sufficient studies and that the planning of those that are available may not have enough depth to give credibility to any decision. Nevertheless, transgenics are widely applied in such fields as agriculture, floriculture and the wood industry. Several studies mention advantages of transgenics use in improving the economy and mitigating the effects of global warming. Also, claimed benefits include an improved wood quality, resistance to stress and disease, and more appealing plants in terms of color, texture and format. On the other hand, claimed disadvantages range from the dissemination of transgenes to rupture of ecological equilibrium to unknown consequences in the long term. Here we review some of the scientific literature that supports both perspectives, with particular focus on the environmental aspects.

Poster #25

Dimeric naphthoquinones from *Diospyros chamaethamnus* Dinter ex. Mildbr. inhibit cholinesterases

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Alzheimer´s disease (AD) is a neurodegenerative disorder, characterized by a reduction of acetylcholine (ACh) in the brain. Inhibitors of cholinesterases (ChE), enzymes responsible for ACh hydrolysis, are one of the possible treatments in AD. At this moment, we know two ChE: acetylcholinesterase (AChE) – its activity is unchanged or even decreased in AD - and butyrylcholinesterase (BChE) – its activity is normally increased in AD [1]. In this work, we studied the AChE and BChE inhibitory activity of natural occurring naphthoquinones (NQ).

Tested NQ were menadione (MND), plumbagin (PLB), naphthazarin (NTZ), juglone (JGL), and the dimeric NQ, diosquinone (DQN) and diospyrin (DPR). Dimeric NQ were extracted from root barks of *Diospyros chamaethamnus* Dinter ex. Mildbr. The enzymes used were AChE and BChE from *Electrophorus electricus* (electric eel) and AChE from human neuroblastome cell line (SH-SY5Y). Galantamine was used as positive control.

The dimeric NQ were the more active compounds and for concentrations lower than galantamine. Inhibition induced by galantamine was (IC_{50}±SEM in µM): human AChE - 40.7±6.48; eel AChE – 5.37±0.41; eel BChE- 48.7±3.53. DQN was the only NQ with activity against human AChE (IC_{50}=32.8±2.4µM) and eel BChE. However, the maximal inhibition of BChE induced by DQN was 46.4±1.5%. DPR had the highest activity towards eel AChE (IC_{50}=5.01±0.65) (**Fig. 1**).

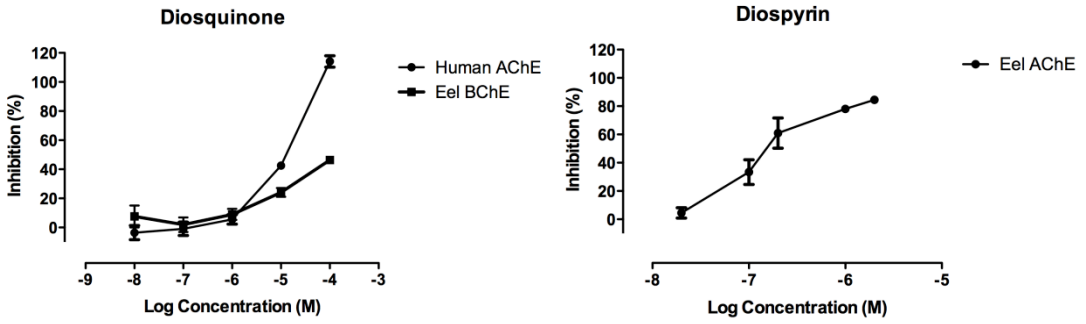

Fig.1. Cholinesterases inhibition by diosquinone and diospyrin. Data are shown as the mean±SEM (n=3).

The results obtained for human and eel AChE inhibition by DPR (higher activity for eel AChE and lower activity for human AChE) showed the importance to evaluate the ChE inhibition of human enzymes. DQN is a promising compound, since it has activity against both human AChE and eel BChE, whereby the next step is the study of the inhibition of human BChE by DQN.

