Programa Inter-Universitário de Doutoramento
em Biologia de Plantas Fundamental e Aplicada

2º WorkShop Anual / *Annual*

18 e 19 de Abril de 2011 / *April 18th and 19th, 2011*

Universidade do Minho / *University of Minho*

PROGRAMA / PROGRAMME
Livro de resumos / *Book of abstracts*

Universidade do Minho

universidade de aveiro

U.PORTO

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Bem-vindos ao *2º Workshop Anual* do Programa Inter-Universitário de Doutoramento em Biologia de Plantas (BioPlant), um projecto de formação de 3º ciclo que reúne esforços de docentes e investigadores de três Universidades (Minho, Aveiro e Porto). Este encontro, ao promover a interacção entre investigadores seniores, universitários e do mundo empresarial, com os que se encontram a iniciar a sua carreira científica, constitui um local privilegiado de partilha de conhecimento e de experiências em várias áreas de interesse científico.

Este ano, o Workshop tem como tema principal a produção e utilização de energias alternativas. Pretende-se abordar formas de como o conhecimento em Biologia Vegetal pode ser utilizado na produção e utilização de materiais de origem vegetal como fonte renovável de obtenção de energia (e.g. biofuel) e evidenciar o seu contributo positivo para a manutenção do equilíbrio ambiental e para a economia. Nesta edição do Workshop serão também abordados outros temas actuais e de interesse em Biologia Vegetal, sendo o programa completado pela apresentação de comunicações orais de curta duração proferidas por jovens investigadores.

Welcome to the 2nd Annual Workshop of the Joint Doctoral Programme in Plant Biology (BioPlant), a training project of 3rd cycle that brings together the efforts of professors and researchers from three universities (Minho, Aveiro and Porto). This meeting promotes the interaction between senior researchers, academics and businessmen, with those that are starting their scientific career, and constitutes a privileged place for sharing knowledge and experiences in various areas of scientific interest.

This year the Workshop has as the main theme the production and use of alternative energies. It is proposed to discuss the ways the plant biology knowledge can be used in the production and use of plant materials as renewable energy sources (e.g. biofuel) and highlight their positive contribution to the maintenance of environmental balance and to economy. This edition of the Workshop will also discuss other topics of current interest in plant biology, being the programme completed by the presentation of short oral presentations made by young researchers.

Alberto Dias
Director do BioPlant 2010/2011

Organização da WorkShop / *Organization of the workshop*

Alberto Dias - Departamento de Biologia, UM
Teresa Lino Neto - Departamento de Biologia, UM
Carmencita Lino - EPALMO

Comissão Directiva BioPlant / *Executive Committee BioPlant*

Alberto Dias - UM (Director do BioPLant 2010/2011)
Conceição Santos - UA
Mariana Sottomayor – UP

Comissão Científica BioPlant / *Scientific Committee BioPlant*

Alberto Dias - UM (Director da CC 2010/2011)
Conceição Santos – UA
João Serôdio - UA
Mariana Sottomayor – UP
Paula Melo - UP
Teresa Lino Neto - UM

Apoios / *Support*

O BioPlant tem o privilégio de usufruir de um financiamento da Fundação Calouste Gulbenkian ao abrigo do seu Programa de Reforço da Capacidade Científica para Projectos Inter-Universitários de Doutoramento, obtido em concurso nacional em que apenas dois programas foram subsidiados. Este financiamento dotou o BioPLant com 180.000 EUR destinados a financiar exclusivamente despesas de funcionamento.

Instituições Promotoras / *Promoting Institutions*

Departamento de Biologia da Universidade do Minho (DB-UM)
Instituição de acolhimento 2010/2011
Departamento de Biologia da Universidade de Aveiro (DB-UA)
Departamento de Biologia da Faculdade de Ciências da Universidade do Porto (DB-FCUP)

Programa / *Programme*

18 Abril / *April 18th*

09:00 Registo e entrega de documentação / *Registration and documentation delivery*

09:30 **Cerimónia de Abertura /** *Opening Ceremony*
Coro Académico da Universidade do Minho / *Academic Choir from University of Minho*
Prof. Doutor Alberto Dias, DB-UM/CITAB, Director do Bioplant

10:00 ***Design and engineering of photosynthetic cell factories for direct biofuel production***
Prof. Doutor Peter Linbdlad, Department of Photochemistry and Molecular Science, Sweden Uppsala University

11:00 Coffee Break e/ *and* Posters

11:30 ***Multiple energy vectors from algae processing***
Prof. Doutor Klaus Hellgardt - Imperial College, UK

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

14:30 **Implementação de sistemas de Bioenergia: integração dos resíduos com a agricultura**
Prof. Doutor Santino Eugénio Di Berardino - LNEG, Unidade de Bioenergia, Lisboa

15:30 **O projecto dos biocombustíveis na Galp Energia/** ***Galp Energia's Biofuels Project***
Prof. Doutor Fernando Bianchi de Aguiar - Unidade de Desenvolvimento de Biocombustíveis da GALP ENERGIA

16:00 **Algae for Energy and CO2 mitigation**
Doutora Joana Silva - Necton, S.A./AlgaFuel, S.A.

16:30 ***Feedstock purification for biodiesel transesterification***
Dr. Manuel Baltazar Barreto Vasconcelos, BB-DIESEL, SA.

17:00 ***Overproduction of fatty acids in Saccharomyces cerevisiae***
Prof. Doutor Björn Johansson - Departamento de Biologia da Universidade do Minho / CBMA

17:30 **Seaweed mariculture for biofuels**
Professora Doutora Isabel Sousa Pinto - CIMAR, Faculdade de Ciências, Universidade do Porto

17:45 Posters

19 Abril / *April 19th*

09:30 **Regulação do crescimento polarizado do tubo polínico através da modulação de endocitose e secrecção membranar/** ***The regulation of pollen tube growth and polarity through modulation of endocytosis and membrane secretion.***
Prof. Doutor Rui Malhó - BioFIG, Universidade de Lisboa, Faculdade de Ciências de Lisboa

10:30 **Characterisation of a new family of MYB-like transcription factors and its role in flower asymmetry**
Dr. João Raimundo - BioFIG, Departamento de Biologia, Universidade do Minho

10:45 ***Bio-waste strategy. Compost - product quality & applications***
Engª Susana Lopes - LIPOR – Serviço Intermunicipalizado Gestão Resíduos, Porto

11:15 Coffee Break e / *and* Posters

12:00 **Analysis of DNA damage and repair in Saccharomyces cerevisiae using the comet assay in the characterization of antigenotoxicity of plant extracts and phytochemicals**
Prof. Doutor Rui Oliveira – Departamento de Biologia, Universidade do Minho / CBMA

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

14:30 **Climate Change: Using Modeling to Predict Potential Effects on Crops**
Prof. Doutor João A. Santos - Universidade de Trás-os-Montes e Alto Douro / CITAB

15:30 **Barcoding of entomopathogenic fungi from olive tree pests: prospects and limitations**
Dr. Ivo Oliveira - CIMO, Instituto Politécnico de Bragança

15:45 **The pollen content of a river beach for forensic purposes**
Drª. Áurea Carvalho- Ambiente e Sociedade, Centro de Geologia da Universidade do Porto

16:05 **Inorganic matter associated to airborne pollen: characterization by Electron Microprobe Analysis (EMPA)**
Drª. Laura Duque - Ambiente e Sociedade, Centro de Geologia da Universidade do Porto

16:20 **Anti-inflammatory and antimicrobial activities of phlorotannins purified extracts from brown seaweeds collected in the Portuguese coast**
Drª. Graciliana Lopes - REQUIMTE, Faculdade de Farmácia da Universidade do Porto

16:35 **Evaluation of the protective effect of *Hypericum perforatum* phenolics compounds, in the toxicity induced by heterologous expression of α-synuclein**
Drº Pedro Vieira - Departamento de Biologia, Universidade do Minho

17:00 Coffee Break e / *and* Posters

17:30 Analysis of ROS production and homeostasis in an *Arabidopsis thaliana* knockout mutant involved in post-translational modification
Drº Daniel Couto - BioFIG, Departamento de Biologia, Universidade do Minho

17:45 Airborne and Allergenic Patterns of *Dactylis* and *Plantago* Pollen: A Comparative Study in Porto
Drª.Raquel Sousa **-** Ambiente e Sociedade, Centro de Geologia da Universidade do Porto

18:00 Endpoints of cadmium cytotoxicity, genotoxicity and clastogenicity in lettuce (*Lactuca sativa* L.)
Drª.Cristina Monteiro - Departamento de Biologia, Universidade de Aveiro / CESAM

18:20Sessão de encerramento / *Closing Session*

Resumos das Comunicações Orais / *Abstracts of Oral Communications*

O1 *Design and engineering of photosynthetic cell factories for direct biofuel production*

Peter Lindblad- Department of Photochemistry & Molecular Science, Microbial chemistry, Uppsala University, Box 523, SE-751 20, Uppsala, Sweden. E-mail Peter.Lindblad@fotomol.uu.se / +46 70 425 0498 / Skype: peter.lindblad3

There is an urgent need to develop sustainable solutions to convert solar energy into energy carriers used in the society. In addition to solar cells generating electricity there are several options to generate solar fuels. The presentation will outline and discuss the design of natural and artificial photosynthetic factories for direct generation of biofuels from solar energy. Specific focus will be on the engineering of cyanobacterial cells, using a standardised synthetic biology approach, to be developed as photosynthetic cell factories for direct biofuel production.

Agervald, Zhang, Stensjö, Devine, Lindblad. 2010. CalA, a CyAbrB protein, interacts with the regulatory region of *hyp*C and acts as a repressor of its transcription in the cyanobacterium *Nostoc* sp. strain PCC 7120. Applied and Environmental Microbiology 76: 880-890.

Angermayr, Hellingwerf, Lindblad, Joost Teixeira de Mattos. 2009. Energy biotechnology with cyanobacteria. Current Opinion in Biotechnology 20: 257-263.

Dasgupta, Gilbert, Lindblad, Heidorn, Borgvang, Skjånes, Debabrata. 2010. Recent trends on the development of photobiological processes for the improvement of hydrogen production. International Journal of Hydrogen Energy 35: 10218-10238.

Heidorn, Camsund, Huang, Lindberg, Oliveira, Stensjö, Lindblad. 2011. Synthetic Biology in Cyanobacteria: Engineering and Analyzing Novel Functions. Methods in Enzymology 497: in press. Editor Voigt. ISBN: 978-0-12-385075-1.

Magnuson, Anderlund, Johansson, Lindblad, Lomoth, Polivka, Ott, Stensjö, Styring, Sundström, Hammarström. 2009. Biomimetic and Microbial Approaches to Solar Fuel Generation. Accounts of Chemical Research 42 : 1899-1909.

O2 *Multiple energy vectors from algae processing*

K. Hellgardt, G. Maitland, F. Zemichael, B. Tamburic, B. Patel
REaCT Group, Department of Chemical Engineering, Imperial College London, UK

3rd generation biofuels based on algae systems appear to be emerging as an interesting alternative to other sources of biofuel.

This is mainly due to the potentially high theoretical yields available combined with the relatively low cost of cultivation (marginal land, salt water etc.).

A number of energy vectors can be derived from algal systems including hydrogen, lipids and biomass. In this presentation, these three energy vectors will be explored and the opportunities and shortcomings associated with their amplification and utilisation will be identified. The main discussion will centre around the science and technology of hydrogen production in this context. Lipid production and transformation as well as biomass gasification will be mainly evaluated from an engineering point of view.

O3 Implementação de sistemas de Bioenergia: integração dos resíduos com a agricultura

Doutor Santino Eugénio Di Berardino)- Unidade de Bioenergia, Laboratório Nacional de Energia e Geologia I. P., LNEG Lisboa. *Email:* Santino.diberardino@lneg.pt

A evolução da nossa sociedade está cada vez mais dependente duma mudança do uso dos recursos naturais e do estilo de vida, que deverá ser baseada numa maior parcimónia e eficiência, na redução de emissões de gases com efeito estufa, na promoção de fontes de energias renováveis. Hoje em dia já se reconhece que os resíduos da nossa civilização, outrora encarados como fonte poluidora e colocados sem proveito em aterro, possuem um valioso potencial químico, energético e fertilizante que, pode ser aliado a culturas agrícolas, quer alimentares, quer energéticas, fechando o ciclo dos nutrientes e proporcionando emprego e desenvolvimento local.

