3rd Forest Genomics Meeting:

Regulation of genome expression dynamics in forest trees,

3rd December 2014 – Oeiras, Portugal

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The third edition of Forest Genomics Meeting (FGM) will be held in Oeiras, Portugal, at 3rd December 2014, at ITQB/iBET Auditorium.

Forest trees are long-lived, woody perennial plants, and continuously challenged by ontogenetic and environmental changing conditions. Complex transcriptional and post-transcriptional networks control genome expression, with subsequent phenotypic variation.

During the previous editions, FGM has been an opportunity to discuss the state of the art on Eucalyptus genomics (1st edition) and transgenic forest trees potential (2nd edition).

The 3rd edition of FGM year will be dedicated to the progress on the understanding of genome expression regulation, in particular the role of transcription factors, smallRNAs, DNA methylation and histone modifications in forest trees.

The 3rd Forest Genomic meeting will be organized within the collaboration of different Portuguese and international institutions, and in the frame of PKBBE-TREEFORJOULES project and TRANSBIO program.

By the Organizing Committee,

Jorge A. P. Paiva

3rd Forest Genomics Meeting:

Regulation of genome expression dynamics in forest trees

3rd December 2014
Oeiras, Portugal
http://forestgenomicsmeeting2014.wordpress.com/

Schedule		Presentation Title	Speaker
09:00	09:20	Opening session	Cláudio Soares (ITQB Director), Pedro Fevereiro (representing iBET Direction, Jorge Paiva (iBET)
09:20	10:20	Systems genetics unravels genetic variation for wood cell wall chemistry in *Eucalyptus* hybrids.	Zander Myburg (Univ. Pretoria, South Africa)
10:20	10:40	Break	
10 :40	11:10	Transcription regulation in Eucalyptus xylem	Jacqueline Grima- Pettenati (LRSV, France)
11:10	12:00	*Selected oral presentations:* * Thermospermine and auxin levels in xylem tissues of *Populus* are balanced by a negative feedback loop mechanism	Ana Milhinhos (iBET/ITQB, Portugal)
		* Effects of flavonoid supplementation on Eucalyptus wood: Increased S/G ratio and saccharification, reduced extractive content, and differential expression of genes involved in cell wall formation a lignin metabolism	Jorge Lepikson-Neto (Univ. Campinas, Brazil)
		* Epigenetic regulation of cork development	Vera Inácio (ISA-UTL, Portugal)
12:00	13:30	Lunch	
13:30	14:30	How DNA methylation can participate to poplar phenotypic plasticity in response to variations in water availability	Stephane Maury (Univ. Orleans, France)
14:30	15:00	Post-translational regulation by miRNA in woody species	Jorge Paiva (IBET, Portugal)
15:30	16:00	Winter dormancy in woody plants: circadian and epigenetic control	Isabel Allona (UPM, Spain)
16:00	16:30	Break	
16:30	17:00	Contribution of P-KBBE – TREEFORJOULES for bioenergy production	Jacqueline Grima-Pettenati (LRSV, France)
17:10	17:30	General Discussion and Closure	

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Organizing Committee

Jorge Paiva – iBET (Portugal)

Susana Araújo – ITQB (Portugal)

Pedro Fevereiro – ITQB~(Portugal)

Rita Costa – INIAV (Portugal)

Jacqueline Grima-Pettenati – LRSV (France)

Olga Serra – Univ. Girona (Spain)

Acknowledgements:

TRANSBIO SUDOE

SUDOE
Programa de Cooperación Territorial
Programme de Coopération Territoriale
Programa de Cooperação Territorial
Territorial Cooperation Programme

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA EDUCAÇÃO E CIÊNCIA

CiB centro de informação de biotecnologia

EuropaBio
The European Association for Bioindustries

Invited Oral Communications

Systems genetics unravels genetic variation for wood cell wall chemistry in *Eucalyptus* hybrids.

A.A. Myburg

Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), and Genomics Research Institute (GRI), University of Pretoria, Private Bag X20, Pretoria, 0028, South Africa.

The completion of the genome of Eucalyptus grandis has generated a rich reference for the genus and a starting place for understanding genome diversity underlying variation in the growth and development of eucalypt trees. Segregating interspecific hybrid pedigrees provide an opportunity to study the genetic architecture and differentiation of quantitative traits such as fibre cell wall chemistry. We have collaborated with forestry companies in South Africa to develop experimental tree populations and genomic resources for genetic dissection of wood properties in Eucalyptus hybrids. Whole-transcriptome sequencing and metabolite profiling together with high-throughput DNA genotyping and genetic mapping in 283 F2 backcross progeny of an interspecific hybrid of E. grandis and E. urophylla has allowed us to begin modelling wood development as a biological system with the aim of identifying key regulatory factors and pathways modulating this process.