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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Poster #26

Anti-allergic properties of natural naphthoquinones: Menadione inhibits leukotrienes production and naphthazarin inhibits degranulation

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Allergy is an abnormal immune response against non-infectious environmental substances, named allergens. Allergic disorders affect roughly 25% of people in the developed world and may induce long-term changes in the structure and function of the affected organs. As so, there have been efforts to search additional anti-allergic drugs or strategies in order to induce immunological tolerance to allergens [1]. In this work, we explored the anti-allergic properties of six natural occurring naphthoquinones (NQ).
We evaluated the hyaluronidase and soybean lipoxygenase inhibition (enzymes involved in allergic response) by NQ, using *in vitro* assays. The ability of NQ to inhibit mast cell degranulation was assessed by quantification of histamine and β-hexosaminidase release after stimulation of basophilic leukemia cell line (RBL-2H3) with calcium ionophore (A23187) or with antibody-antigen complex. The lipoxygenase inhibition was confirmed by quantification of leukotrienes produced by antibody-antigen stimulated RBL-2H3 cells. Tested NQ were both commercially available [menadione (MND) and juglone (JGL)] and extracted from root barks of *Diospyros chamaethamnus* Dinter ex. Mildbr. [plumbagin (PLB), naphthazarin (NTZ), diosquinone (DQN) and diospyrin (DPR)].
Several NQ showed lipoxygenase inhibitory activity (IC_{50}±SEM in μM): DPR - 30.3±2.7 < DQN - 90.3±7.2 < MND - 139±5. DQN was the only one that totally inhibited the lipoxygenase activity (100% of inhibition) at 192 μM (**Fig. 1**). However, only MND was able to decrease leukotrienes production by stimulated cells. No NQ had ability to inhibit hyaluronidase. NQ were not able to avoid or reduce the degranulation induced by calcium ionophore; nevertheless, NTZ, at 0.1uM, decreased the β-hexosaminidase release when antibody-antigen complex was used as stimulus.

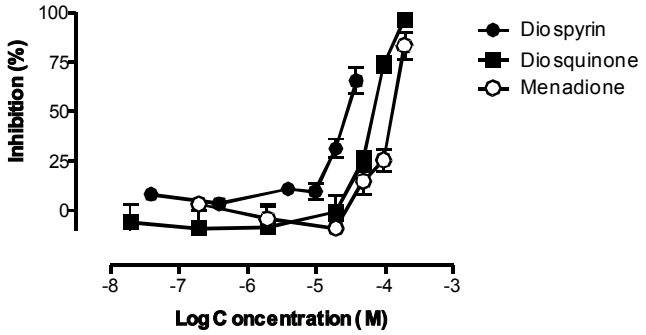

Figure 1. Lipoxygenase inhibition by diosquinone, diospyrin, and menadione. Data correspond to mean±SEM (n=3-5).

Dimeric structure of NQ seems to favor the lipoxygenase inhibition, since DPR and DQN were the most active NQ in lipoxygenase inhibitory assay. However only menadione induced a decrease of leukotrienes production by stimulated RBL-2H3 cells, probably due to structural differences among soybean lipoxygenase and mammal 5-lipoxygenase. Attending to inhibition of degranulation, NTZ is a promising NQ with anti-allergic properties.

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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Poster #27
Transgenics in food safety: an update of negative implications

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Nowadays, the implications of genetically modified organisms (GMO) in our society and environment still remain controversial within the scientific community. Here we discuss some relevant data concerning GMO's impacts on food safety. For example, a literature review supports that some aspects of biosafety policies remain unclear to the large public. Also some research papers highlight that GMO may be at the basis of allergenic reactions in animals and humans. Moreover, other scientific data points at a correlation between GMOs and the development of diseases (e.g, development of tumors, hepatic and renal toxicity, as well as growth changes). The available data reporting allergenic potential of GMOs combined with an increasing feeling of unclearly addressed policies of products labeling is becoming a primary concern of consumers (e.g., SERC 2005). We highlight here some case studies: for example the impact of a GMO-based diet on mice survival and body weight. This and other studies raised the concern of several advisory committees and the large public (eg, German Journal of Consumer Protection and Food Safety). Finally we highlight some concerns reported by other authors about the GMO putative risks and advances using animal feeding authors (e.g., Flachowsky et al. 2012).

