BAM

BIO ALUMNI MEETING

II ENCONTRO ALUMNI DE BIOLOGIA DA UNIVERSIDADE DE ÉVORA

PROGRAMA / RESUMO DE COMUNICAÇÕES

ÉVORA, 2 DE ABRIL DE 2013
COLÉGIO DO ESPIRITO SANTO

Morpho-physiological responses of two Jatropha curcas accessions under water stress and recovery

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Abiotic stresses such as drought or salinity are responsible for important crop losses. Species with high plasticity response to extreme environmental conditions may help to unveil new stress tolerance mechanisms. *Jatropha curcas* has been described to be adapted both to drought and poor soils, although its major interest is due to the quality of its oil-seed for biofuel production. The reason of *J. curcas* tolerance to water-limiting conditions has not yet been explored. To address this, we have studied the response of two *J. curcas* accessions from different climate origins (wet tropical and semi-arid climate) to water stress. Potted plants with 36 days old were subjected to continuous well watered conditions or to water withhold followed by recovery. Soil and plant water status, growth and biomass partitioning, leaf gas exchange and chlorophyll a fluorescence were assessed. We found no marked differences between accessions in the response to water stress. Both *J. curcas* accessions presented a dehydration-avoidance strategy, maintaining a high water status due to a strict stomatal control and reduced transpiration area. Moreover, despite the strong reduction on leaf gas exchange parameters under stress, the plants fully recovered after rehydration, suggesting a high photoprotection of the photosynthetic apparatus during severe water stress. The results presented suggest that *Jatropha curcas* accessions rapidly respond to drought, withstanding severe water stress and rapidly recovering after re-hydration, independently of its provenance. This reinforces this species plasticity to unfavorable conditions.
We acknowledge funding from FCT through project PTDC/AGRGPL/101435/2008.

P6.32

HOS1 is necessary for maintaining the period of the *Arabidopsis* circadian clock

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HOS1 is an E3 ubiquitin ligase required for regulating the low temperature signal transduction pathway in plants that promotes tolerance to freezing. There is a strong interaction between cold signal transduction and the circadian clock, as a correctly functioning clock is required for the magnitude and gating of gene expression in response to a cold stimulus. Here we show that *HOS1* is necessary for maintaining the 24-hour period of the *Arabidopsis* circadian clock.

Our assays demonstrate that *hos1* mutants exhibit a long period phenotype that is consistently observed in multiple light and temperature regimes, suggesting *HOS1* has a general role in the functioning of the central oscillator. The *hos1* clock phenotype cannot be explained by the previously published changes in the expression of cold signalling or flowering genes, as loss- and/or gain-of-function mutants in these genes do not recapitulate a long period phenotype, showing that known functions of *HOS1* are not causative of the circadian phenotype. *HOS1* has recently been suggested to be part of the plant nuclear pore, and HOS1-cyan emitting GFP fusions confirm the localization of HOS1 to the nuclear periphery. As with other nuclear pore mutants, we show that *hos1* exhibits an increase in poly(A) mRNA retained in the nucleus. RNA analysis shows the timing and amplitude of the core clock machinery is altered in *hos1*, in some cases dramatically.

Together, these data indicate that *HOS1*, perhaps through its ability to regulate mRNA export, is necessary for the circadian clock to run with correct periodicity.

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Poster Session – Sunday 1st July 2012

P6.33

The LHY transcription factor couples abscisic acid signalling to the *Arabidopsis* circadian clock

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The complex regulatory network underlying the oscillatory mechanism of the plant circadian clock is now well characterized, but we still know relatively little about how downstream processes are controlled. In order to investigate this question we identified genes that are under direct control of one of the oscillator components, the MYB transcription factor LATE ELONGATED HYPOCOTYL (LHY). Genome-wide binding sites for LHY were identified by chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-Seq).

Our results show that LHY regulates the transcription of several thousands of genes. Among these targets were a number of genes involved in the production and perception of the hormone abscisic acid (ABA) as well as downstream components of signal transduction pathways. Furthermore abscisic acid regulated elements were overrepresented within LHY-binding regions, suggesting that LHY acts to modulate the expression of ABA-responsive genes. Interestingly, ABA was also found to regulate expression of the *LHY* gene. A number of ABA-responsive transcription factors were found to bind the *LHY* promoter in yeast one-hybrid and chromatin immunoprecipitation assays. Furthermore, expression of the *LHY* mRNA was increased upon spraying of plants with ABA.