Transcriptional regulation of secondary cell walls in *Eucalyptus*

Marcal SOLER[1], Anna PLASENCIA[1], Hong YU[1], Victor CAROCHA[1,2,3], Raphael PLOYET[1], Jorge LEPIKSO-NETO[1], Eduardo L. CAMARGO[1], Hélène SAN CLEMENTE[1], Bruno SAVELLI[1], Nathalie LADOUCE[1], Alexander MYBURG[4,5], Jorge PINTO PAIVA[2,3], Fabien MOUNET[1], Hua CASSAN-WANG[1], Isabelle TRUCHET[1] & Jacqueline GRIMA-PETTENATI [1]

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Eucalyptus, the most planted hardwood worldwide, is also the second forest tree whose genome has been recently sequenced (Myburg *et al*, 2014). With the final goal of improving wood properties relevant to pulping or bioethanol production, we attempted to identify new transcription factors regulating the biosynthesis of lignified secondary cell walls (SCW) in *Eucalyptus* and *Arabidopsis* (Cassan-Wang *et al*, 2013). Although there have been recent major breakthroughs in identifying regulatory mechanisms involved in SCW formation in *Arabidopsis*, many unknown regulatory players remain to be discovered. Taking advantage of the recently published *E. grandis* genome, we carried out a genome-wide survey of several important gene families (MYB, NAC, Aux/IAA and ARF) and performed comparative phylogenetic analyses (Soler *et al*, 2014; Hussey *et al*, 2014; Yu *et al*, 2014a; Yu *et al*, 2014b). Remarkably, we could highlight woody-preferential and woody-expanded clades in the R2R3 MYB family. Using RNAseq and high-throughput RT-qPCR (Cassan-Wang *et al*, 2012), we examined the expression patterns of these genes in different tissues and organs during normal development and/or in response to environmental cues. This enabled us to identify new candidate genes (not studied before in any plant species) with preferential and/or specific expression in cambium and/or differentiating wood cells undergoing SCW thickening. Functional characterization of the most promising candidate genes is underway using over-expression and dominant repression constructs *in planta*.

Although protein-protein interactions are known to be crucial to regulate the activity of transcription factors, their roles in the regulation of SCW formation has been largely underexplored. With the goal of getting insights in this important post-transcriptional mechanism during SCW formation, we first constructed a yeast-two-hybrid library using *Eucalyptus* xylem RNA and screened it for partners of *Eg*MYB1, a R2R3 MYB transcription factor able to repress the lignin biosynthesis and SCW formation (Legay *et al*, 2010). We found several candidate partners, among which a histone linker (*Eg*H1). We validated the interaction between *Eg*MYB1 and *in planta* using FRET-FLIM assay. To further investigate the biological role of that interaction, we constructed *Arabidopsis* transgenic lines, either over-expressing *Eg*MYB1 alone, *Eg*H1 alone, or both proteins together. Histological analyses of these plants showed a dramatic reduction of SCW thickness and lignin levels in the *Eg*H1-*Eg*MYB1 overexpressing plants, much stronger than in *Eg*MYB1 plants alone. Finally, we performed a microarray comparing the transcriptomes from the stem base of these plants, which allowed us to highlight transcriptome remodelling in response to the *Eg*MYB1-*Eg*H1 interaction.

Altogether our results allowed the identification of new SCW regulators and interactions, increasing the complexity of the network regulating SCW but also opening new avenues to ultimately improve SCW composition for biofuel production.

Camargo et al, 2014, BMC Plant Biol, 14:256; Cassang-Wang *et al* (2013) Front Plant Sci 4 : 189; Cassan-Wang et al (2012) PCP 53: 2101–2116; Hussey et al, 2014, New Phytol , in Press; Legay et al. (2010) New Phytol., 188, 774-86.; Myburg et al. (2014) Nature, 2014.510: 356-362; Soler et al, 2014; New Phytol, in press; Yu et al, 2014a, Plos one, in press; Yu et al, 2014b, PCP in press

Acknowledgements; TreeForJoules Project

How DNA methylation can participate to poplar phenotypic plasticity in response to variations in water availability

Anne-Laure Le Gac, Clément Lafon-Placette, Alain Delaunay, Isabelle Le Jan, Régis Fichot, Franck Brignolas, Stéphane Maury

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Plant response to abiotic stress is a main challenge in agriculture. This is particularly relevant in a context of global climate change. The understanding of physiological as well as genetic/molecular processes controlling plant's response to abiotic stress will help to improve plant breeding. Recently, epigenetic mechanisms such as DNA methylation have been shown to participate to the control of plant development and their adaptation to environment through modifications of chromatin compaction and gene expression profiles. Phenotypic plasticity defines as the different phenotypes for a given genotype in distinct environments is a key process for plant to adapt to their changing environment. This is particularly relevant for perennial plants such as trees that are exposed to repeated fluctuations of their living conditions. In this context, drought is a significant threat to forest health and agro-ecosystem productivity. With the availability of its genome and its important natural genetic and phenotypic variations, Populus became a model tree. Poplars (Populus spp.) are among the fastest growing trees in temperate latitudes. Their high productivity is associated with large water requirements. The concept of water deficit tolerance, when applied to cultivated tree species such as poplars, has been defined as the ability to limit the decrease of biomass production in response to a moderate water deficit. Variations of DNA methylation have been reported between genotypes, tissues but also in response to drought and geographic location origin. Nevertheless, the relationships between gene body DNA methylation, gene expression and the phenotypic plasticity still need clarification. This is the objective of my work in the LBLGC laboratory at the University of Orléans. Our experimental data have been obtained using poplar hybrids grown in greenhouse, nursery and pedoclimatic sites under different water availability conditions. We have focused our epigenomic approach on the shoot apical meristem that is the center of the shoot morphogenesis. This may ultimately help to improve the actual predictive phenotypic models based on genetic variations for selection.