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Poster #28
Evaluation of molecular polymorphism in selected *Pinus* genotypes by microsatellite markers

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The genus *Pinus* from *Pinaceae* family comprises about 100 taxonomically distinct species. *P. elliottii var. elliottii x P. caribaea var. hondurensis* is a *Pinus* hybrid that combines the enhanced growth characteristics of parent *P. caribaea var. hondurensis* with the improved wood density of parent *P. elliottii var. elliottii*. These species have important ecological and economic value for wood and resin industry. Various molecular markers can be found linked with genes controlling traits, which renders these markers relevant predictors of plant phenotype. In addition, many specific molecular markers may provide insight into the genetic stability of plant individuals that are regenerated from a single original clone, since stress conditions in tissue culture techniques could also be responsible for the DNA changes observed in micropropagated plants. The purpose of this study is to analyze hybrids and parental genotypes and to assess genetic stability of plants regenerated by in vitro culture methods, using microsatellite markers. For this, a set of available nuclear microsatellite markers for *Pinus species (P. taeda, P. strobus, P. radiata, P. caribaea, P. elliottii, P. elliotti x P. caribaea)* will be analyzed. These microsatellites are predicted to be located at different genetic loci and to be discriminative. Our final goal is to identify single microsatellite markers or combinations of these which distinguish different genotypes, e.g. for the assessment of daughter plant genetic stability after regeneration or for phenotype prediction.

Poster # 29

Analysis of the bioactivity of flavonoids in different osteosarcoma cell lines

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More than 4000 unique flavonoids have been identified in plant sources. These molecules are found in fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers, as well as tea and red wine. They are prominent components of citrus fruits and other food sources and are consumed regularly with the human diet. They are usually subdivided according to their substituents into anthocyanidins, flavonols, flavones, flavanols, flavanones, isoflavone, chalcones, dihydrochalcones and dihydroflavonols.
Flavonoids have been shown to demonstrate diverse biological activities and epidemiological studies suggest that a diet rich in vegetables and fruit protects against chronic diseases such as cancer, cardiovascular and neurodegenerative diseases due to their antioxidant properties. Human clinical trials also indicate that flavonoids have important effects on cancer chemoprevention and chemotherapy in diverse cell systems.
In this work we are interested in investigating the role of phytochemicals as potential chemicals against osteosarcoma (OS). OS is a lethal form of muscle skeletal cancer which develops from the cells responsible for forming the bone matrix. It is the most common type of primary bone cancer in children and adolescents, with approximately 1-3 cases per million annually, and 70-75% of patients being 10 to 25 years of age.
As a first experimental approach, pre-screening of flavonoids such as, Fisetin, Hesperetin, Naringenin, and Prunetin will be performed both in OS cell lines (MG-63, Saos-2 and U2OS) and in normal human osteoblasts (hFOB3.1). The pre-screening will consist of assays on cell viability (MTT). After pre-screening, analysis will be conducted on the cell cycle, the proportion of nuclear aberrations (CBMN assay), and cell migration (scratch assay). The osteosarcoma cell lines most responsive to the selected flavonoids will be analysed in depth for future studies as therapeutic agents.