These results uncover a novel regulatory feedback loop between LHY and ABA signalling. ABA plays a role in regulating seed dormancy. It also acts to regulate growth in adult plants and is an important component of environmental stress responses. Current work aims to uncover temporal patterns of gene expression that are mediated by this interaction between ABA signalling and the clock, and their relevance to plant physiology.

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Poster Session – Sunday 1st July 2012

P6.34

Analysing the structural error and parameter uncertainty of two *Eucalyptus* models differing in representation of autotrophic respiration

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There is lively discussion about representation of autotrophic respiration (R_{aut}). An empirical approach is to model plant respiration, R_{aut}, as a constant fraction of gross primary production (P_G). In more mechanistic models, photosynthesis and respiration are simulated as partly independent processes. The aim of this work was to analyse the structural error and parameter uncertainty of two versions of a process-based model that differed in autotrophic respiration modelling. The original version (3PGN) was based on a constant ratio between net and gross primary production, while in a new version (3PGN*) R_{aut} was modelled as a function of temperature and biomass.

The two models were calibrated and evaluated using a comprehensive dataset consisting of forest growth experimental data and eddy-covariance measurements. The analysis was performed under a Bayesian framework consisting of model calibration, model comparison and analysis of model-data mismatch. In addition, global sensitivity analyses of the models were carried out by means of Morris screening.

Our results support the view that a more mechanistic approach to modelling photosynthesis and respiration as partly independent processes improved model performance.

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Poster Session – Sunday 1st July 2012

P6.35

Morphophysiological responses of two *Jatropha curcas* accessions under water stress and recovery

Margarida Oliveira (ITQB, Universidade Nova de Lisboa [UNL], Portugal), Helena Sapeta (ITQB, UNL, Portugal), Tiago Cruz (ITQB, UNL, Portugal), Tiago Lourenço (ITQB, UNL, Portugal) and Miguel Costa (ITQB, UNL, Portugal)

Abiotic stresses, such as drought or salinity, are responsible for important crop losses. Species with high plasticity response to extreme environmental conditions might help to unveil new stress tolerance mechanisms.

Jatropha curcas has been described to be adapted both to drought and poor soils, although the main interest in this plant is due to the quality of its oil-seed for biofuel production. The reason for *J. curcas* tolerance to water-limiting conditions has not yet been explored. To address this, we have studied the response of two *J. curcas* accessions from different climate origins (wet tropical and semi-arid climate) to water stress.

Thirty-six-day-old potted plants were subjected to continuous well watered conditions or to water withhold followed by recovery. Soil and plant water status, growth and biomass partitioning, leaf gas exchange and chlorophyll a fluorescence were assessed.

We found no marked differences between accessions in the response to water stress. Both *J. curcas* accessions presented a dehydration-avoidance strategy, maintaining a high water status due to a strict stomatal control and reduced transpiration area. Moreover, despite the strong reduction in leaf gas exchange parameters under stress, the plants fully recovered after rehydration, suggesting high photoprotection of the photosynthetic apparatus during severe water stress.

The results presented suggest that *J. curcas* accessions rapidly respond to drought, withstanding severe water stress and rapidly recovering after rehydration, independently of its provenance. This reinforces this species plasticity to unfavourable conditions.

We acknowledge funding from FCT through project PTDC/AGRGPL/101435/2008.

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Poster Session – Sunday 1st July 2012

P6.36

Shoot development controls variations in plant leaf area for *Medicago sativa* grown at contrasted densities in pure and mixture stands

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Understanding the factors affecting leaf area (LA) expansion is critical to predict changes in light interception and crop production. In *Alfalfa*, LA is usually described as the product of a leaf number, resulting from plant development, by an average leaf size resulting from leaf tissue expansion. Such a representation has yielded good results in pure stands, assuming that shoot development follows a fixed predetermined pattern. In mixtures with grasses, however, the prevailing light conditions can change a lot from pure stands and can vary significantly over the season as a result of the change in dominant species. The aim of this work was to rank the components of LA in their response to light availability in order to set up the basis of a generic LA model.