Winter Dormancy In Woody Plants: Circadian And Epigenetic Control

Isabel Allona

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Trees from temperate and cold regions have developed an adaptive mechanism called winter dormancy aimed to survive the extreme temperatures that take place during this season. In our laboratory we try to understand the circadian and epigenetic control of this process, that at the same time determine the geographical distribution of woody perennials.

The circadian clock is involved in the transduction of daylength and temperature signals that regulate the winter dormancy. In chestnut in response to cold, circadian clock genes lose their rhythmic expression and become constitutively activated (Ramos et al., 2005; Ibáñez et al., 2008). This molecular response to cold also occurs in Arabidopsis but only when light/dark cues are absent. We are interested in decipher how this specific molecular response is controlled in order to shed light on the biological meaning of the constitutive activation of circadian clock genes in response to cold.

Global changes in 5mC DNA methylation have been shown in the transition of developmental stages in plants such as chestnut bud set and burst, flowering in azalea, aging in pine trees among other. In our laboratory we have observed that methylation was significantly higher in poplar stem during dormancy compared to the growing season (Conde et al., 2013). However, the mechanism and the enzymes involved in the modification of the methylome and its control over those development processes remain to be identified. We have identified and characterized the first 5mC demethylases found in trees.

Post-Translational Regulation In Forest Trees

Victor Carocha[1], Ana Carvalho[2], Clara S. Graça[3], Susana Pera[4], Grégoire Le Provost[5] and Jorge A P Paiva[3],

(1)ITQB-UNL, Oeiras - Port, Portugal, (2)Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology (IBB/CGB), Vila Real, Portugal, (3)Instituto de Biologia Experimental e Tecnologica, Oeiras, Portugal, (4)Instituto de Investigação Científica Tropical, Lisboa, Portugal, (5)UMR 1202 BIOGECO - INRA / Université Bordeaux 1, Site de Recherches Forêt Bois de Pierroton, Cestas, France

Pines (*Pinus* spp) and *Eucalyptus globulus* are important species in the Portuguese forest plantations, being used for timber, pulp, and paper production. Due to their economic and ecological importance several transcriptomic and proteomic resources were made available and allowed to identify major molecular players involved in xylogenesis and biotic and abiotic responses. However, the understanding of molecular mechanisms regulating xylogenesis and these responses, including the post-regulation mediated by miRNAs, are still incipient. MicroRNAs are small non-coding RNAs (21-24bp), being key post-transcriptional regulators of gene expression in different developmental and stress-related biological processes. In plants miRNAs act by down-regulating mRNA expression either by cleavage or by translational repression, through direct base-pairing to target sites in mRNAs.

In order to get new insights on these post-transcriptional regulation mechanisms we are developing resources to study the post-transcriptional regulation mediated by miRNAs of *Pinus pinaster, P. sylvestris* and *Eucalyptus globulu*s. A catalog of miRNA of xylem, phloem, and leaves tissues were produced, using smallRNA-Seq data and appropriated bioinformatics analysis methodologies. In addition, degradome libraries for those tissues were prepared and used to identify their target genes. Here we present the results of our work, shedding light on identification of miRNAs and their targets in these important tree species in Europe.

Invited Speakers

JACQUELINE GRIMA-PETTENATI

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Academic Qualifications:
Habilitation Degree (HDR), 1995/ PhD Thesis in Pant Physiology, 1985-University Toulouse, France
Appointments: Sept 04 to Sept 05, Invited Professor at Laval University (Québec, Canada)/1997-present, Research Director CNRS (DR2), Toulouse/1988-1996 CNRS scientist/1987-1988 EEC Postdoctoral fellowship Imperial Chemical Industries (Runcorn, UK)

Research Projects

J Grima-Pettenati (JGP) has a long-standing experience in molecular biology of lignin biosynthesis in woody species (Eucalyptus, poplar). She participated in pioneer work aimed at modifying lignin content in plants of economic importance by genetic engineering in the frame of two European projects (OPLIGE & TIMBER). The current objective of her team is to get a better understanding of the genetic control of wood formation and quality in Eucalyptus. Functional genomic approaches are being developed to identify and characterise major players involved in wood formation with a particular emphasis on transcriptional and post-transcriptional regulators. This on going work has great relevance to the current proposal and the team has all necessary expertise to complete the project objectives. JGP participated in more than 20 projects (European, National, bi-tri lateral...), is currently involved in the FP7 project RENEWALL “Improving plant cell walls for use as renewable industrial feedstock”. She is the coordinator of the running ERAPG project EUCANET [2007-2010], “Eucalyptus genomics research network for improved wood properties and adaptation to drought” (8 partners, 3 countries). JGP has already successfully collaborated/published with several partners involved in TreeforJoules (P2, P3, P4, P8, P9, P10). JGP
has supervised 11 PhD theses including 2 in co-supervision with Laval University (Canada), has more than 70 publications including 55 in peer-reviewed international journals, one patent based on biotechnical applications of improved plant biomass production for pulp and paper industry, one public database EUCAWOOD (eucalyptus xylem unigenes: http://polebio.scsv.ups-tlse.fr/eucalyptus/eucawood/). She is member of the scientific committee of the 2011 IUFRO meeting in Brazil.