A recente directiva comunitária (Directiva 2009/28/EC) aponta direcções claras sobre a evolução energético-ambiental da Europa. Estabelece metas concretas para a substituição dos combustíveis tradicionais com fontes renováveis nos transportes e na produção de energia, para a redução emissões de CO_2 e para a eficiência da utilização dos recursos energéticos, constituindo um marco importante para a difusão de sistemas integrados de valorização energética ambiental dos resíduos.

Por outro lado a directiva comunitária 1999/31/EU sobre o destino final de lamas e resíduos orgânicos, transposta em 2006 para o direito nacional, restringe a disposição em aterro de resíduos orgânicos e lamas. Desta forma, hoje em dia, são ambientalmente aceites para os resíduos orgânicos apenas dois destinos finais: a termovalorização (incineração, gasificação e pirólise) e a aplicação no terreno, para fins de produção agrícola ou de protecção dos solos. A primeira, que proporciona a valorização térmica dos resíduos e, de acordo com novos processos térmicos, a produção de biocombustíveis, é uma solução geralmente viável em grande escala. A segunda pode ser aplicada em qualquer situação e dedicada a todo o tipo de culturas, podendo fomentar uma cadeia de produção e valorização da biomassa para fins industrias, alimentares e energéticos.

A reciclagem na agricultura adapta-se bem a sistemas mais pequenos e pode estimular a valorização de solos incultos ou pouco aproveitados, a produção de novas culturas agrícolas, a produção descentralizada de energia e, consequente a economia e emprego local. Enfim proporciona um novo modelo de desenvolvimento socioeconómico e, talvez, riqueza, dando início a uma cadeia bioenergética/biotecnológica, além de proteger recursos naturais e contribuir para diminuir a dependência dos combustíveis fósseis.

Os resíduos destinados à agricultura devem ser previamente tratados antes da aplicação, destacando-se o uso da digestão anaeróbia, uma tecnologia conhecida há mais de 150 anos, que produz o biogás, um combustível renovável com uso versátil, que pode gerar energia eléctrica ou substituir o gás natural, que pode alimentar os dispositivos mais diversos, nomeadamente as células a combustível, as turbinas a gás ou pode ser convertido em biometano.

Nesta última década a aplicação da digestão anaeróbia, inicialmente dedicada essencialmente a resíduos e lamas, tem visto alargar a sua aplicação, com a inserção de culturas energéticas, especialmente plantadas para o efeito, em alguns países da Europa, onde este conceito foi facilmente aceite e posto a funcionar (Biogas barometer 2008), beneficiando da disponibilidade de excedentes agrícolas, elevado preço dos combustíveis e tarifas de remuneração da energia eléctrica produzida, bastante favoráveis.

O resíduo digerido possui geralmente propriedades fertilizantes adequadas para a aplicação agrícola e até, possui propriedades superiores ao do substrato original, em termos de contaminação bacteriana e de disponibilidade dos compostos nutrientes, que se encontram num estado reduzido.

O biogás produzido por digestão anaeróbia a partir dos resíduos, é um importante vector energético potencial, reconhecido pelo Parlamento Europeu (resolução 2009/C 66 E/05 de 12 de Março de 2008):

"Constitui uma fonte de energia vital que promove o mercado energético renovável, a reciclagem de matérias-primas nutrientes das plantas e a redução das emissões, conduzindo, consequentemente, à protecção ambiental e climática, ao desenvolvimento rural e a novas perspectivas de rendimento".

Pode-se, assim, concluir que existem excelentes condições, quer legislativas quer tecnológicas, para a disseminação de sistemas de digestão anaeróbia no tratamento dos resíduos orgânicos do lixo, das indústrias agro-alimentares e da agropecuária, num esquema de integração agrícola com o território, procurando a valorização do potencial fertilizante do efluente digerido. Este esquema integrado proporciona energia, matérias-primas e fertilizantes. Pode dar origem à produção agrícola de biocombustíveis (álcool, biodiesel) e uma cadeia de materiais e bioenergia (conceito da bio-refinaria). Os resíduos da produção de biocombustíveis podem ser tratados pela Digestão anaeróbia, misturados ou não com outros resíduos (codigestão) e podem ser combinados com processos térmicos (pirólise ou gasificação) gerando mais combustíveis e energia térmica. Esta

Em suma pode surgir uma nova fileira industrial, baseada em novos produtos tecnológicos e em biocombustíveis que valoriza áreas territoriais deprimidas e pode criar lugares de trabalho.

De acordo com a recente experiência europeia, a digestão anaeróbia e a produção de energia têm sido a solução para tirar vantagens da agricultura e da gestão de resíduos. As culturas agrícolas associadas aos resíduos em codigestão, produzem consideráveis quantidades de biogás, contribuindo para reduzir as emissões de efeito estufa. Podem facilmente ser ensiladas, constituindo uma forma de armazenar energia e contribuir para o controlo da tensão da rede eléctrica.

As primeiras experiências de projectos de agricultura integrada com os resíduos usavam as culturas alimentares para a produção de biogás, resultando numa má e contestada solução. As práticas mais recentes estão direccionadas para a produção de culturas secundárias, geralmente as culturas de inverno, frequentemente deixadas sobre o solo, para adubação e controlo de pragas.

Tendo em conta as disponibilidades de áreas cultiváveis da C. E., estima-se, que o potencial de biocombustíveis se situa na gama dos 20-25 % do consumo nos transportes, se forem aplicados critérios de sustentabilidade no uso do terreno e na escolha das colheitas, e se for previsto o uso dos resíduos florestais, subprodutos e efluentes de agro-industriais e agro-pecuários e a fracção orgânica dos lixos domésticos. Estimativas recentes apontam que o contributo potencial da bioenergia na comunidade europeia em 18 % das necessidades energéticas, salvaguardando os efeitos sobre os solos e os ecossistemas naturais em que são produzidos. Um montante que não resolve as necessidades, mas constitui um contributo significativo. Em países da C. E. de pequena dimensão com clima favorável, tal como Portugal, esta percentagem poderá ser superior, tornando o País mais eficiente e menos dependente do exterior.

A agricultura é hoje a opção de destino final mais promissora para os resíduos orgânicos e a sua aplicação está devidamente regulamentada pelo recente decreto-lei, em Portugal, tornando a gestão dos resíduos e a agricultura interdependentes. Mas as práticas agrícolas devem ser adaptadas a esta oportunidade, a fim de poder receber mais resíduos e gerar colheitas valiosas.

O cultivo de terrenos com plantas herbáceas com resíduos digeridos, pode substituir vantajosamente as culturas de alto rendimento e ocupar e valorizar terrenos marginais. Estas culturas secundárias, definidas hoje "culturas energéticas", podem ser colhidas e utilizadas para a digestão anaeróbia, contribuindo com a sua absorção de nutrientes para acomodar mais resíduos no mesmo terreno, oferecendo um excelente e seguro destino final. Executando culturas nos ciclos de outono/inverno, em terrenos não utilizados entre os ciclos,a presença de biomassa vegetal oferece devida protecção contra o risco de erosão causada pela chuva e pelo vento. Em situações de inundações enchentes a terra cultivada propicia uma melhor absorção de água, reduzindo a anaerobiose no solo submerso e a consequente produção e libertação de

CH_4. A agricultura a praticar deve ser fundamentada em rigorosos critérios de sustentabilidade e respeito dos habitats naturais, assegurando também a permanência da biodiversidade.

Planeando o sistema em todas as suas vertentes, adoptando uma estratégia de investimento prudente e criando sistemas bem organizados, este sector poderá dar retorno económico moderado mas seguro e interessante, para além de contribuir para a evolução da nossa sociedade.

As soluções que integram a valorização energética e ambiental dos resíduos com base na Digestão Anaeróbia e práticas agrícolas "secundárias", proporcionam vantagens adicionais: o desenvolvimento rural, a oportunidade de emprego, o aumento da eficiência da agricultura, e podem constituir uma oportunidade de negócios e de desenvolvimento do meio rural. A tecnologia existente consegue satisfazer, vantajosamente, as aplicações práticas, existindo propostas inovadoras que poderão permitir maiores rendimentos.

Apesar de existirem muitas e evidentes potenciais vantagens estes sistemas sendo ainda pouco utilizados e têm dificuldades em avançar, em alguns países da Europa e em Portugal, pois existem barreiras técnicas, não técnicas e económicas, que dificultam a sua implementação e que devem ser detectadas e removidas. É necessário introduzir medidas que estimulem o interesse e a cooperação entre todos os actores (produtores de resíduos, agricultores, etc.), que devem dialogar uns com os outros.

No plano tecnológico, a implementação viável destes sistemas requer a disponibilidade de dados técnicos relevantes sobre as culturas agrícolas e a degradação de resíduos. É importante conhecer o rendimento da biodegradação e de produção de metano dos resíduos orgânicos envolvidos no projecto, na utilização como substrato único ou em codigestão (lamas, as chamadas "lamas equivalentes" e resíduos agrícolas de culturas energéticas). É essencial determinar o rendimento agrícola de culturas seleccionadas, cultivadas em campo experimental, no intuito de definir a espécie adequada e o crescimento, a composição, a absorção de nutrientes e os efeitos sobre o solo.

Também é relevante, avaliar a possibilidade de aumentar o desempenho do digestor. Novas tecnologias baseadas na hidrólise térmica e na temperatura termofílica, no ataque mecânico, químico ou enzimático podem aumentar a produção de energia e a eficiência, tornando o efluente digerido adequado para aplicação no solo, de acordo com a legislação em vigor.

No LNEG, foram efectuados trabalhos de Investigação dedicados à valorização de resíduos orgânicos na produção de culturas agrícolas e sua biodegradação em instalações de codigestão. O objectivo do trabalho foi avaliar três silagens de culturas, com potencial de uso para a produção de biogás em codigestão com resíduos industriais.
Foram experimentadas culturas nacionais utilizadas pela sua capacidade de fixação do azoto, reduzindo a adubação no ciclo da cultura de sucesso, e culturas eficazes contra os nematodos, e actuam na desinfecção do solo. Os rendimentos de produção de biogás foram de 300 e 400 m^3 de CH_4/tonMO, respectivamente, um resultado encorajador.

Estão previstos novos estudos envolvendo diversas áreas, nomeadamente: a melhoria da degradação, a avaliação do desempenho de pré-tratamentos (mecânicos, térmicos e enzimáticos) que aumentam a hidrólise e o potencial de produção de metano do substrato, a recuperação de compostos valiosos, a aplicação do efluente digerido num campo agrícola experimental, com solos seleccionados, semeados com culturas com potencial energético, a fim de encontrar as estirpes mais adequadas para as condições climáticas nacionais. Os estudos são complementados pela análise do ciclo de vida. Esta actividade pretende constituir uma prática positiva e contribuir para a implementação de sistemas sustentáveis baseados em resíduos e agricultura, em Portugal.

O4 O projecto dos biocombustíveis na Galp Energia / *Galp Energia's Biofuels Project*

Eng. Hugo Pereira e Eng. Fernando Bianchi de Aguiar
Unidade de Desenvolvimento de Biocombustíveis da GALP ENERGIA, Lisboa, Portugal/ GALP ENERGIA Biofuels,, Lisbon, Portugal.

A estratégia da Galp Energia para os biocombustíveis inclui a presença em toda a cadeia de produção para garantir a sustentabilidade social, ambiental e económica. Em 2007 iniciaram-se os trabalhos de prospecção de terrenos e parceiros em Moçambique e no Brasil estando hoje em dia os projectos em plena fase de instalação. Os projectos centram-se na produção de óleos vegetais para a produção de biodiesel em resposta às necessidades do mercado.
Utilizamos culturas plurianuais extensivas, exploradas em regime de sequeiro, com um impacte sensível no desenvolvimento rural das regiões onde se realizassem. Em Moçambique plantamos *Jatropha curcas* Linn. (vulgo Purgueira), no quadro de uma campanha do governo de divulgação da cultura, posteriormente consagrada em legislação nacional. No Brasil a cultura da Palma (*Elaeis guineensis,* Dendém), igualmente num quadro de um programa nacional para a promoção da cultura no estado do Pará e em parceria com a Petrobrás Biocombustíveis. Partimos para a avaliação da sustentabilidade ambiental com um crédito importante de emissões em relação às metas mínimas fixadas pelas Directivas Comunitárias. As tecnologias de produção são acompanhadas pela análise do ciclo de vida (LCA) na assumpção de uma utilização do óleo em Portugal e a sua transformação em biodiesel hidrogenado (HVO). / *Galp Energia's strategy for biofuels' projects development is based on presence throughout the chain of custody thus guaranteeing social, environmental and economic sustainability. Land surveys, alongside our business partners, began in Mozambique and Brazil in 2007 after which projects, in both countries, are currently being developed. Both projects are focused on the vegetable oils' production for further transformation into biodiesel thus responding to the future market needs. Rainfed permanent crops are extensively employed providing the conditions for rural development in the involved areas. In Mozambique physic nut (Jatropha curcas Linn.) trees are planted in support of the government's promotion initiative that contemplated the plant in previous legislation. Similarly and in partnership with Petrobras Biofuels (Petrobrás Biocombustíveis), palm trees (Elaeis guineensis) were planted in the Pará state of Brasil, where government has been promoting this crop as a means of boosting regional development and family farming. The projects' environmental sustainability is highlighted as an important asset towards meeting the carbon reduction targets of European Union directives. Production techniques are strongly linked to Life Cycle Assesement (LCA) assuming hydrogenation of the vegetable oils at Galp Energia's refineries in Portugal.*

O5 *Algae for Energy and CO2 mitigation*

Doutora Joana Silva
Necton, S.A. / AlgaFuel, S.A.