Two experiments were carried out outdoors with fertirrigated pots. The first studied LA and potential shoot development on isolated plants. The second studied modification of LA induced by plant spacing in pure stands (LD; HD) and mixture stands with tall fescue (LD_{mix}; HD_{mix}). Shoot number, shoot development (including branches) and leaf size were recorded.

The potential shoot development was stable in function of thermal time, showing a linear appearance of branches and leaves. Competition for light reduced plant LA expansion. At low light competition, both leaf size and shoot development contributed to explain variations in LA. At high density, shoot number and shoot development explained most of the variations. The different components of LA presented different responses to light availability.

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Poster Session – Sunday 1st July 2012

P6.37

Systems biology of root responses to nitrogen: Insights from nodulation and natural variants

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Plant development is remarkably plastic in response to changing environmental conditions. The ability of a plant to rapidly modify lateral root outgrowth in response to changes in nitrogen status in the soil is a dramatic example of such adaptation. If we could understand more about this, we could enhance crop productivity. We are analysing nitrogen responses in *Arabidopsis* natural variants (ecotypes). In an approach akin to expression quantitative trait loci mapping, this has involved combining genome-wide association studies in which 96 different ecotypes are associated with root architecture traits, with the association of transcriptome profiles from the response to nitrogen-treatment in a subset of the ecotypes.

We are also investigating the evolution of nitrogen-fixing nodulation. Transferring this ability to our staple crop species could provide a solution to environmentally detrimental and costly nitrogen fertilisers. We are using comparative genomics to compare gene expression responses during nodulation and to nitrogen influx in the legume *Medicago truncatula* to the non-legume *Arabidopsis thaliana* over multi-point time courses. Fluorescence-activated cell sorting is used to isolate individual root cell types relevant to these developmental pathways (pericycle and cortex) for expression analysis. By separating signals from different cell types we can deconvolute the genomic programs mediating cell identity from those conferring environmental responses and this will be able to gain new perspectives on the evolutionary origin of nodulation.

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Poster Session – Sunday 1st July 2012

P6.38

The role of the rice transcription factors OsEREBP1 and OsEREBP2 in the responses to abiotic stress

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Rice (*Oryza sativa* L.) is an important crop and it is affected by adverse environmental conditions, such as salt and drought stress. High salinity causes reduced growth and yield. To survive, plants need to perceive and transduce stress signals to activate the tolerance mechanisms. In rice, the salt-induced *Root Meander Curling* (*OsRMC*) encodes a receptor-like kinase proposed to be involved in salinity perception and described as a negative regulator of salt stress responses. In order to better understand the regulation of *OsRMC*, we have identified (by yeast-one hybrid) and characterized the function of two Ethylene Response Factor transcription factor binding to the *OsRMC* promoter. We have shown that both OsEREBP1 and OsEREBP2 bind to a GCC-like motif and negatively regulate *OsRMC* gene expression. Under salt stress, *OsEREBP1* transcript level was not affected but its protein level increased due to higher protein stability, indicating that post-translational modifications might be involved. Supporting this hypothesis, *Arabidopsis* plants over-expressing OsEREBP1, when subjected to high salinity, showed a response similar to wild-type. These transgenic plants also revealed that OsEREBP1 plays a role in abscisic acid and drought stress responses. The OsEREBP2 was shown to be a multifunctional transcription factor putatively involved in drought, salt, abscisic acid and cold responses. In addition, the abnormal phenotype displayed by *Arabidopsis* plants over-expressing OsEREBP2 and the deregulation of development-related genes suggested that OsEREBP2 has also a role in rice developmental processes.