List of relevant publications:

1. Foucart C, Jauneau A, Gion JM, Amelot N, Martinez Y, Panegos P, Grima-Pettenati J, Sivadon P (2009) Overexpression of EgROP1, a Eucalyptus vascular-expressed Rac-like small GTPase affects secondary xylem formation in Arabidopsis thaliana. New Phytol 183: 1014-1029 (IF =5.25)
2. Rengel D, San Clémente H, Servant F, Ladouce N, Paux E, Wincker P, Couloux A, Sivadon P, Grima- Pettenati J (2009). A new genomic resource dedicated to wood formation in Eucalyptus. BMC Plant Biology 9:36 (IF=4.03)
3. Leplé JC, Dauwe R, Morreel K, Storme V, Lapierre C, Pollet B, Naumann A, Kang KY, Kim H, Ruel K, Lefèbvre A, Joseleau JP, Grima-Pettenati J, De Rycke I, Andersson-Gunneråas S, Erban J, Fehrle I, Petit-Conil M, Kopka J, Polle A, Messens E, Sundberg B, Mansfield S, Ralph J, Pilate G, Boerjan W (2007) Down-regulation of cinnamoyl-coenzyme A reductase in poplar (Populus tremula x P.alba); multiple level phenotyping reveals effects on cell wall polymer metabolism and structure. Plant Cell 19:3669-3691 (IF =9.653)
4. Goicoechea M, Lacombe E, Legay S, Milhaevic S, Rech P, Jauneau A, Lapierre C, Pollet B., Verhaegen D, Chaubet-Gigot N, Grima-Pettenati J (2005) EgMYB2, a new transcriptional activator from Eucalyptus xylem, regulates secondary cell wall formation and lignin biosynthesis. Plant J 43:553-567(IF = 6.75)
5. Paux E, Carocha V, Marques C, Mendes de Sousa A., Borralho N, Sivadon P, Grima-Pettenati J (2005) Transcript profiling of Eucalyptus xylem genes during tension wood formation. New Phytol 167:89-100
6. Soler , Marçal; Camargo, , Eduardo Leal Oliveira; Carocha, Victor; et al. 2014. The Eucalyptus grandis R2R3-MYB transcription factor family: evidence for woody growth-related evolution and function . New Phytologist In press http://onlinelibrary.wiley.com/doi/10.1111/nph.13039/pdf
7. Myburg, Alexander A.; Grattapaglia, Dario; Tuskan, Gerald A.; et al. 2014. The genome of Eucalyptus grandis.: Nature Volume: 510 Issue: 7505 Pages: 356-362 DOI: 10.1038/nature13308

ZANDER MYBURG

Prof Alexander (Zander) Myburg
Chair in Forest Genomics and Biotechnology
Department of Genetics
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Zander Myburg is a professor enetics at the University of Pretoria (UP) and holds the Chair in Forest Genomics and Biotechnology at UP. His research programme in the Forestry and Agricultural Biotechnology Institute (FABI) and Genomics Research Institute (GRI) focuses on the genetic control and molecular breeding of wood formation in fast-growing forest trees and, in particular, the genetic regulation of cellulose biosynthesis in wood fibre.

The Forest Molecular Genetics (FMG) Programme is supported by forestry industry partners (FMG Consortium), THRIP, NRF en DST. His research team is pioneering the use of population genomics and systems genetics approaches to unravel the genetic control of growth and wood formation in Eucalyptus trees. He was also the lead investigator of the US Department of Energy (DOE) funded Eucalyptus Genome Project which generated the reference genome for the genus recently published in the journal Nature. This information is being used to develop biotechnology applications for tree improvement including DNA fingerprinting, genome-assisted breeding and genetic engineering. He has supervised 34 postgraduate (MSc and PhD) students and is author of 58 peer-reviewed papers and book chapters in the field of plant molecular genetics and genomics.

STÉPHAN MAURY

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My research activities aimed at a better understanding of the relationships between phenotypic plasticity and the epigenetic control mediated by DNA methylation. Our model is focused on poplar, the model tree, and variations of water availability. Our objectives are to increase fundamental knowledge and to develop epigenetic biomarkers for tree breeding.

EDUCATION

- **Authorisation Direction of Research (HDR)** 2006 University Orléans, Faculty of Sciences (Plant Physiology)
- **Ph.D.** 2000 University Louis Pasteur Strasbourg (France), Institute of Plant Molecular Biology (Plant Genetic Engineering and Phytopathology)
- **Master** 1996 University Louis Pasteur Strasbourg, Faculty of Sciences (Cellular and Molecular Biology)
- **License** 1994 University Louis Pasteur Strasbourg, Faculty of Sciences (Biochemistry)

RESEARCH AND PROFESSIONAL EXPERIENCE

09/2012-present **Professor** in Plant Physiology at University of Orléans
09/2011-09/2012 **Member of CNU** section 66 (Physiology)
06/2008-present **Assistant Director** of Laboratory of Woody and Crop Plants University Orléans
01/2003-2011 **Member** of national jury for high school professors recruitment
09/2004-09/2009 **Assistant Director** of Teaching Department of Biology University Orléans
10/2000-08/2012 **Assistant Professor** in Plant Physiology at University of Orléans