A4F, AlgaFuel S.A., é uma empresa que resulta de um spin-out da Necton, S.A., dedicando-se ao desenvolvimento e implementação de projectos de bioengenharia para a produção industrial de microalgas. Oferece soluções biotecnológicas de produção industrial de microalgas que, fixando CO2, produzem biomassa com elevado potencial económico.
A empresa possui mais de 20 anos de experiência no cultivo de microalgas e mais de 10 anos na produção industrial de microalgas em Raceways, Fotobioreactores de placas e Fotobioreactores tubulares.

A abordagem da A4F, AlgaFuel S.A. à indústria passa por 4 fases com reavaliação contínua nomeadamente Estudo, Protótipo, Piloto e Scale-up. Esta tecnologia permite obter biomassa em grandes

quantidades e, através de um processo economicamente produtivo, poderão trazer vantagens às indústrias nomeadamente na comercialização da biomassa para; alimentação humana (F2), rações para animais Premium, corantes alimentares, entre outros.
É de realçar a potencialidade da biomassa microalgal numa perspectiva de biorrefinaria para obtenção de bioetanol, biodiesel e biometano. Contudo, é importante, considerar rentabilizar todos os componentes da biomassa, alguns muito mais valiosos do que os biocombustíveis.

O6 *Feedstock purification for biodiesel transesterification*

Author: Manuel Vasconcelos
Company: BB-DIESEL

The feedstock quality is directly related to the biodiesel quality, hence an appropriate feedstock treatment is as important as the biodiesel processing. The main problems presented in the biodiesel feedstock is the content in phosphorous resulted from the vegetable cell walls, and the free fatty acids resulted from the oil or animal fats degradation. After the mechanical extraction, the crude vegetable oil has impurities like phospholipids, water and free fatty acids (FFA). The phospholipids also known as gums are basically fats with a phosphate group associated. Crude vegetable oils have 0.5% to 1.5% as typically gums value. The phosphate group present in gums makes these molecules anphypatic, having chemical affinity to the fatty phase and to the aqueous phase, making them emulsifying agents. These compounds are inhibitors of transesterification reaction restraining good biodiesel conversions and difficult the biodiesel / glycerine separation. Degumming process consists of adding phosphoric acid to convert liposoluble phospholipids in water soluble phospholipids to be easily washed in oily phase. The feedstock degradation during the extraction process produces 0.8% to 2% of free fatty acids (FFA). In the biodiesel production process, the FFA has negative effect consuming catalyst and making emulsifying agents like soaps. For low FFA concentration, the FFA are removed by saponification, reacting caustic soda with the crude vegetable oil to produce soaps from the FFA, after removed by water washing. For feedstock with high free fatty acids content, commonly found in animal fats and jatropha or castor crude oils, the FFA are directly converted into biodiesel reacting the raw material with an acid methanol based solution, in a process called acid esterification.

O7 *Overproduction of fatty acids in Saccharomyces cerevisiae*

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Production of renewable liquid biofuels that can substitute fossil fuel has emerged as a major challenge for applied biology.
Biodiesel, in the form of fatty acid esters, produced by oleaginous organisms could be an alternative, since the utilization of diesel fuel is more efficient than (bio)ethanol.
Oleaginous organisms such as certain yeasts, plants and algae can accumulate very high (>60%) levels of intracellular lipids but two drawbacks are a relatively complicated extraction process and the subsequent

transesterification with the accompanying glycerol by-product formation.
The objective of this work is to apply metabolic engineering of fatty acid synthesis and secretion to the model yeast S. cerevisiae in order to create a microorganism able to produce and secrete free fatty acids or fatty acid esters. S. cerevisiae is the model of choice, since its lipid metabolism hasbeen studied

extensively and all genes encoding enzymes directly involved in lipid synthesis are known. Further, S. cerevisiae can acquire oleaginous properties by as few as three genetic modifications (1).
In the yeast S. cerevisiae, activation of exogenous long-chain fatty acids to coenzyme A derivatives, prior to metabolic utilization, is mediated by the fatty acyl-CoA synthetases Faa1p and Faa4p. It has been shown that free fatty acids are secreted from a FAA1,4 double mutant (2).
Further engineering of a pyruvate dehydrogenase bypass, in order to enhance the supply of acetyl-CoA to the fatty acid biosynthesis pathway, will be performed by overexpression of acetyl-CoA synthetase and acetaldehyde dehydrogenase and deletion of pyruvate dehydrogenase. The fatty acid production of the modified strains was analyzed by gas chromatography and results will be discussed.
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O8 Seaweed mariculture for biofuels

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O9 Regulação do crescimento polarizado do tubo polínico através da modulação de endocitose e secrecção membranar. / *The regulation of pollen tube growth and polarity through modulation of endocytosis and membrane secretion.*

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Pollen tube growth involves many interactions, including activation of complex signaling networks, rapid synthesis and release of specific molecules. We will discuss recent findings from our lab showing the involvement of phosphoinositides, lipid kinases and SNAREs in this process using Arabidopsis and tobacco as biological models.
In Arabidopsis studies we resort to a reverse genetic approach and the characterization of homozygous mutant plants. Tobacco was used as a heterologous system for the transient expression of Arabidopsis proteins and the study of their sub-cellular localization and putative role in polarity. Our experimental methodology involves also the use of dominant negative mutants, protein co-expression and the effect of specific inhibitors.
The results obtained so far support a model for membrane secretion and recycling where the apical and sub-apical region are a functional area containing the components required to promote and sustain growth. We will integrate these findings in a more comprehensive model for tip growth.

O10 Characterisation of a new family of MYB-like transcription factors and its role in flower asymmetry

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Floral asymmetry is a trait that has evolved many times independently in different angiosperm lineages from a radially symmetrical ancestral condition. Therefore, it provides a good system to study how a pathway that is responsible for the establishment of a new trait has evolved.
In Antirrhinum majus, floral asymmetry requires the combined activity of four transcription factors: CYC, DICH, RAD and DIV. CYC, DICH and RAD are expressed dorsally in floral primordia and promote dorsal petal identity. DIV is expressed all round the floral primordium, even though it only has a phenotypic effect in more ventral regions. Genetic and molecular studies have revealed that RAD is a direct target of CYC and antagonises the activity of DIV. This was further explored by using yeast two-hybrid screens, which led to the identification of two novel related MYB-like proteins (RIPs, RAD-interacting proteins) that interact with both RAD and DIV. Therefore, the RIP proteins might also be involved in the antagonism that RAD has on DIV activity, essential for the establishment of flower asymmetry in Antirrhinum.
In order to establish a role of the RIP gene family in these species we initiated the characterisation of the function of the RIP genes in Antirrhinum. Furthermore, the function of the RIP homologs in Arabidopsis, the RIP-like genes (RIPLs), was also studied in order to give insights into an ancestral role of the RIP family, before dorsoventral asymmetry of flowers has evolved.

O11 *Bio-waste strategy. Compost - product quality & applications*

Engª Susana Lopes - LIPOR – Serviço Intermunicipalizado Gestão Resíduos

O12 Analysis of DNA damage and repair in *Saccharomyces cerevisiae* using the comet assay in the characterization of antigenotoxicity of plant extracts and phytochemicals

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In this work we used the model organism *Saccharomyces cerevisiae* to characterise the biological activity and the mechanism of action of phytochemicals. We have assessed DNA damage and repair using the comet assay, evaluated as “comet tail length” and used this system to assess the antigenotoxic properties of a leaf extract from *Ginkgo biloba* (GBE). Typical experiments involved incubation of yeast cells, or spheroplasts, with GBE before and during oxidative shock with hydrogen peroxide. Our results show that DNA damage was significantly decreased upon GBE treatment in a dose-dependent manner. In addition, DNA repair kinetics was significantly improved in cells incubated with GBE. However, in the mutant strain affected in *CDC9*, encoding a DNA ligase involved in the mechanisms of nucleotide excision repair and base excision repair, oxidative DNA damage repair kinetics was unchanged with GBE, suggesting that the

activity of this extract involves one of these mechanisms, or both. Hydrogen peroxide-induced cell cycle arrest in G2 was abolished when cells were incubated with GBE after oxidative shock, suggesting that the improved repair kinetics allows progression of the cell cycle and/or GBE can have a direct effect on its regulation. As expected, GBE treatment improved survival of yeast cells when challenged with oxidative shock with H_2O_2 and intracellular oxidation was considerably decreased upon pre-treatment with GBE as revealed by flow cytometry.

O13 Climate Change: Using Modeling to Predict Potential Effects on Crops

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The main aim of this presentation is to provide some fundamental concepts related to climate and to its inherent variability, which might be considered an essential tool for a better judgment of the large amounts of information currently available regarding this topic. This is particularly pertinent for agricultural research, as agronomic systems tend to be largely regulated by climate and by atmospheric parameters. Some elementary concepts on the nature of the climate system are first presented; its components and coupling processes are succinctly described, giving particular emphasis to the spatial and temporal time scales relevant for agro-systems research. Both internal and forced climate variability are discussed from a physically-based perspective with a special focus on the anthropogenic forcing of the climate system. The discussion of these topics is followed by some essential ideas on atmospheric modeling and on downscaling strategies. The likely impacts of a changing climate on several agronomic sectors are also referred, giving particular emphasis to the impacts on viticultural zoning in Europe. A number of relevant bioclimatic indices are used for this purpose and changes in their spatial patterns under human-driven climate change are discussed. An overview of the threats and challenges imposed by climate change is given and possible mitigation measures are pointed out.

O14 Barcoding of entomopathogenic fungi from olive tree pests: prospects and limitations

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From all the barcoding initiatives in progress, fungal barcode is probably the one where more difficulties have been encountered. While for plants and animals the barcode regions were easy to define, for fungi the choice was not so straightforward. The internal transcribed spacer (ITS) region was one of the proposed DNA regions for barcoding fungi. This is an extensively used region, for molecular systematic and identification of species, being probably the most widely sequenced DNA region of fungi. This is due to the simplicity of the amplification, related to the multicopy nature of the rDNA; the possibility of using universal primers; and the high level of sequence variation that occurs even between closely related species. Furthermore, a significant number of identified sequences for comparison are available in the GenBank database. Although the ITS region of rDNA was chosen for some groups of fungi, the use of this

region presents very limited application for others, especially for Ascomycetes. As some of the most important entomopathogenic fungi are Ascomycetes, belonging to genera *Beauveria, Cordyceps, Isaria, Lecanicillium* and *Paecilomyces,* the use of the ITS region for barcoding purpose are being complemented with other regions. This work, based on the identification of fungal entomopathogens isolated directly from cadavers of one of the major pests in olive groves, the olive moth (*Prays oleae* Bern.), intends to illustrate the application of the ITS region to identify these fungal species. The use of this region proved to be useful for the identification of most of the entomopathogenic fungi found in dead larvae and pupae of *P. oleae.* However, the use of ITS region for barcode purposes did not allow the identification of several isolates, proving the requirement of using a second barcoding region, to enable full fungal identification

O15 The pollen content of a river beach for forensic purposes

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Palynology has applications in many areas of science, including forensics. Pollen grains are microscopic structures transported from the anthers to the stigma of the same species by abiotic and biotic agents.
Pollen adheres to different natural, artificial and human surfaces, being a silent proof due to its invisibility. They are resistant to physical, chemical and biological degradation and can be preserved for several years. Morphologically exhibit diversity in size, shape, symmetry, ornamentation and number of apertures in its wall. This heterogeneity is a good taxonomic parameter to identify species. Therefore they may provide information on the association of a suspect, victim, subject or location at a given crime scene because they are characteristic of each region.
Our work was based on the study of the pollen content in Areinho, a fluvial beach in Vila Nova de Gaia in order to establish the autochthonous and allochthonous pollen knowing the diversity of surrounding vegetation.
The sampling was performed in a profile with eight points, spaced 15 meters, along a transect perpendicular to the river side. At the laboratory, the samples were dried at 40°C and pollen analysis was conducted.