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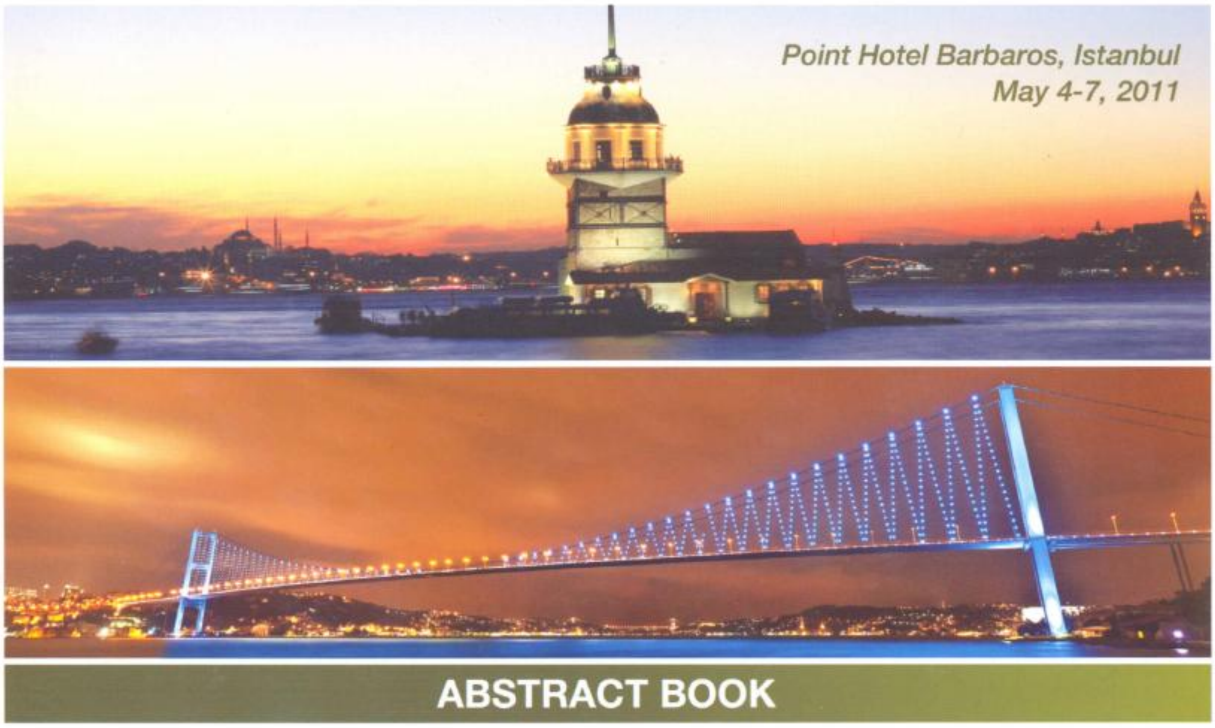
Poster Session – Sunday 1st July 2012

9.

Plant GEM Istanbul 2011
Plant Genomics European Meetings

Istanbul

The Plant Genomics European Meeting (Plant GEM) meets global challenges.



Point Hotel Barbaros, Istanbul
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ABSTRACT BOOK

P03

DROUGHT STRESS TOLERANCE IN TWO JATROPHA CURCAS ECOTYPES

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Species better adapted to extreme environmental conditions may help to unveil new tolerance mechanisms to transfer to specific crops. Jatropha curcas is quite well adapted to drought conditions and to poor soils, although its major interest relies on the quality of its oil-seed for biofuel production. Aiming to understand J. curcas tolerance to water-limiting conditions we are following physiological and molecular approaches to characterise the behaviour of two different ecotypes (adapted to arid or to wet tropical regions) when submitted to drought stress. Seeds from both ecotypes were germinated in a growth chamber with controlled conditions, for stress application and periodic analyses. At the physiological level we have monitored leaf gas exchange, water relationships and growth under water deficit and during recovery. To gain insight into the molecular mechanism of drought tolerance in J. curcas, putative key genes were targeted. In a first approach, target candidates described as involved in drought tolerance (regulating gene expression, osmolyte accumulation and signal transduction) were selected from heterologous species. Fragments of Jatropha homologous genes were obtained (300-600bp), sequenced, confirmed for homology, and extended by RACE-PCR. When targeting specific Jatropha sequences, specific primers were designed and used in expression analyses along the drought-stress period. Preliminary experiments suggest that the two ecotypes use discriminating strategies to cope with water stress, especially after re-hydration. The changes observed in the gene expression profiles of the target genes are not enough to justify the different plant behaviour. New experiments are being conducted using longer stress and recovery periods. The new data is providing a more clear idea of the most important time points for RNA collection, aiming to identify genes differentially expressed in the two ecotypes and putatively responsible for the differential adaptation.