5 RECENT PUBLICATIONS

1. Lafon-Placette C, Faivre-Rampant P, Delaunay A, Street N, Brignolas F, **Maury S** (2013) Methylome of DNase I sensitive chromatin in Populus trichocarpa shoot apical meristematic cells: a simplified approach revealing characteristics of gene-body DNA methylation in open chromatin state. New Phytologist 197, 416-430. doi 10.1111/nph.12026.
2. Hébrard C, Trap-Gentil M-V, Lafon-Placette C, Delaunay A, Joseph C, Lefebvre M, Barnes S, **Maury S** (2013) Identification of differentially methylated regions during vernalization revealed a role of RNA METHYLTRANSFERASE in bolting. Journal of Experimental Botany 64, 651-663. doi 10.1093/jxb/ers363.
3. Bräutigam K, Vining K, Lafon-Placette C, Fossdal CG, Mirouze M, Gutiérrez MJ, Fluch S, Fernández Fraga M, Guevara MÁ, Abarca D, Johnsen Ø, **Maury S**, Strauss SH, Campbell M, Rohde A, Díaz-Sala C, Cervera MT (2013) Epigenetic regulation of adaptive response of forest tree species to the environment. Ecology and Evolution 3, 399-415. doi 10.1002/ece3.461.
4. Zhu J, Shevchenko O, Ma C, **Maury S**, Freitag M, Strauss SH (2013) Poplars with a PtDDM1-RNAi transgene have reduced DNA methylation and show aberrant post-dormancy morphology. Planta 237, 1483-1493. doi 10.1007/s00425-013-1858-4.
5. Teyssier C, **Maury S**, Beaufour M, Grondin C, Delaunay A, Le Metté C, Ader K, Cadene M, Label P, Lelu-Walter MA (2014) In search of markers for somatic embryo maturation in hybrid larch (Larix × eurolepis): global DNA methylation and proteomic analyses. Physiologia Plantarum 150: 271–291. doi 10.1111/ppl.12081.

ISABEL ALLONA

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I am an Associate Professor in the Biotechnology Department of the Universidad Politécnica de Madrid (UPM) and the Principal Investigator of the Group of Molecular Biology of Winter Dormancy and Cold Acclimation in Woody Plants at the Centre for Plant Biotechnology and Genomics CBGP-UPM since 2008. My laboratory studies the molecular basis of cold acclimation and winter dormancy in woody plants, mainly chestnut and poplar. Briefly, the laboratory is focused on understanding how the environmental signals influence the molecular networks regulating specific phases of dormancy, leading to the identification of new targets to modulate vegetative growth and reduce economics cost in forest management. I have more than 20 years' experience in molecular biology, biochemistry, physiology, agronomy and biotechnology. After my graduation as Forestry Engineer I received my PhD in Forest Biotechnology in 1991 at the Universidad Politécnica de Madrid, directed by Dr. Aragoncillo. Then I worked as Postdoctoral fellow with Dr. Paz-Ares at the CIB-CSIC and as Assistant Professor at the Universidad Politécnica de Madrid from 1992 to 1995. During this period I developed pioneer work in biochemistry and molecular biology methods for the analysis of proteins involved in seed defence in forestry species. From 1995 to 1998, I was granted as Fulbright Postdoctoral fellowship at the Forest Biotechnology Group (NCSU, USA) with Dr. Sederoff, where I developed soft skills on mechanism of transcriptional regulation of xylem development in forestry species, highlighting my work on the application of cutting-edge technology for gene expression analyses in Pinus taeda xylem formation. In 1999 I got an Associate Professor position at the Universidad Politécnica de Madrid, establishing with Dr. Aragoncillo a molecular biology research group in woody plants. Our pioneer work in chestnut showed some specific features observed in the circadian clock of woody plants apparently unknown in annual herbaceous plants. During these years I achieved high level of scientific experience and international recognitions. I have been involved in 13 National and International projects, 5 as PI. I am the author of 24 papers in SCI-indexed journals, two of them in the journal PNAS, USA., with a h index of 14. I have been the coordinator of the Spanish Forest Genomics Network and member of the COST Action FP0905 Biosafety of forest transgenic trees, for improving the scientific basis for safe tree development and implementation of EU policy directives. I have been reviewer of international journals included in the JCI, of national and international projects and I have been in the panel of several grant and fellowship programs. I have supervised 4 PhD theses and directed five master and DEA works. I belong to the UPM Ethics Commission, I am the responsible of the UPM research group "Biotecnología de Proteínas Vegetales" and I belong to the Scientific Committee of the CBGP.