This beach is in a specific geological environment marked by different lithologies, being a very busy spot with water sports and bathing season.
This pollen characterization enhances its use in solving crimes contributing to the clarification of cases and reasoning of judicial decisions.

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O16 Inorganic matter associated to airborne pollen: characterization by Electron Microprobe Analysis (EMPA)

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Aerosol is a combination of liquid droplets and small solid particles, suspended in a gas or a mixture of gases. Particles from atmospheric aerosol, also called particulate matter, have different sources, sizes and compositions. Sea-salt, mineral dust, biological particles (e.g. pollen grains) and particles derived from carbonaceous combustion are some examples of particles that make up atmospheric aerosol. Particulate matter is believed to enhance pollen allergenicity and negatively affect human health.
The aim of our study was to describe inorganic matter associated to pollen grains present in the atmosphere of Porto.

Airborne pollen sampling was performed using a 7-day volumetric spore trap. Control pollen samples of selected plants were collected in public gardens or sidewalks. Quantitative analysis and X-ray map analysis were performed with a Field Emission Electron Probe Microanalyser (EMPA).
Our results showed that during its "flight", pollen acquires an external coating, becomes heavier and its wall composition is significantly changed. Control pollen showed mainly the presence of C, O, N, P, K, Mg, S, Cl and Ca in different quantities, depending on the considered species. Airborne pollen consistently revealed a higher content of Mg and Cl than control pollen, while S, Na, Ca and Si contents varied differently according to the samples. These alterations were confirmed by quantitative analysis and X-ray dot maps. This coating seems to be related to sea spray, since Mg^{2+}, Cl^{-}, Na^{+} and $SO4^{2-}$ represent about 95% of sea water salinity. The Ca and Si content can be related to local resuspension or long-distance transport of crustal components. In addition to this film covering the pollen, we identified several particles, from different sources, adhering to its wall.

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O17 Anti-inflammatory and antimicrobial activities of phlorotannins purified extracts from brown seaweeds collected in the Portuguese coast

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The Portuguese coast is characterized by the presence of several seaweed species. Phlorotannins are polyphenols restricted to Phaeophyta species, suggested to have multiple ecological roles. Diverse effects of phlorotannins have been reported on biological systems. Thus, the bioactivity of phlorotannins purified extracts of ten seaweeds was assessed.

Anti-inflammatory capacity was assessed *via* inhibitory effect on nitric oxide (NO) production by lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. *Cystoseira tamariscifolia* extract significantly inhibited nitrite formation. At the highest concentration NO production was reduced to 25% (Fig. 1).

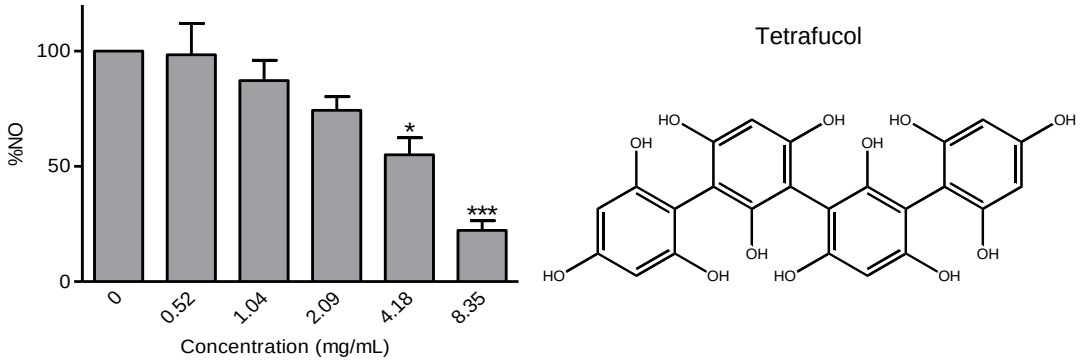

Fig. 1: Effect of *C. tamariscifolia* phlorotannins extract on LPS-induced nitrite production in RAW 264.7 cells.

Regarding antibacterial capacity, the different extracts were more effective against Gram-positive bacteria, being *Enterococcus faecalis* the most susceptible one (MIC: 2.5-10 mg/mL, dry wheight). In what concerns to antifungal potential, different results were obtained. At the studied concentrations, *Aspergillus fumigatus* was resistant to all seaweeds extracts. *Trichophytom rubrum* and *Candida albicans* were the most susceptible studied fungi, being *Cystoseira nodicaulis* the most effective seaweed (MIC=3.9-7.8 mg/mL, dry wheight).

All our results point to the genus *Cystoseira* as the most promising for the future development of a new natural drug, with both anti-inflammatory and antimicrobial potential.

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O18 Evaluation of the protective effect of Hypericum perforatum phenolics compounds, in the toxicity induced by heterologous expression of α-synuclein

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Parkinson's disease (PD) is a neurodegenerative disorder with high prevalence, affecting 2% of the human population over the age of 60 years. PD is characterized by the loss of dopaminergic neurons and the presence of cytoplasmic eosinophilic inclusions named Lewy bodies, in which α-synuclein is the major constituent. Recent work implicates abnormal protein accumulation, protein phosphorylation, mitochondrial dysfunction and oxidative stress as common pathways implicated in PD pathogenesis. Polyphenolic compounds are commonly found in both edible and medicinal plants, and they have been reported to have multiple biological effects, including antioxidant activity. The budding yeast Saccharomyces cerevisiae has been used as a model to study several neurodegenerative diseases, including biological function of α-synuclein, as well as its toxicity. The heterologous expression of wild-type and A53T mutant form of α-

synuclein causes toxicity in cells. Therefore, the aim of this study was to evaluate the possible protective effect of Hypericum perforatum phenolic compounds (quercetin, kaempferol and biapigenine), in the toxicity induced by the heterologous expression of α-synuclein, using the yeast Saccharomyces cerevisiae as a model. Preliminary results indicate that the presence of these phenolic compounds decrease the toxicity observed in cells expressing α-synuclein. We concluded that these phenolic compounds apparently have beneficial biological properties that consequently could have a potential use in preventing Parkinson's disease.

O19 Analysis of ROS production and homeostasis in an *Arabidopsis thaliana* knockout mutant involved in post-translational modification

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Adverse environmental factors, such as high light, drought, salinity or temperature fluctuation, negatively affect plant growth and development. The resulting disruption of cellular homeostasis often leads to

increased production of reactive oxygen species (ROS). These species are generated from energy or electron transfer to ground state oxygen, being extremely toxic to the cell by easily reacting with proteins, lipids and nucleic acids. In addition to their role as toxic by-products of unbalanced metabolism, ROS are also key signalling intermediates linking abiotic stress perception to stress adaptive responses. Considering the implications of ROS accumulation in plant cells, an integrated network of ROS-detoxifying agents must be able to efficiently adjust ROS to desirable levels. Recently, post-translational modifiers have been implicated in several responses to abiotic stress. In this work we show that an *Arabidopsis thaliana* insertion mutant involved in protein modification and previously associated with abiotic stress resistance, displays altered ROS homeostasis, possibly due to altered activity of ROS generating centres.

O20 Airborne and Allergenic Patterns of *Dactylis* and *Plantago* Pollen: A Comparative Study in Porto

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Orchard grass (*Dactylis glomerata*) and english plantain (*Plantago lanceolata*) plants have overlapping flowering seasons, producing large amounts of pollen that trigger respiratory allergies. We intended to study the allergenic profiles of *Dactylis glomerata* and *Plantago lanceolata* pollen in sensitized patients, and the affinity of recombinant maize profilin 3 polyclonal antibody. Airborne pollen was monitored by a Hirst-type volumetric trap to determine their flowering seasons in the city of Porto. *Dactylis glomerata* or *Plantago lanceolata* pollen were collected from plants in sidewalks and public gardens in Porto. After total pollen soluble protein extraction, we performed a SDS-PAGE and an immunoblotting, probing each pollen extract with sera from plantain or grass polysensitized patients or recombinant maize profilin. Plantain and grass pollen are present in the atmosphere of Porto from March to September. All plantain-sensitized sera presented 3 set of IgE-reactive bands of ~50 and ~30 kDa and of 14-15 kDa in some sera, which do not

correspond to plantain allergens already characterized. Grass-allergenic patients showed high IgE-binding proteins to 4 prominent groups of bands of 55-61, 32-33, 24-26 and 13-15 kDa. Both pollen allergenic profiles showed IgE-binding affinity to recombinant maize profilin 3. There are evidences that both pollen types are also strongly recognized by different atopic individual sera, sharing some IgE-binding protein groups.

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O21 Endpoints of cadmium cytotoxicity, genotoxicity and clastogenicity in lettuce (*Lactuca sativa* L.)

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Key words: Cadmium, toxicity, endpoints, lettuce

Cadmium (Cd) is considered a priority pollutant by Environmental Protection Agency (EPA), due to its high toxicity, mutagenicity and carcinogenicity. High contents of Cd come from anthropogenic activities like mining, production of fertilizers, fungicides and pesticides. As the metal may accumulate in edible crops as lettuce, it leads to potential ecological and health impacts. From its accumulation, Cd may indirectly induce

oxidative stress, cytotoxicity, genotoxicity and clastogenicity. In this work a set of endpoints were selected to evaluate Cd cytotoxicity in lettuce (*Lactuca sativa* L.) based on the assessment of protein oxidation (by

carbonyls quantification), lipid peroxidation (by malondialdehyde (MDA) content), and cell cycle impairment (by flow cytometry (FCM)), in roots and leaves. Cd genotoxicity and clastogenicity were evaluated by Comet and micronuclei (MN) assays, and by FCM, in the both organs.
Lettuce seeds were germinated in $Cd(NO_3)_2$ solutions (0, 1, 10 and 50 µM) and then transferred to hydroponic culture with equivalent Cd concentrations. After 28 days of culture, Cd induced an increase in lipid peroxidation in both roots and leaves, however in leaves it decreased for the highest dose. Concerning protein oxidation, Cd exposure induced an increase in carbonyl content for the doses of 10 and 50 µM in both organs. DNA damage assessed by Comet assay only increased at the lowest Cd concentration, in both roots and leaves. In roots MN were found from 10 µM, and mostly at this concentration, while in leaves MN were only found at 50 µM. FCM analysis showed no changes in cell cycle phases and it was found a clastogenic effect in roots of plants exposed to 10 µM Cd.
The array of biomarkers used in the present work allowed a comprehensive understanding of Cd toxicity in *L. sativa* L.

Resumo de Posters / *Abstracts of Posters*

P1 Characterization of Quercus spp. pollen potential allergens: profilin and Bet v 1-homologues

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Bet v 1- homologues belong to a multigene family of pathogenesis-related proteins (PR). These proteins are expressed in gymnosperms and angiosperms in response to biotic and abiotic stress, assuming an important mechanism of defence. Profilin is a ubiquous protein involved in cell development, cytokinesis, membrane trafficking and cell motility. Bet v 1 related proteins and profilin are conserved proteins and have been described as pollen allergens [1 - 2].
The aims of this work were to identify and characterize profilin and Bet v 1 homologues of Quercus spp pollen. Samples of pollen from different Quercus species (Q. suber, Q. robur, Q. rubra, Q. faginea, Q. imbricaria) were collected during the Spring of 2010. Soluble proteins extracts were assayed by SDS-

PAGE. Immunoblotting was performed using these extracts and specific antibodies to Zea mays profilin and to Betula pendula Bet v 1. The results revealed the presence of profilin-like and Bet v 1 related proteins in Quercus spp. pollen extracts.
Total RNA from Quercus spp. pollen were analysed in agarose gel by RT-PCR using degenerate primers for profilin and for Bet v 1 based on the sequences of other genes preferentially belonging to Fagales order. There were cDNA amplifications of ~ 400 bp and ~ 500 bp for profilin and Bet v 1- homologues, respectively. The amplified cDNA of profilin and Bet v 1- homologues of Quercus spp. will be sequenced. Subsequently, the sequences obtained will be submitted to bioinformatic analysis.
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P2 Induction of cellular stress responses by phytochemicals for nutritional applications toward anti-aging intervention

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Aging is an important risk factor for the development of age-related diseases and is associated with decreased cellular antioxidant defenses. Under the scope of the undergoing NaturAge project, our group is currently investigating the ability of some plant extracts and isolated phytochemicals to induce antioxidant defenses through Nrf2/ARE signaling. That, will be associated with possible anti-aging effects using normal human skin fibroblasts undergoing aging in vitro.