P04

CHARACTERIZATION OF THE NITROSATIVE STATUS OF CITRUS PLANTS UNDER VARIOUS ABIOTIC STRESS CONDITIONS

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Nitric oxide (NO) is a key signaling molecule in plants. However, little is known about the metabolism of endogenous NO in plants under abiotic stress conditions. Using citrus plants (Citrus aurantium L.) exposed to six different abiotic stress conditions (high light intensity, continuous dark, low and high temperature, drought and salinity), several aspects of the NO-related metabolism were investigated. Abiotic stress treatments induced the de novo production of NO in the leaves of plants, particularly in the case of high temperature and high light conditions. Increased NO production was localized in different leaf tissues by confocal laser scanning microscopy using the fluorescent probe 4,5-diaminofluorescin diacetate. Salinity and drought resulted in strong nitrosative DNA damage induced by peroxynitrite, further indicating that abiotic stress situations elicited nitrosative challenges in citrus plants. S-nitroso glutathione reductase (GSNOR) appeared to play a signaling role in the high light, dark and high temperature responses. Protein Tyr-nitration targets were also characterized in leaves of citrus subjected to abiotic stress. Gene expression profiling experiments revealed that a wide array of NO-associated genes in citrus plants (including AOX, XO, GSNO, NOS, NiR and NR) were regulated by abiotic stress conditions. These results indicate that nitrosative responses are key components in the plant's behavior against environmental stimuli and provide further insights into NO-mediated signaling.

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HOTEL PORTO PALÁCIO

membrane. Additionally, proteomic analysis of the plasma membrane of CSB cells, allowed the identification of several proteins up-regulated in response to high temperature.

Conde C, Agasse A, Glissant D, Tavares R, Gerós H e Delrot S (2006) Pathways of glucose regulation of monosaccharide transport in grape cells. Plant Physiology 141, 1563-1577

POSTER PRESENTATION (P H16)

Unveiling new drought stress tolerance genes in *Jatropha curcas*: an integrative approach

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Environmental changes are occurring in the last decades. These changes have lead to a decline in the available arable land while food demand is increasing. This is partly due to crop losses resulting from abiotic stresses such as drought or salinity. In this context, species particularly well adapted to extreme environments may help to unveil new stress tolerance genes to aid in breeding programs. *Jatropha curcas* is attracting much attention due to the quality of its oil-seed for the production of biofuel. This species is also interesting due to its capacity to grow on marginal soils. This results from a natural tolerance to water limiting conditions. However, the reason for this tolerance has not yet been explored. Our goal is to extend the basic knowledge on the biology of *J. curcas* drought tolerance, combining molecular biology and morphophysiological analyses.

Putative key genes responsible for the drought tolerance mechanism of *J. curcas* were targeted from several candidates known from heterologous species as being involved in drought stress response (e.g. *ABF*, *ABI*) using degenerate primers. The obtained fragments were extended by RACE for full-lenght cDNA. Specific primers were designed for the *Jatropha* sequences and used in expression analyses during the drought-stress treatment. Other molecular techniques are also envisaged aiming to identify putative new genes responsible for drought stress tolerance (e.g. SSH library).

One-month old seedlings of two different ecotypes (adapted to arid or to wet tropical regions) were subjected to control and stress conditions (two weeks of drought, followed by one week of recovery). Morphophysiological measurements (e.g. photosynthesis efficiency) and RNA extractions for molecular analyses were performed weekly.

In control conditions both accessions presented similar physiological and growth pattern (stem diameter, number of leaves, stomatal index, etc). However, differences were observed in water use efficiency and photosynthetic rate. Under drought stress conditions, the wet tropical region line arrests growth and strongly invests in biomass production during the recovery period. On the other hand, the arid region line maintains a low growth rate during the drought period and presents a lower biomass production during the recovery period. Both photosynthesis rate and stomatal conductance reached values close to zero after eight days of water stress. Differences in these parameters could be found again during the recovery period where the wet tropical region line shows a faster recovery. The transcript profile of selected drought related genes during the stress treatment were also analyzed by RT-PCR.

In conclusion, the two ecotypes showed discriminating strategies to cope with water stress especially after re-hydration. Although gene expression profiles of the selected genes do not explain these differences alone, some correlations can be established between gene expression and plant responses.