1- M. Johansson1, J.M. Ramos-Sánchez1, D. Conde1, C. Ibáñez, N. Takata, I. Allona*, M.E. Eriksson* (1Joint first authors; *corresponding authors). "Role of the circadian clock in cold acclimation and winter dormancy in perennial plants" .
Libro: Advances in Plant Dormancy. Editorial: Springer. In press.
2- Daniel Conde, Pablo González-Melendi and Isabel Allona "Poplar stems show opposite epigenetic patterns during winter dormancy and vegetative growth" Trees-Structure and Function (2013) 27: 311 - 320
3- Alicia Moreno-Cortés, Tamara Hernández-Verdeja, Paloma Sánchez-Jiménez, Pablo González-Melendi, Cipriano Aragoncillo and Isabel Allona "CsRAV1 induces sylleptic branching in hybrid poplar" New Phytologist (2012) 194: 83-90
4- C. Ibáñez, A. Ramos, P. Acebo, A. Contreras, R. Casado, I. Allona y C. Aragoncillo "Overall alteration of circadian clock gene expression in the chestnut cold response" PLoS ONE (2008)3 (10): e3567
5- A. Ramos, E. Pérez-Solís, C. Ibáñez, R. Casado, C. Collada, L. Gómez, C. Aragoncillo e I. Allona "Winter disruption of the circadian clock in chestnut" Procceding of the National Academy of Science USA (2005) 102: 7037 – 7042

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The main focus of his research is on transcriptional and post-transcriptional (miRNAs) dynamic during wood formation and secondary cell wall traits variation as response to developmental and environmental conditions, in forest species. Major contributions for the advance of forest genomics: the sequencing of *E. grandis* chloroplast genome (Paiva et al 2011), co-authoring of the E. grandis genome sequence paper (Myburg et al, 2014), participation at European Pine sequencing project (PROCOGEN), and generation of several gene catalogs for pine, eucalyptus and cork-oak (Paiva et al, 2008ab; Pereira-Leal et al, 2014).

Education & professional positions:

2006, PhD in Biology , ITQB, New University of Lisbon, Lisbon, Portugal
2006, PhD in Cellular and Molecular Biology, University of Bordeaux II, Bordeaux, France,
2009- Present, Researcher at IICT, Lisboa Portugal (Ciência2008 Program)
2006 - Present, Researcher at IBET, Oeiras, Portugal

Five most relevant publications in the context of Application (**corresponding author)

1. Capitão C , Jorge AP PAIVA, Dulce M Santos and Pedro Fevereiro. 2011. In Medicago truncatula, water deficit modulates the transcript accumulation of components of small RNA pathways. BMC Plant Biology 2011, 11:79 doi:10.1186/1471-2229-11-79
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Posters

Thermospermine and auxin levels in xylem tissues of *Populus* are balanced by a negative feedback loop mechanism*

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Polyamines, that are low molecular weight polycationic amines ubiquitous in living organisms, have stepped into plant xylem development biology as it was shown that the gene encoding for thermospermine synthase, *ACAULIS5* (*ACL5*) is essential for xylem cell specification and to delay cell death in differentiating xylem vessels in *Arabidopsis*. To elucidate the role of thermospermine in secondary xylem formation of *Populus* the *ACL5* ortholog, *POPACAULIS5*, was ectopically expressed under the 35S constitutive promoter. While attempting to overexpress *POPACAULIS5* we became aware that excessive accumulation of thermospermine in xylem is prevented by a negative feedback control mechanism that maintains steady-state levels of thermospermine. 35S driven expression of *POPACAULIS5* in transgenic *Populus* trees resulted in strong upregulation of *POPACAULIS5* expression and elevated thermospermine levels in leaves but not in the secondary xylem tissues of the stem of 2 month-old trees. In stem tissues it was also obvious that 35S::*POPACAULIS5* expression had a negative effect on indole-3-acetic acid (IAA) accumulation while exogenous auxin treatments had a positive effect on the expression of the *POPACAULIS5* transgene and on *PttHB8*, a Class III homeodomain-leucine zipper (HD-ZIP III). Further, overexpression of *PttHB8* positively affected *POPACAULIS5* expression, whereas increased *POPACAULIS5* expression had a negative effect on *PttHB8* expression (Milhinhos et al. 2013). These results support that a negative feedback control of *POPACAULIS5* transcript levels happens through suppression of IAA levels, and that *PttHB8* is involved in the transcriptional control of *POPACAULIS5*. We here propose that this auxin-thermospermine negative feedback loop functions to maintain thermospermine homeostasis to ensure proper xylem development.

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* selected for oral presentation

Epigenetic regulation of cork development*

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Cork oak (Quercus suber L.) is one of the most important Mediterranean forest trees with high ecologic and economic value in Portugal due to the exploitation of its cork for several industrial uses, especially for the production of stoppers. The cork can be harvested from the same tree every 9-10 years for more than 200 years and is the result of the activity of a secondary meristem, the cork cambium or phellogen consisting of multiple layers of cells which differentiate through the inner deposition of suberin in their walls. This process is controlled by an irreversible developmental program that ends with senescence and cell death. The ontogenetic development, ageing and maturation are characterized by altered patterns of cell differentiation and organ formation processes. The potential role of DNA methylation in other developmental processes has been evaluated in some tree species, however, the epigenetic processes underlying cork biosynthesis and differentiation are totally unknown.

With this work we aim to comprehend the epigenetic regulation associated with cork development by analysing the DNA methylation patterns of individual nuclei of different tissues: cortex, phellogen and phellem with one, two and three years of age and reproduction cork. Branches and reproduction cork were included in glycol methacrylate (GMA), and thin sections were used for immunodetection of 5-methylcytosine (5-mC). Densitometric analysis revealed that in the first year no differences were found in the methylation level of cortex and epidermis nuclei however a marked DNA methylation increase was detected as phellem cells are maturing and becoming more differentiated until complete loss of the cellular content. These observations are consistent with the epigenetic changes associated to cellular differentiation in programmed cell death, and clearly show that cork development is under strong epigenetic control.