Poster # 30

Sulforaphane present in plants induced antioxidant defenses decrease in human osteosarcoma cell line

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Today is used medicinal plants and bioactive phytocompounds worldwide. The discovery of the curative properties of certain plants must have sprung from instinct, since primitive peoples already used certain plants as food and as therapeutic drug. In the early 1980s, was a resurgence of interest in the use of natural substances, such as phytocompounds presents in vegetables namely Sulforaphane (SFN). This interest can be easily understood in the light of questions concerning the safety, cytotoxicity, and side-effects of synthetic compounds, and the need to find new medicines, including new substances to treat diseases such cancer [1].
SFN is an isothiocyanate mainly found in Brassicaceae such as broccoli and a compound extensively studied for its putative anticancer properties [2]. This compound has been used in diverse types of cancers such as Osteosarcoma (OS)
OS is the most common bone sarcoma and is most frequently observed in children [3,4]. Diverse studies have been developed to study OS and the natural bioactive compound SFN is a potential candidate for OS therapy. However, the effects of SFN are still poorly understood in OS cell lines.
In this work our goal was to study the oxidative stress effects of SFN in the MG-63 OS cell line. For this, *in vitro* cultured MG-63 cell line was exposed to increasing concentrations (0 to 20 µM) of SFN for 24 and 48 h. Afterwards, the enzymatic activities was analyzed, namely glutatione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) activities. Enzyme activity was analyzed by different colorimetric assays.

SFN treatment was associated with decreased enzyme activities for GPx, GR and SOD in a concentration and time-dependent manner. For the higher SFN concentration tested i.e. 20 µM, almost no GR and GPx activity was observed. This suggests that SFN decreases the antioxidant defenses in osteosarcoma cells. Since SFN decreases osteosarcoma cell defenses against oxidative damage, it is a potential drug for chemoprevention and chemotherapy in this type of cancer.

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Poster #31

Transient transformation of *Arabidopsis* seedlings by vacuum infiltration: a fast and simple method for cardosins expression

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In our lab, cardosins trafficking studies (Duarte *et al*. 2008; da Costa *et al*. 2010) have been relying on several heterologous systems such *Nicotiana tabacum*, Bright-yellow-2 (BY-2) tobacco suspension cells and *Arabidopsis thaliana* stable constitutive expression. In addition to this stable expression in *Arabidopsis thaliana*, a high-throughput method for transient expression in Arabidopsis seedlings has been optimized in our lab. The main advantage of this system is the rapid screening of protein fusions, the transformation of several seedlings at the same time with different constructs and the easiness in applying drugs or blockage agents. The protocol was established by Marion and co-workers (Marion *et al*., 2008) and here we present the optimization of the method in the context to the cardosins studies using a plasmid of fluorescence-accumulating seed technology (pFAST-G with OLE1-GFP) (Shimada *et al*., 2010) for *Arabidopsis* optimal expression. This protocol uses conventional binary vectors and a conventional *Agrobacterium* strain, and is compatible with a large diversity of fluorescent reporters. Therefore, it is a powerful pre-screening and characterization assay before stable transformation. Transient expression in *Arabidopsis* seedlings is thus a fast and simple method that requires minimum handling and potentially allows medium-to high-throughput live imaging analyses of chimeric proteins subcellular localisation in *Arabidopsis* cotyledons.

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Shimada T. L., Shimada T. and Hara-Nishimura I. (2010) A rapid and non-destructive screenable marker, FAST, for identifying transformed seeds of *Arabidopsis thaliana*. *The Plant Journal*, 61: 519–528.
Da Costa D.S., Pereira S., Moore I. and Pissarra J. (2010). Dissecting cardosin B trafficking pathways in heterologous systems. *Planta*, 232(6), pp.1517-30.
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MAP Presentations

MAP-OP #1 .
Cloning, expression and localization of VvSIP1 and functional studies of the purified protein in artificial phosphatidylethanolamine liposomes

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Univ. Minho, Braga

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). Here, we describe the localization, expression and functional characterization of a VvSIP from the grape berry. VvSIP1 is expressed in the early stages of berry development, and colocalizes at the ER in transformed yeast cells with a 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.