Recently, we have shown the ability of the polyphenol curcumin to induce cellular antioxidant defenses through induction of a stress response in normal human skin fibroblasts, affording protection from a further oxidant challenge with tert-BOOH [1]. Curcumin incubation for 24h induced heme oxygenase-1 (HO-1) protein levels, GST activity, GSH levels and GSH/GSSG ratio. These effects were preceded by induction of oxidative stress as shown by increased levels of ROS and DNA damage, and impairment of the cells' GSH redox state. The induction of antioxidant defenses in human fibroblasts was shown to be redox and PI3K/Akt dependent [1]. In conclusion, these results support the view that phytochemical-induced hormetic stimulation of cellular antioxidant defenses can be a useful approach toward anti-aging intervention.

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P3 Cellular distribution and regulation of intestinal SGLT1

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Diabetes is achieving epidemic proportions in many countries. In addiction to high blood glucose it is associated with increased intestinal expression of the sodium-glucose cotransporter (SGLT1). This transporter is located in brush-border membrane (BBM) of the enterocytes and is responsible for transporting glucose and galactose from the intestinal lumen into the cytosol, using the inward Na^+ gradient maintained by the basolateral Na^+/K^+-ATPase. Our previous results show that the adaptive response to increase dietary carbohydrates also involves increased intestinal expression of SGLT1 at the BBM. This raise does not seem to reflect changes in mRNA suggesting an involvement of posttranscriptional mechanisms in SGLT1 BBM expression. In Caco-2 cells, the intracellular SGLT1 resides in endosomes and the abundance of the transporter at BBM seems to be affected by the cellular endocytic pathway. Currently, we are focusing our studies on the regulation by glucose, insulin and other dietary factors on the cellular distribution of SGLT1 and the mechanisms of its traffick to the plasma membrane in Caco-2 cells.

Acknowledgements: FCT supported CMS (SFRH/BD/42566/2007), as well as the work (POCI/AGR/62040/2004).

P4 Autophagy triggered by ursolic acid synergistically enhances 5-fluorouracil induced cell death in HCT15 (MSI p53 mutant) colorectal cancer cells

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Colorectal carcinoma (CRC) is a common cause of cancer-related death and tumors with microsatellite instability (MSI) and p53 mutations have been shown to be resistant to chemotherapy with 5-fluorouracil (5-FU). Therefore, it is essential to find compounds that could contribute to treatment efficacy by increasing the sensitivity to 5-FU. HCT15, a MSI human CRC derived cell line that harbours a p53 mutation, was incubated with the triterpenoid ursolic acid (UA) at a concentration that induces approximately 50% cell death (measured by PI stainning) after 48h. A synergistic enhancement of apoptosis was observed when co-incubating 5-FU with UA (measured by TUNEL assay). UA induction of apoptosis was totally abolished by the JNK inhibitor SP600125 (SP), but not by the caspase inhibitor zVAD-fmk. Apoptosis did not account

for all the observed cell death induced by UA. Thus, we asked whether UA was also inducing autophagy. We observed that UA induced accumulation of autophagossomes (using fluorescent dyes) as well as of LC3-II (assessed by western blot), which was also significantly inhibited by SP. These results suggest that UA induction of apoptosis and autophagy is JNK dependent. A decrease in mutated p53 and phospho mTOR, which are associated with an induction of autophagy, were also observed. In conclusion, UA showed anticancer activity by inducing apoptosis and autophagy, which was JNK-dependent in HCT15 cells. In addition, in these resistant cells, UA synergistically cooperate with 5-FU to induce cell death

P5 DNA DAMAGE PREVENTION AND SIGNALING PATHWAY REGULATION BY SAGE IN A COLON CANCER MODEL

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Colorectal cancer (CRC) is a common malignancy and significant cause of mortality in Western societies. It develops through an accumulation of genetic and epigenetic alterations, transforming normal colon cells and giving them growth advantage. Many food plants are rich in bioactive compounds and have shown to posses anticancer properties.
We proposed to explore the effects of sage (*Salvia officinalis* (SO)) water extract (herbal tea) drinking on colon cancer prevention and modulation of epigenetic events. F344 rats were used to study the effects of sage tea drinking on pre-initiation (SO treatment before AOM exposure) and post-initiation (SO after AOM exposure) phases of carcinogenesis. We found a chemopreventive effect of SO in the pre-initiation group, but not in the post-initiation. We then investigated if SO affected AOM metabolism, searching for effects on CYP2E1 expression and activity. We found that AOM decreased CYP2E1 activity when compared with control, but SO treatment before AOM prevented this effect. The capacity of SO in vivo treatment to protect colonocytes from H_2O_2 damage induced in vitro was also investigated. SO decreased significantly the oxidative H_2O_2-induced DNA damage. We also are searching for alterations in expression of key proteins involved in signalling pathways important for cell proliferation or apoptosis and proteins involved in DNA repair.
Sage water extract seems to have the ability to prevent CRC and studies to further explore this potential are ongoing.

P6 PREPARATION OF *JATROPHA CURCAS* OIL AS FEEDSTOCK FOR BIODIESEL PRODUCTION

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Jatropha curcas plant can grow in arid soils and produce high non edible oil yields. *Jatropha curcas* oil is considered as one of the most important feedstock for biodiesel production. Preparation of this oil must meet the specification of feedstock that could ensure the highest quality of biodiesel. Adjustment of Free Fatty Acid (FFA) content is one of the main steps before the transesterification process using base as

catalyst. FFA can react with base catalyst to form soap colloids that are hard to remove from biodiesel product. Removal of dispersed material (such as phospholipids and waxes) in raw jatropha oil is also needed during the pretreatment process, usually known as refining process. Refining process could be done by physical or chemical methods. The main objective of this study was to reduce the initial FFA content in raw jatropha oil for a further used as a feedstock for biodiesel production. The refining process included cracking of phospolipids with phosphoric acid, settling, adsorbing with fuller's earth or bentonite, and esterification of FFA to triglycerides using alcohol catalyzed by sulphuric acid.The initial jatropa oil used in our study was cloudly and dark brown with FFA value of 28%. Results showed that a clear light yellow oil with FFA value less than 4% can be obtained by treating the raw oil with 0.5% vol. of 4% phosphoric acid, followed by adsorption using fuller's earth and a later esterification with 0.61 % vol. ethanol, 0.17% v/v sulphuric acid at 54 ºC during 79 min.

P7 HPLC-PAD-ESI-MS^n screening of an insect/plant system: the case of *Spodoptera littoralis*/*Lycopersicon esculentum* phenolics and alkaloids

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Spodoptera littoralis is one of the most deleterious pests, representing a major challenge to Solanaceae plants. *S. littoralis*/*Lycopersicon esculentum* system was studied for the first time concerning glycoalkaloids and phenolics. Using HPLC-PAD-ESI-MS^n we were able to characterize fifteen phenolic compounds in *L. esculentum* leaves, being nine compounds reported for the first time. Some differences were found between leaves of *cerasiforme* and "Bull's heart" varieties (Fig. 1).

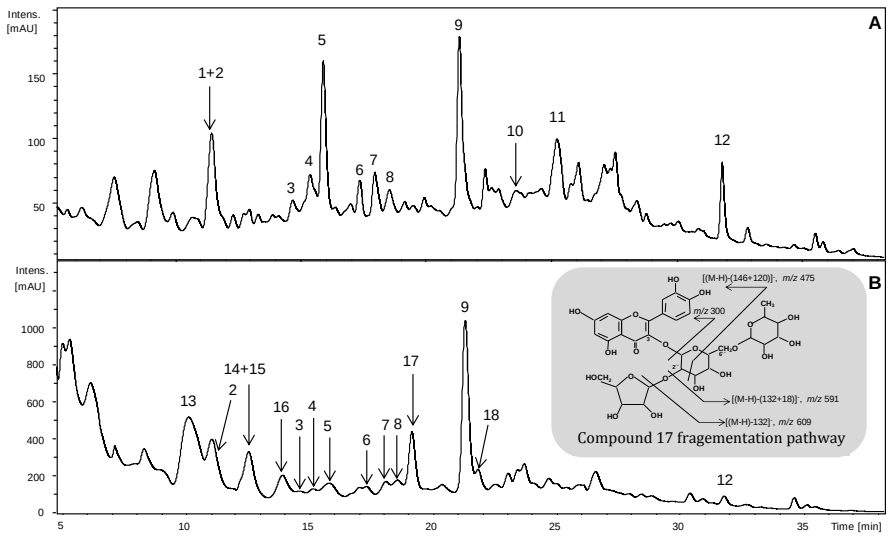

Fig. 1. HPLC phenolic profile of *L. esculentum* var. *cerasiforme* (A) and "Bull's heart" (B) leaves. Peaks: (**1**) caffeoyl-hexoside acid, (**2**) *p*-coumaroyl-hexoside acid, (**3**) *p*-coumaroyl-quinic acid, (**4**) sinapoyl-hexoside acid, (**5**) 5-feruloyl-quinic acid, (**6**) *p*-coumaroyl-quinic acid isomer, (**7**) unknown, (**8**) feruloyl-quinic acid

isomer, (**9**) quercetin-3-rutinoside, (**10**) kaempferol-3-rutinoside, (**11**) unknown, (**12**) unknown, (**13**) 3-caffeoyl-quinic acid, (**14**) feruloyl-hexoside acid, (**15**) sinapoyl-hexoside acid isomer, (**16**) 5-caffeoyl-quinic acid, (**17**) quercetin-3-pentosyl-rutinoside, (**18**) sinapoyl derivative.However, in *S. littoralis* materials (larvae, adults, exuviae and excrements), reared in both *L. esculentum* leaves, no phenolics were identified. α-Tomatine was the main glycoalkaloid in the host plant. The glycoalkaloids composition of the different *S. littoralis* materials was distinct, with α-tomatine and dehydrotomatine being the main detected compounds in larvae and excrements. These results indicate that *S. littoralis* has the capacity to detoxicate α-tomatine, which constitutes a defence of *L. esculentum*. **Acknowledgments**: M. Taveira (SFRH/BD/62662/2009) is indebted to FCT for the grant. T. Teixeira (M3.1.6/F/041/2009) is grateful to FRCT for the grant.

P8 Viability assays to evaluate *F. carica* leaf effects on V79 cells

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Fig (*Ficus carica* L.) is a seasonal food, representing an important constituent of Mediterranean diet. *F. carica* leaves are commonly consumed as infusion and are traditionally used as laxative, stimulant, antitussive, and emmenagogue.

Cell cultures play an important role in the evaluation of biological parameters and / or therapeutic *vs* toxicological effects of natural matrices. In this work the biological activity of three *F. carica* leaves extracts (methanolic, ethyl acetate and aqueous lyophilized extracts) were evaluated by measuring MTT reduction, using hamster lung fibroblast (V79 cells) under quiescent conditions.

In order to assess possible relations between chemical composition and cell response, the phenolic profile of the extracts was established by HPLC-DAD. Methanolic extract presented the same kind of phenolic compounds of the aqueous one (Fig.1).

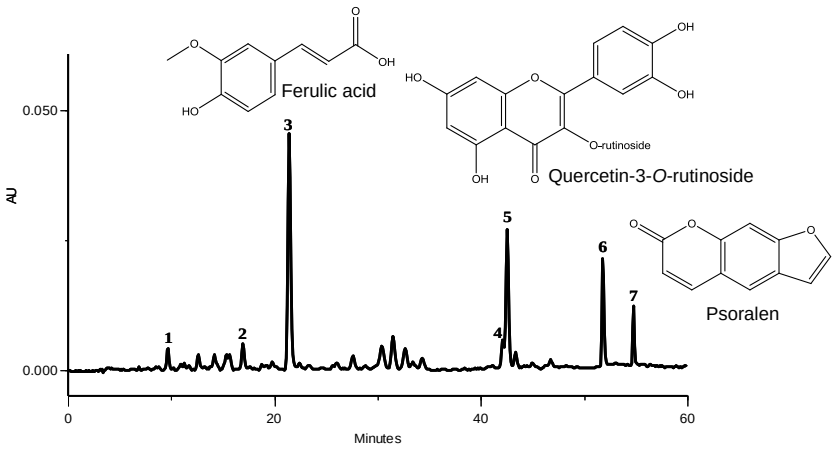

Fig.1. HPLC phenolic profile of *Ficus carica* leaves aqueous lyophilized extract. Detection at 320 nm. Peaks: (1) 3-*O*-caffeoylquinic acid; (2) 5-*O*-caffeoylquinic acid; (3) ferulic acid; (4) quercetin 3-*O*-glucoside; (5) quercetin 3-*O*-rutinoside; (6) psoralen and (7) bergapten.