*selected for oral presentation

Keywords: cork cambium, phellem, 5-methylcytosine

Effects of Flavonoid supplementation on Eucalyptus wood: Increased S/G ratio and saccharification, reduced extractive content, and differential expression of genes involved in cell wall formation a lignin metabolism*

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Eucalyptus species are the most widely planted hardwood species in the world and are renowned for their rapid growth and adaptability. In Brazil, one of the most widely grown Eucalyptus cultivars is the fast-growing *Eucalyptus urophylla x Eucalyptus grandis* hybrid. The flavonoids, naringenin-chalcone and narigenin, are intermediates in phenylpropanoid metabolism in plants, occupying the central position as primary intermediates in flavonoid biosynthesis and are synthesized by chalcone synthase (CHS) and chalcone isomerase (CHI) respectively. Our group has previously demonstrated that flavonoid supplementation reduces extractives content and increases S/G lignin monomeric ratio on *E. urograndis* plantlets. In this work, we report the transcriptional responses occurring in these trees that may be related to the observed chemical differences. Gene expression was analyzed through mRNA-sequencing, and notably, compared to control groups, the treated trees display differential down-regulation of cell wall formation pathways such as phenylpropanoid metabolism as well as differential expression of genes involved in sucrose, starch and minor CHO metabolism and genes that play a role in several stress and environmental responses. We also performed enzymatic hydrolysis of wood samples from the different treatments, and the results indicated higher sugar contents and glucose yields in the flavonoid-treated plants, proving that flavonoid supplementation not only alters lignin and cell wall content, but also changes it´s solubility. Our results further illustrate the potential use of flavonoids as a nutritional complement for modifying Eucalyptus wood, altering its chemical composition, gene expression and increasing saccharification.

*selected for oral presentation

A comparative transcriptome analysis of *Quercus suber* flower development

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Monoecious species have been long considered unique tools to study the developmental programs involved in the formation of separate male and female flowers. However, for the majority of these species, insufficient or inexistent genomic and transcriptomic data availability has hampered functional studies. Advances in next generation sequencing technologies have made possible to perform a rapid and cost-effective compilation of large RNA sequence data sets in non-model organisms with no or little prior genomic data available. In this context, the main goal of this study was to identify differentially expressed genes during the development of male and female flowers of the monoecious species *Quercus suber*, an economically important Mediterranean tree. Total RNA was extracted from different developmental stages of *Q. suber* flowers and non-normalised cDNA libraries, representing early and late stages of female and male flowers, were obtained using 454 pyrosequencing technology. A comparative analysis of the transcriptomes revealed flower type and stage specific ESTs. Differential gene expression between female and male flowers was validated by qRT-PCR. As expected, genes involved in pollen exine formation (LAP3, LAP5, LAP6 and MS2) and in the tapetal cell development (AMS), had high number of reads and were detected in high abundance in the male flowers. Conversely, genes involved in stigma-specific recognition (STIG1), in the recognition of pollen (At4g27290), in ovule formation (EDA17) and in fruit growth and development (CYP78A9, PG1) were more expressed in the female flowers. Moreover, differentially expressed genes that have not yet been characterised and others that have not been previously shown to be implicated in flower development were also identified. This transcriptomic analysis may therefore contribute to uncover sex-specific regulatory networks and serve as a platform to future studies in model and non-model species.

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Deciphering the *Quercus suber* root response to drought, an RNA-Seq approach

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Cork oak (*Quercus suber* L.) is a significant Mediterranean forest species, and due to its high economic value, it has been considered a national species of interest. Given that climate changes directly affect the development of plants and reduce their productivity, two-month-old *Q. suber* seedlings were subject to different water stress regimes to impose drought stress. Subsequently, the root transcriptome in response to moderate and severe drought stress was established by Next Generation Sequencing (Roche 454 technology). Data was assembled into 21012 unigenes, and reads were subsequently mapped to the assembly, in order to identify differential expression. This allowed us to recognize 546 differentially expressed genes. These genes were subjected to subsequent *in silico* analysis, including their annotation and assembly into functional categories. Genes were then annotated against the genome of the model plant *Arabidopsis thaliana*, allowing for ortholog identification. This information was used to establish networks of GO functional assignment, gene co-expression, known protein-protein interactions and *cis*-element enrichment, thus establishing functional relationships between differentially expressed genes. Ultimately, we demonstrated the induction, in drought stressed *Quercus suber* roots, of a complete, ABA-dependent signaling cascade, ranging from ABA-sensing components to transcription factors and then to effector genes involved in the drought response.