MAP-OP #2
Characterization, micropropagation and preservation of *Pinus* genotypes

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Slash Pine (*Pinus elliottii var elliottii*), Caribbean Pine (*Pinus caribaea var hondurensis*) and the resulting hybrid (*Pinus elliottii var elliottii x Pinus caribaea var hondurensis*) have a great economic value due to their high growth ratio and resin production. Therefore, it is important to achieve a strategy to propagate these selected genotypes more rapidly maintaining their characteristics. This project aims to preserve/enlarge the *Pinus* germplasm collection, provided by the company Klón, by micropropagation and cryopreservation techniques; to characterize genetically these genotypes and the putative changes of the micropropagated plants by flow cytometry; to study plant survival rates, growth and photosynthetic performance during acclimatization.

MAP-OP #3
Genetic characterization of *Pinus* genotypes with high oleoresin production: molecular and functional studies

Diana Sousa (MAP-PhD Student)
José Miguel Oliveira*, Vanessa Pereira* Conceição Santos* (*supervisors)
Department of Biology University of Aveiro

The genus *Pinus* from *Pinaceae* family comprises about 100 taxonomically distinct species. *P. elliottii var. elliottii* x *P. caribaea var. hondurensis* is a *Pinus* hybrid that combines the enhanced growth characteristics of parent *P. caribaea var. hondurensis* with the improved wood density of parent *P. elliottii var. elliottii*. These *Pinus* species industrial value relies mostly on wood and oleoresin production. We'll use these genotypes/populations previously selected as not-improved and as excellent resin producers. Selected genotypes/populations will be characterized for their variation: a) intraspecific and interspecific through polymorphisms in microsatellites; b) in transcriptional regulation of genes involved in MVA pathway (eg.,HMGR,MDC) and MEP pathway (eg.,DXS,DXR,HDR) among:

i) genotypes/populations;
ii) different organ/tissues (eg.,needles,wood,root)
iii) considering propagation type [in vivo vs. in vitro conditions (eg., calli, and micropropagated vs. zygotic derived-plants)].

The genetic characterization of populations of these species and the hybrid is very important, both to distinguish as to assist in the certification the populations and to certificate regenerants derived from micropropagation and cryopreservated regenerants. Moreover, the identification of genetic functional markers of resin is of extreme importance.

MAP-P #4
Flavonoids bioactivity in different osteosarcoma cell lines

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José M.P.F.Oliveira (main supervisor), Conceição Santos (co-supervisor)
Department Biology , University Aveiro

Phytochemicals may prevent tumour proliferation by modulating critical cellular pathways, such as those involved in the cell cycle and apoptosis.
In particular for osteosarcoma (OS), specific molecular targets of phytochemicals remain unclear.
We hypothesize that different classes of phytochemicals have different effects on apoptosis, on the cell cycle and metabolism in OS cell lines vs. normal osteoblasts. These effects may be modulated by epigenetic regulation, e.g. through the action of micro RNAs (miRNAs).
Using in vitro OS vs. normal osteoblast-cell systems, our project will focus on critical steps of apoptosis, cell cycle pathways and metabolism regulated by phytochemicals, from the point of view of miRNA regulation. Data will open perspectives for targeting multiple signalling pathways that are active in OS

MAP-P #5
The response to stress of *Melia azedarach*

Maria da Costa (MAP-BioPlant Student)
Gloria Pinto*, Conceição Santos* (supervisors)
Department of Biology and CESAM, University Aveiro

Abstract not available

MAP-P #6
The effect of high temperature on sugar transport in grape cells.

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Sugar status is directly related to the final alcoholic content of wine, and regulates the development of its aromatic and organoleptic properties. High temperatures affect berry set and development and alter the normal sugar content of the fruit. Peaks of high temperature, nowadays more and more frequent, may stop the ripening progress. We have been exploring the mechanisms involved in sugar import and compartmentation into the berry. In particular, the regulation by sugars of the expression of the monosaccharide transporter VvHT1 was investigated at transcriptional, translational, and protein activity levels. Recently we have found that this protein is down-regulated by high temperature, through a decrease of the protein amount in the plasma membrane that correlates with the observed decrease in the uptake rates of radioactive glucose. We aim at the unlocking of the biochemical/molecular mechanisms behind the regulation of grape berry sugar status by absolute temperature and day/night temperature differences.

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