On the other hand, *F. carica* leaf ethyl acetate extract was only composed by psoralen and bergapten.

Preliminary results with aqueous lyophilized extract point to an increase on V79 cell viability, at the highest tested concentration (1.5 mg/mL). In addition, methanolic and ethyl acetate extracts were not cytotoxic.

Acknowledgments: A. P. Oliveira (SFRH/BD/47620/2008) is indebted to Fundação para a Ciência e a Tecnologia (FCT) for the grant.

P9 An omic approach to unravel the metabolism of the highly valuable medicinal alkaloids from Catharanthus roseus

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Catharanthus roseus accumulates in low levels the terpenoid indole alkaloids (TIAs) vinblastine and vincristine, used in cancer quimiotherapy, as well as ajmalicine, used as an antihypertensive, and serpentine, used as sedative. Although much is known about the biosynthesis and regulation of TIAs, gene/enzyme characterization is still lacking for many biosynthetic steps, the membrane transport mechanisms of TIAs are basically uncharacterized, and no effective master switch of the TIA pathway has been identified.

In *C. roseus* leaves, the first steps of TIA biosynthesis occur in the epidermis, while the late steps and TIA accumulation occur in differentiated mesophyll cells, the idioblasts, characterized by a conspicuous blue fluorescence. Therefore, transport of TIA intermediates is thought to occur between the two cell types. Here, we implemented a targeted strategy involving the isolation of idioblast protoplasts from those of common mesophyll cells by Fluorescence Activation Cell Sorting (FACS), followed by differential transcriptomic analysis in order to discover new candidate genes involved in the biosynthesis, regulation and transport of *C. roseus* TIAs. The fraction of idioblast cells obtained by FACS showed a high purity, and cDNA-AFLP-based transcript profiling was performed for roots, leaves, leaf protoplasts and sorted idioblast protoplasts. At this moment, we have identified 6 sequence tags of putative ABC transporters which are differentially expressed in idioblasts, and are candidate genes either for cell extrusion, cell uptake or vacuole uptake of TIAs. Further *in silico* analysis should lead to the identification of more candidate genes.

P10 *Capsicum annuum* seeds: new source of bioactive compounds

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Peppers belong to *Capsicum* genus, being *Capsicum annuum* reported as the most widely cultivated species. Recently more attention has been focused on the use of food-processing by-products and wastes,

as well as underutilized agricultural products. The use of seed waste of pepper fruit can be a new, alternative and cheap source of bioactive phytochemicals [1, 2].The aim of this work was to compare the chemical composition and antioxidant activity of red and green pepper seeds. Fatty acids and volatile compounds were analyzed by GC-MS, sterols by HPLC-DAD and organic acids by HPLC-UV. Antioxidant activity was evaluated by DPPH, nitric oxide and superoxide radical scavenging assays.

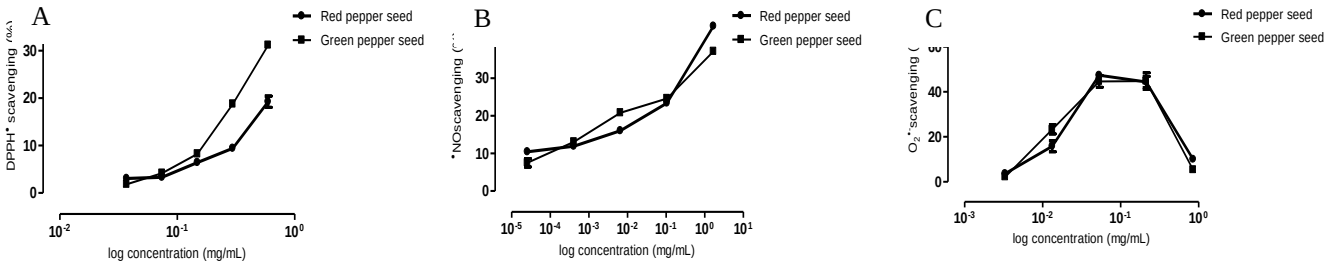

Figure 1. Effects of *Capsicum annuum* seeds aqueous lyophilized extracts against: (A) DPPH, (B) nitric oxide and, (C) superoxide radical. Values show mean ± SE from three experiments performed in triplicate.

The chemical profile of pepper seeds was distinct except in sterols. Green seeds exhibited higher diversity and amounts of fatty acids, namely oleic acid. Regarding volatiles, green pepper seeds have aldehydes, alcohols, esters, and sesquiterpenes in higher levels. Overall, green pepper seeds exhibited the highest contents in bioactive compounds. In antioxidant activity green pepper seeds extract revealed to be more active than the red pepper seeds one (Fig.1).Thus, the results are very promising, constituting a base for the possible application of this waste matrix in food, cosmetic and pharmaceutical industries.References:[1] Firatligil-Durmus, E., Evranuz, O. (2010), *Response surface methodology for protein extraction optimization of red pepper seed (Capsicum frutescens)*, Food Science and technology, 43, 226-231.[2] Sim, K.H., Sil, H.Y. (2008), *Antioxidant activities of red pepper (Capsicum annuum) pericarp and seed extracts*, International Journal of Food Science and Technology, 43, 181.

P11 *Glycine max, Vigna radiata* and *Medicago sativa* sprouts: a natural source of bioactive compounds with antioxidant potential

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Sprouts are thought to be rich in health-promoting phytochemicals that are known to prevent a number of chronic and degenerative diseases. Germination may cause changes in nutrients and removes antinutrients, such as enzyme inhibitors from seeds, thus making sprouts safe for the diet [1]. However, despite the popularity of sprouts as a healthy food, very little is known about their health-promoting qualities

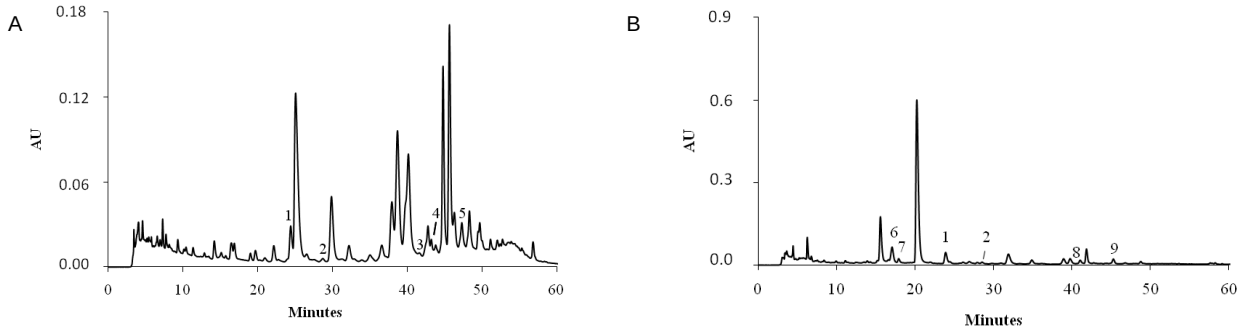

Figure 2 - HPLC phenolic compounds profile of *Medicago sativa* (A) and *Glycine max* (B) sprouts. Detection at 320 nm. Peaks: (1) *p*-Coumaric acid; (2) Ferulic acid; (3) Cinnamic acid; (4) Luteolin-7-*O*-glucoside; (5) Luteolin-4-*O*-glucoside; (6) 5-Caffeoylquinic acid; (7) Caffeic acid; (8) Kaempferol-3-*O*-rutinoside; (9) Kaempferol-3-*O*-glucoside.

or about factors that may affect their phytochemical composition [2].The aim of this work is to establish the metabolic profile and to evaluate the antioxidant capacity of sprouts of species used in human diet: *Glycine max, Vigna radiata* and *Medicago sativa*. Phenolic compounds and sterols were analyzed by HPLC-DAD, organic acids by HPLC-UV, fatty acids and volatile compounds by GC-MS. Antioxidant activity was assessed by DPPH, nitric oxide and superoxide radical scavenging assays.The results obtained reveal some differences among the three species, particularly concerning volatile and phenolic compounds, organic acids and sterols. Also their antioxidant potential was distinct, but good activities were observed for all of them. Thus, this study showed a good potential of these matrices as sources of bioactive compounds consumed in human diet.References:[1] Pei-Yin, L. and His-Mei, M. (2006), *Bioactive Compounds in Legumes and Their Germinated Products,* J. Agric. Food Chem., 54, 3807−3814.[2] Myung-Min, O. and Rajashekar, C.B. (2009), *Antioxidant content of edible sprouts: effects of environmental shocks*, Journal of the Science and Food and Agriculture, 89, 2221-2227.

P12 *In vivo* toxicity profile of naphthoquinones in zebrafish embryos

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Naphthoquinones (NQ) are mostly plant-derived compounds displaying promising antibacterial, anti-inflammatory and antiparasitic activity in cell-based assays. Currently, little is known about NQ chronic exposure at both organ and whole-organism levels.
We exposed zebrafish (*Danio rerio*) eggs from 4-80h post-fertilization (hpf) to the following NQ (Fig.1): atovaquone (ATV), diosquinone (DQN), juglone (JGL), menadione (MND), naphthazarin (NTZ) and plumbagin (PLB). Embryos were scored for mortality, teratogenicity, heart and hatching rates. LC_{50} and heart rate values are reported at 80hpf. Valproic acid (VPA) and trichostatin A (TSA) were used as positive controls.
Lethality was (LC_{50} in µM): PLB-0.36 > DQN-0.47 > ATV-0.50 > JGL-0.57 > TSA-0.75 > MND-2.03 > NTZ-2.38 > VPA-4900. Teratogenicity was (minimal teratogenic concentration in µM): PLB-0.3 > TSA-0.5 > MND-1 > VPA-1000; Heart rate in beats/min (concentration in µM): Solvent-173 > DQN-155 (1) > JGL-153 (0.3) >NTZ-150 (1) > PLB-142 (0.3) > MND-138 (1) > TSA-132 (0.5) > VPA-82 (1000). MND typically caused haemolysis, cardiac oedema and bradycardia at 1µM.
NQ structure analysis suggests that OH group in C5 (PLB, DQN and JGL) or in C3 (ATV) influences lethality. Interestingly, two OH in NTZ attenuate toxicity, possibly due to double keto-enol tautomerism. The teratogenicity may be attributed to CH_3 group present in C2 of the teratogenic NQ (PLB and MND).

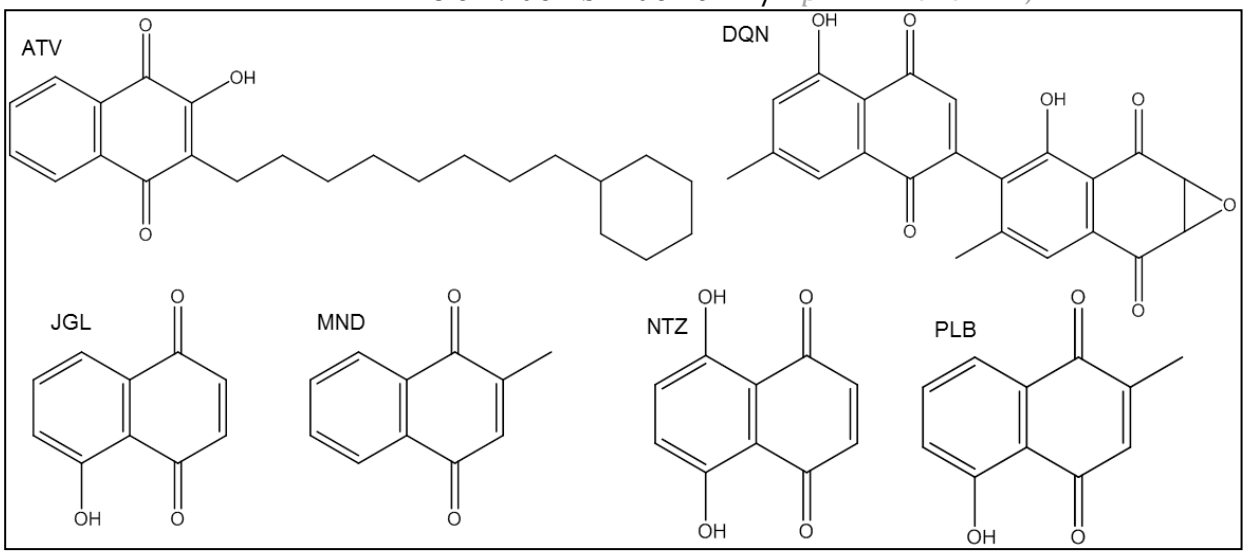

Fig.1: Chemical structures of NQ.

ACKNOWLEDGMENTS: FCT SFRH/BD/63852/2009 PhDGrant: Pinho B; IJUP2010#195: Oliveira JM

P13 Cloning of a mannitol transporter (VvMaT1) and a mannitol dehydrogenase (VvMTD1) from *Vitis vinifera*: evidences for a mannitol role in salt/drought stress tolerance

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Mannitol is the most widely distributed sugar alcohol in nature, and has been reported in more than 100 species of vascular plants of several families, including the Rubiaceae, Oleaceae and Apiaceae. In the moderately salt tolerant plant *Olea europaea*, we have found that mannitol is an important carbon and energy source and an osmoprotectant. So far, such functions for mannitol were not demonstrated in grapevine. In the present study, the gene encoding a putative *Vitis vinifera* Mannitol Transporter (VvMaT1) and a gene encoding a putative *V. vinifera* Mannitol Dehydrogenase (VvMTD1*)* were cloned from grape berry mesocarp and from grape cultured cells, respectively. Both cDNAs display high similarities to the respective homologs in olive and celery. A primary characterization of mannitol dehydrogenase activity in crude extracts from cells cultivated in the presence of mannitol allowed the estimation of a K_m = 30.1 ± 2.36 mM mannitol and V_{max} = 0.63 ± 0.01 µmol h^{-1} mg $protein^{-1}$. Mannitol oxidation was almost 5-fold lower in cells cultivated in the absence of mannitol. Incubation of cultured cells with 100 mM NaCl considerably decreased mannitol dehydrogenase activity (11-fold), suggesting an intracellular accumulation of this osmoprotectant in salt stress (or drought) conditions. Localization, functional analysis and regulation studies are being undertaken to elucidate the coordination between membrane transport of mannitol and its intracellular oxidation on grapevine defense against drought. *Conde C, Delrot S, Geros H. 2008. Physiological, biochemical and molecular changes occurring during olive development and ripening. J Plant Physiol. 165:1545-62. Conde C, Silva P, Agasse A, Lemoine R, Delrot S, Tavares R and Gerós H. 2007. Utilization and Transport of Mannitol in Olea europaea and Implications for Salt Stress Tolerance. Plant Cell Physiol. 48: 42–53.* *This research was supported by Fundação para a Ciência e Tecnologia (PTDC/AGR-ALI/100636/2008; to Artur Conde grant ref. SFRH/BD/47699/2008)*

P14 A 2D in vivo approach to study photosynthesis in grape berry

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Is argued that fruit photosynthesis serves mainly as a respiratory CO_2 refixation mechanism [1] but its contribution to growth and metabolism, localization and dynamics during fruit development are poorly known. Unlike the leaves, fruit volume imposes a constraint to photosynthesis by limiting light penetration. However, the patterns of chlorophyll distribution are apparently independent of a light intensity gradient. Microscopic observations of transversal slices of green stage grape berries (6-8 weeks after fruit set) of Alvarinho cultivar, revealed that exocarp cells, mesocarp cells next to vascular bundles, and seed coat cells present higher chlorophyll contents than inner mesocarp cells. The photosynthetic activity was determined on this material by Imaging-PAM fluorometry, a powerful tool for 2D mapping of *in vivo* photosynthesis. In 2 mm-thick grape berry discs, chlorophyll fluorescence parameters were estimated (F_v/F_m and Φ_{II}), and rapid light curves (RLC) were performed. Exocarp and seed coats of green berries showed the highest F_v/F_m values (*ca.* 0.6-0.7), and mesocarp cells around 85% of that value. Exocarp from mature grapes maintained F_v/F_m values during maturation, but in mesocarp and seed coats this value strongly decreased. ETR_r were very sensitive to increasing light intensities and decreased with grape berry maturation. Our future prospects include the implication of photosynthesis on grape berry solute contents (sugars, acids), fruit and seed development.

[1] Guido Aschan & Hardy Pfanz, (2003): Non-foliar photosynthesis-a strategy of additional carbon acquisition-Flora **198**, 81-97

P15 Contribution to the knowledge of Middle Holocene forests of Northern coast of Portugal

Helena Ribeiro[1], Astrid Bernal[2], Deolinda Flores[1],José Pissarra[2,3], Ilda Abreu[1,2], Juan Vidal Romani[4], Fernando Noronha[1]

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Coastal zones are particularly vulnerable to global climate changes. Through paleobotanical studies in samples recovered from these areas it is possible to withdraw evidence of how sea level fluctuations influenced past vegetation and reconstruct existing palaeoenvironments. In this study, one organic-rich formation found in the coastal zone of Castelo do Neiva (Viana do Castelo), where the actual beach is present, was studied. Pollen and wood macrofossil remains were analyzed as well as its petrographic characteristics. The formation was dated, by ^{14}C, with 5880±60 BP, corresponding to middle Holocene. At this time sea level was lower and climate conditions would correspond to a period of colder winters and hotter summers than present. Pollen results show a clear dominance of oak woodland forest mixed with

Corylus spp. and *Alnus* spp., as well as the presence of hydrophytes species, such as *Myriophyllum* spp.and *Typha angustifolia*, and the Filicale *Isoetes* spp. The macroremains analysis was coherent with pollen results, showing the presence of *Quercus robur* L. wood. The organic matter of this organic-rich mud is at an early evolutionary stage compatible with a peat stage. Our results showed the presence of a fresh water swamp forest occupying the area of the actual beach, suggesting different climatic conditions relatively to present times.

P16 COPPER TRANSPORT IN GRAPE BERRY VACUOLES: CLUES OF A Cu^{2+}/H^+ ANTIPORTER INVOLVEMENT

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Mineral elements play pivotal functions in all living organisms; however, their excess may be a cause for toxicity. Plants are major targets for heavy metal stress and have developed a number of mechanisms to withstand the elevated metal levels. In grapevine, copper toxicity is a major topic of concern due to the intensive and long term use of copper based fungicides against diseases caused by pathogens such as downy mildew and botrytis. In this study, purified intact vacuoles isolated from grape berry cells (cv. Cabernet Sauvignon) cultivated under different copper availabilities were used as a model system to study the activity of $V\text{-}H^+$-PPase and the involvement of Cu^{2+} compartmentalization into the vacuole as a mechanism of tolerance in *Vitis vinifera*. Cultured cells were able to grow with up to 100 µM $CuSO_4$ in the culture medium but with a substantial modification of the final population size when compared to the control cells grown without copper. At 500 µM $CuSO_4$, cells were unable to grow. Pyrophosphate (PPi)-dependent H^+-transport was higher in intact vacuoles from 40 µM Cu^{2+}-grown cells than in intact vacuoles from control cells. Cu^{2+}-dependent dissipation of a pre-established pH gradient was used to measure Cu^{2+}/H^+ exchange in intact vacuoles. The initial rates of H^+ efflux followed Michaelis–Menten kinetics and the V_{max} of H^+ dissipation was higher in 40 µM Cu^{2+}-grown cells when compared to the control. The increase of both pH gradient across the tonoplast and Cu^{2+} dependent H^+ dissipation in response to increased availability of the heavy metal suggests that the sequestration of the cation into the vacuole contributes to Cu^{2+} tolerance in *V. vinifera* via a putative implication of a Cu^{2+}/H^+ antiporter.

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P17 Evaluation of the conditions for photoconversion and imaging of Kaede and EosFP by laser scanning confocal microscopy in transformed plant cells

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The possibility to locally change the emission wavelength by focused UV light makes Eos fluorescent protein (FP) a superb marker for experiments aimed at tracking the movements of biomolecules within the living cell [1]. Monomeric EosFP-based probes retain all the qualities of single-colored fluorescent proteins while providing the additional capability of photoconversion. Both green and red fluorescent forms of mEosFP are stable and thus provide the highly desirable intracellular controls during prolonged live

imaging [2]. Kaede, another photoconvertible fluorescent protein that changes from green to red upon exposure to violet light, has been used as an intracellular optical marker to monitor cellular and intracellular

movements. Here we propose to study the conditions of conversion and imaging of Kaede and mEosFP in *Nicotiana tabacum* leaf epidermis transient expression. mEos and Kaede fused with a Golgi marker - sialyl-transferase (ST-Eos/ST-Kaede) were engineered and tested simultaneously with a mEos version with a signal peptide for endoplasmic reticulum (SP-Eos). *Nicotiana tabacum* leaves were Agrobacterium-infiltrated with the three constructs and imaged in a confocal laser-scanning microscope (SP2 LSCM, Leica), with a 488 excitation laser for green fluorescent detection and a 568 excitation laser for red fluorescent detection. mEosFP is easily photoconverted to a red color following an approximate 10s exposure to UV illumination, while the images of ST-Kaede taken 3 days after infiltration were inconclusive. More testes are in progress using plant aspartic proteinases cardosin A and cardosin B which have been extensively studied in our laboratory either in native plant *Cynara cardunculus* or in genetic transformed models such as *Nicotiana tabacum*. Therefore, fusions of mEos and Kaede will be performed to investigate the use of photoconvertible fluorescent proteins as an important tool to explore the trafficking pathways of these proteins in living cells.

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P18 Study of cardosin B Plant Specific Insert in protein processing and transport to the vacuole

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Cardosin B is an aspartic proteinase (AP) abundant in cardoon (*Cynara cardunculus*, L.) flowers, where it has been found in the cell walls of transmitting tissue, in the vacuoles of petals and associated with programmed cell death in the ovule nucellus and anther tapetal cells. Interestingly, cardosin B accumulates in vacuoles when expressed in *Nicotiana tabacum* leaves. The PSI (Plant Specific Insert) domain is quite common among plant APs and it is thought to possess other functions than vacuolar sorting within the cell, such as interactions with cellular membranes or defence mechanisms. However, regarding the maturation and trafficking of cardosin B, the function of PSI is still uncertain. To uncover the real implications of PSI in this matter, several mutagenic PCR techniques were performed in order to obtain mutant cardosin B constructs: cardosin B with mutations on PSI's cleavage sites, and cardosin B with cardosin A's PSI. Constructs were expressed in transiently transformed *Nicotiana tabacum* leaves and to confirm the protein synthesis extracts from leaves and vacuoles were analyzed by Western Blotting. Furthermore, a fluorescent protein (mCherry) was incorporated in one of the constructs, allowing its sub-cellular localisation and tracking by confocal laser scanning microscopy (CLSM). At this point, results showed that cardosin B sorting was not affected by the mutation on the cleavage sites of the PSI. The transport to the vacuole of this truncated form was, however, slower than in the non-mutated form. When the PSI was replaced by cardosin A's PSI, the sorting of the AP was not affected, though the processing was belated. Nevertheless, further studies are still necessary to unveil the role of PSI in the sorting and processing of APs.

P19 Cytostatic activity of sulforaphane in human osteosarcoma lines

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Cruciferous vegetables such as broccoli, cauliflower, cabbage and brussels sprouts, contain many bioactive components and are characterized for their high content of glucosinolates, which enzymatically hydrolized, originate biologically active compounds such as isothyacianates (ITC). These compounds are mainly associated to the chemoprotective effects of high cruciferous vegetables intake. Sulforaphane (SFN), mostly present in broccoli, is the ITC most extensively studied. In the lastest years SFN has received a great deal of attention, focused on its anti-tumoral proprieties. Several studies have shown the ability of SFN to cause cell cycle arrest, depending on the cell line under study. However, regarding osteosarcoma lines, the effects of SFN on cell proliferation and cell cycle progression are still poorly understood. The human osteosarcoma cell line MG-63 was exposed to 5µM and 10µM of SFN. After 48h, the highest SFN dose reduced cell proliferation and viability in about 45% of the control. Cell cycle progression was also affected and an increase in the percentage of cells in the G2 phase was detected for the highest SFN dose. Our results indicate that SFN inhibited proliferation and induced cell cycle arrest in human osteosarcoma cells.

P20 Mutant cardosin A constructs and their expression in *Nicotiana tabacum* for the study of the Plant Specific Insert

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Cardosin A is an aspartic proteinase (AP) abundant in cardoon flowers (*Cynara cardunculus*, L.). Sub-cellularly it accumulates in vacuoles and, like other plant APs, possesses an internal segment called “Plant Specific Insert (PSI). This domain has been implicated in vacuolar sorting both due to its action as a sorting signal and to its ability to interact with lipid membranes. It has also been associated with other cellular processes namely in membrane reorganization and solute leakage during seed germination, and in defense mechanisms. To unveil its true function in both cardosin A maturation and trafficking processes we have applied several mutagenic PCR techniques for constructing mutated forms of cardosin A. Results were analyzed trough Western blotting after protein isolation from leaf vacuoles preparations, in some constructs a fluorescent protein (mCherry) was incorporated. This approach allowed us to visualize its sub-cellular localization and *in vivo* movements through confocal microscopy. Preliminary results showed that point mutations inserted into the amino-acid sequence prevented the cleavage of the PSI and resulted in cell death and plant senescence. Furthermore, the swapping of the PSI from cardosin A with the PSI from another AP – cardosin B – resulted in a mixed western blotting profile, which indicates that the original protein may have gained some of the characteristics of cardosin B processing, such as a slower pattern of accumulation. It also confirms the retention in the ER, observed by confocal microscopy. To better assess the physiological role this domain may play in APs further studies are being undertaken in our lab.

P21 EFFECT OF HIGH-TEMPERATURE ON SUGAR TRANSPORT IN GRAPE CELLS

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Berry sugar content 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. Also, 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. VvHT1 (*Vitis vinifera* hexose transporter 1) is a high affinity plasma membrane H^+-dependent symporter with broad specificity for monosaccharides abundant at early stages of berry development. The expression of this transporter is tightly regulated by sugars at transcriptional and post-translational levels (1). In the present study we aimed at the elucidation of the effect of extreme temperature and temperature fluctuations on sugar transport in grape cells. Results showed that a temperature treatment of 38ºC for 12 h decreased by 40% the V_{max} of ^{14}C-glucose transport in CSB (Cabernet Sauvignon Berry) cells. Contrarily, abscisic and salicylic acid stimulated sugar uptake. The down-regulation of glucose uptake mediated by high temperature corroborated the observed decrease of the VvHT1 levels in the plasma membrane. Additionally, proteomic analysis of the plasma membrane of CSB cells, allowed the identification of several proteins up-regulated in response to high temperature.

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P22 Interaction between two co-occurring fungi present in chestnut orchards

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Hypholoma fasciculare is a wood-decaying basidiomycete with a worldwide distribution, including tropical, temperate and boreal forest ecosystems. In Trás-os-Montes region (Northeast of Portugal) this species is commonly present in soils of several habitats, including chestnut and oak tree forests. The ectomycorrhizal fungus *Pisolithus tinctorius* is another species with high occurrence in those ecosystems. The present work intends to study the *in vitro* interaction between *H. fasciculare* and *P. tinctorius* through evaluation of fungal growth, changes on hyphae morphology, the production of volatile compounds and lytic enzymes. The results obtained showed that *H. fasciculare* inhibited significantly the growth of *P. tinctorius* in 49%, long before hyphal contact of their colonies. This inhibition could be result from the liberation from *H. fasciculare* of volatile compounds and/or diffusible inhibitory substances, such as extracellular enzymes. Alteration in the production of volatile compounds, distributed in several chemical classes (alcohols, ketones, aldehydes, terpenes, among others) was detected over the time course of interaction. In addition, amylase, cellulase, laccase and lipase were produced by *H. fasciculare*. The possible role of these compounds during interaction will be discussed.

P23 Competitive interactions between ectomycorrhizal and saprotrophic fungi on chestnut tree

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In Northeast of Portugal the macrofungal community associated to chestnut tree (*Castanea sativa* is rich and diversified. Among fungal species, the ectomycorrhizal *Pisolithus tinctorius* and the saprotroph *Hypholoma fasciculare* are common in this habitat. The aim of the present work was to assess the effect of the interaction between both fungi on growth, nutritional status and physiology of *C. sativa* seedlings. In pot experiments, *C. sativa* seedlings were inoculated with *P. tinctorius* and *H. fasciculare* individually or in combination. Inoculation with *P. tinctorius* stimulated the plant growth and resulted in increased foliar-N, -P, and photosynthetic pigment contents. These effects were suppressed when *H. fasciculare* was simultaneously applied with *P. tinctorius*. This result could be related to the inhibition of ectomycorrhizal fungus root colonization as a result of antagonism or to the competition for nutrient sources. If chestnut seedlings have been previously inoculated with *P. tinctorius*, the subsequent inoculation of *H. fasciculare* 30 days later did not affect root colonization and mycorrhization benefits were observed. This work confirms an antagonistic interaction between ectomycorrhizal and saprotrophic fungi with consequences on the ectomycorrhizal host physiology. Although *P. tinctorius* is effective in promoting growth of host trees by establishing mycorrhizae, in the presence of other fungi it may not always be able to interact with host roots due to an inability to compete with certain fungi.

P24 Cyanobacteria in the intertidal zones of the Portuguese coast

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

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P25 Identification and analysis of cadmium transporting HMA genes in *Solanum lycopersicum*

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Tomato (*Solanum lycopersicum*) is one of the model plants from the Solanaceae family and the first to have its genome sequenced and publicly available (the ongoing International Tomato Genome Sequencing Project (ITGSP) is available at http://solgenomics.net).

The HMA family (P1B-type Heavy Metal ATPases) is a type of heavy metal transporters responsible for the metals loading to the xylem. The HMA1 to HMA4 genes in *Arabidopsis thaliana* are known to possess Cd transport ability.

Using sequence information from the ITGSP, with data mining and gene prediction techniques, 2 distinct HMAs (HMA1 and HMA2) were identified and characterized in tomato.

HMA1 was identified in the tomato genome and the cDNA prediction program wise2 deduced a coding ORF that has 71% identity with HMA1 of *A. thaliana*.

HMA2, was also identified in tomato with a wise2 predicted coding ORF that has 69% identity with HMA2, 3 and 4 of *A. thaliana*, but only up to the first 2100 bp of the ORF. The remaining sequence has a very low identity to the *A. thaliana* HMAs. An EST BLAST search was conducted with the referred interval, revealing that this identity difference between *A. thaliana* HMA2/3/4 and HMA2 of tomato is consistent in the Brassicaceae family and is characteristic of the Solanaceae family.

We expect that these metal transporters identified in Tomato will be a stepping stone in the understanding of how each one specifically contributes to the metabolism of heavy metals in Solanaceae.

P26 PrimerIdent 2.0: An improved tool for specific conserved primer design

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PrimerIdent is a primer design tool conceived to help designing DNA oligonucleotide primers suitable for amplification of DNA sequences from understudied plant species or other organisms (conserved primers), and primers that can effectively distinguish cDNA sequences from the same organism that share great identity among themselves (such as isozymes).

The program is currently implemented as a web service in a website - http://primerident.up.pt - hosted by the University of Porto.

PrimerIdent 2.0 is a new version that includes extended features, such as support for larger sequence alignments, degenerated primers analysis/design, multiple template sequences for primer design and identity based sorting of the results. Such improvements allow more complex approaches that include specific amplification of individual isogenes, across species.

Thanks to these improvements it was possible to identify, for the first time, the cDNA sequences of the ZIP family of metal transporters in plants from the Solanaceae family, namely Tomato (*Solanum lycopersicum*),

Potato (*Solanum tuberosum*), Eggplant (*Solanum melongena*), Tobacco (*Nicotiana tabacum*) and Black Nightshade (*Solanum nigrum*).

It is expected that the usage of this primer design tool will help to enable the design of primers for specific amplification of individual members of multigenic families, across species and also to evaluate the differential expression of isogenes.

P27 PLASTIDIC AND CYTOSOLIC GLUTAMINE SYNTHETASE ISOENZYMES ARE DIFERENCIALLY REGULATED BY NITRIC OXIDE (NO) IN MEDICAGO TRUNCATULA

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Glutamine Synthetase (GS), is a crucial enzyme in nitrogen metabolism as it catalyses the first step at which nitrogen is brought into cellular metabolism and thus the enzyme must be precisely regulated. The complete understanding of the mechanisms controlling GS activity in plants is of crucial importance and many studies have been devoted to understand how GS is regulated in plants, which have shown that the enzyme is subjected to tight controls operating at many different levels. Although the regulation of GS at the transcriptional level has been well studied, there is clearly a lack of information concerning its posttranslational regulation. Nitric oxide (NO) and its related species can induce important posttranslational protein modifications through S-nytrosylation and nitration. Is this study we evaluated the effect of NO on the activity of two different GS isoenzymes from the model legume M. truncatula, the cytosolic GS1a and plastidic GS2a. In vitro incubation of the enzymes with reactive nitrogen species producers (peroxynitrite or tetranitromethane) induced a dose-dependent loss of GS activity that could be related to an increase in nitrotyrosine immunoreactivity. To investigate whether the loss of activity was related to cysteine nytrosylation or tyrosine nitration, we treated the enzymes with a number of reagents that selectively modify cysteine or tyrosine residues. The results indicate that GS1a inactivation is a result of tyrosine nitration, whereas GS2 inactivation appears to be a consequence of cysteine nitrosylation. Furthermore, incubation of the enzymes with epicatechin, a selective nitration inhibitor, was able to prevent GS1a inactivation by NO, but had no significant effect in preventing the inactivation of GS2 by reactive nitrogen species. The results indicate the cytosolic GS isoenzyme GS1a is post-translationally regulated by tyrosine nitration in *M. truncatula* whereas the plastidic isoenzyme GS2 appears to be regulated S-nitrosylation. Further experiments are underway to investigate the physiological significance of these posttranslational modifications for plant nitrogen metabolism.

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P28 Isolated brain mitochondria are affected by compounds from Hypericum perforatum contributing to protect neurons from excitotoxic insults

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We have shown that *Hypericum perforatum* extracts (HP) effectively protect from neurodegeneration, by mechanisms that involve antioxidant properties. Prolonged N-methyl-D-aspartate (NMDA) receptor activation can result in cell failure to maintain calcium homeostasis that can result from loss of mithocondria function, usually, preceding cell death. Here, we evaluated the mitochondrial involvement in excitotoxic neuroral death and neuroprotection in the presence of isolated compounds from HP.

The present studies strongly indicate that phenolics present in HP have neuroprotective properties against excitotoxic insults. Specifically, Biapigenin prevented calcium deregulation induced by acute excitotoxic

stimulus, possibly due to attenuation of mitochondrial dysfunction by protecting it from oxidative stress and from the loss of mitochondrial membrane potential ($\Delta\Psi_m$), after exposure of mitochondria to high calcium levels.

P29 Hypericum perforatum plant defence kills Agrobacterium and prevent T- DNA transfer

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Agrobacterium-mediated plant transformation is an indispensable tool in modern plant biology. Even though this system has been currently applied to several crops, many plant species including Hypericum perforatum (HP) remain recalcitrant to Agrobacterium-mediated transformation. In this study, we analyzed the basis of HP recalcitrance to Agrobacterium-mediated transformation using cell suspension culture. When co-cultivated with Agrobacterium, HP cells swiftly produced an intense oxidative burst typical reaction of plant defense. Subsequently, Agrobacterium viability started to decline and reached 99% mortality within 12 h, while the HP cells remain viable. Genomic DNA isolated from the co-cultivated HP cells did not show DNA fragmentation indicating that the plant cells did not suffer apoptotic process. Northern blot analysis of total RNA extracted from HP cells revealed that accumulation of phenylalanine ammonia lyase (PAL) mRNA in the HP cells was highly correlated to the time-course mortality rate of Agrobacterium indicating that the activation of phenolic metabolism might have been played an important role in the killing of the bacterium and/or in protecting the HP cells.

From our study, it is clear that a recalcitrant plant like HP recognizes Agrobacterium as a potential pathogen and rapidly evokes its defense response. This leads to the mortality of Agrobacterium before it could transfer its T-DNA to the plant cells which would be the one of the main reason for the recalcitrance of HP towards Agrobacterium-mediated transformation. This is the first evidence showing that the recalcitrance of a plant species involves direct killing of Agrobacterium during co-cultivation.

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