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Castanea resistance EST based genes to *Phytophthora cinnamomi*

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European chestnut, sweet chestnut (*Castanea sativa* Mill.) is a multipurpose tree species of great economic importance not only for fruit and timber but also for its contribution to the landscape and environment. The most serious pathogen affecting chestnut production in Europe is *Phytophthora cinnamomi*, which causes root rot, also known as ink disease. However, Japanese chestnut (*Castanea crenata* Sieb. et Zucc) and Chinese chestnut (*Castanea mollissima* Bl.) show substantial levels of resistance to the disease. Root pathogens such as *P. cinnamomi* and their interactions with hosts are poorly understood and little information has been acquired on the molecular defense strategies against the pathogen. In order to obtain a better understanding of chestnut's specific defense mechanisms, 454 pyrosequenced EST data was generated from four cDNA libraries: *Castanea sativa* and *Castanea crenata* inoculated and non-inoculated with the pathogen.
Twenty differentially expressed genes were selected to validate pyrosequenced data, by quantitative PCR. This selection was based on the diverse levels of *P. cinnamomi* response: pathogen recognition which triggers resistance signaling pathways; transcription factors, involved in the regulation of other defense related processes; hypersensitive response preventing further colonization; enzymes involved in cell wall modifications and anti-fungal enzymes. This transcriptomic approach is integrated in an ongoing chestnut breeding program based on controlled crosses: inter-specific crosses have been established from the resistant species into the susceptible species, and a hybrid population has been created [1] . Response to *P. cinnamomi* was evaluated for replicates of each hybrid progeny revealing a wide range of susceptibility/resistance levels [2] . Expression analyses is conducted for the twenty candidate genes, using root biological triplicates collected at 24h and 48h after inoculation, for *C. sativa* and *C. crenata* and five selected hybrid genotypes. Gene expression profiles obtained for genotypes with a broad-spectrum response will provide new insights about specific chestnut-*P. cinnamomi* molecular interactions.

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Identification and analysis of small RNAs in maritime pine embryogenesis

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It has been suggested that the distinct features of embryo development in angiosperms and gymnosperms result from differential gene regulation. Microarray analysis of P. pinaster zygotic embryogenesis spanning the zygotic embryo development from early developing to mature embryos highlighted several epigenetic regulation mechanisms [1] and showed that functions related to small RNA (sRNA) pathways appeared differentially regulated across all stages of embryo development with a prevalence of micro RNA (miRNA) functions in mid to late embryogenesis.
In this work, small RNA libraries prepared from samples of developing embryos at the same stages of development as previously described [1] were sequenced using Illumina technology. The bioinformatics analysis of the sequencing data allowed the identification of several conserved MIRNA families that corresponded to a small fraction of the total sequenced reads. The precursors of the identified conserved miRNAs (pre-miRNA) were searched against the transcriptome of reference for P.pinaster [2] and a few promising candidates were found. The most expressed conserved miRNA family is the MIR166
which is also the family comprising the largest number of isoforms. MIR166 family is widely found across plant species and is known to be involved in organ polarity and vascular development through HD-ZIPIII regulation [3]. A search for the sRNAs candidates putatively regulating the differentially transcribed genes that had been previously described as being implicated in epigenetic regulation [1] was
performed. The results showed evidences of sRNAs involvement in the regulation of other epigenetic players along the zygotic embryo development of maritime pine.
The analysis of the sRNA transcriptome obtained from P.pinaster developing embryos is currently ongoing but it already allowed complementing some of the aspects highlighted by the microarray analysis of P. pinaster zygotic embryogenesis. It is expected that these results will help to identify the sRNAs essential for P. pinaster embryo development as well as their interplay with other epigenetic pathways.

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EgMYB1 interacts with a histone linker to regulate secondary cell wall formation in xylem

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Eucalyptus species grow very fast and produce high yields of biomass, representing the main wood industrial plantations in the world. *E. grandis* is also the second forest tree whose genome has been sequenced (Myburg *et al*., 2014). To improve wood properties related to pulping or bioethanol production, we are focusing our efforts towards the identification of genes regulating the biosynthesis of secondary cell wall (SCW) polymers in *Eucalyptus*. Many genes thought to act in a transcriptional hierarchical network to regulate SCW formation have been studied these last years, mostly in *Arabidopsis* (reviewed in Zhang *et al*., 2014). However, very little is known about the protein-protein interactions that regulate the activity of the transcription factors involved in this network. Our team has shown that *Eg*MYB1, a R2R2 MYB transcription factor, was able to repress the lignin biosynthesis and SCW formation (Legay *et al*., 2010). Aiming at understanding how this transcription factor activity is regulated, we decided to seek for its potential protein partners. By screening a yeast-two-hybrid library that we constructed using *Eucalyptus* xylem RNA, we found several candidate partners, among them a histone linker. We validated the interaction between *Eg*MYB1 and the histone linker *in planta* using FRET-FLIM assay. To underscore the biological role of that interaction, we constructed *Arabidopsis* transgenic lines, either over-expressing *Eg*MYB1 alone, histone linker alone, or the both proteins together. Histological analyses of these plants showed a dramatic reduction of SCW thickness and lignin levels in histone linker-*Eg*MYB1 plants, much stronger than in *Eg*MYB1 plants alone. Finally, we performed a microarray comparing RNA from the stem base of these plants, which allowed us to identify target genes modulated by this interaction.

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Distribution of arabinogalactan proteins and pectin epitopes in *Quercus suber* female flower

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The evergreen Fagacea cork oak (*Quercus suber*) is a dominant monoecious tree species from the Southern Iberian Peninsula forests. *Q. suber* has an extremely important socio-economic, cultural and an environmental value for Portugal. It presents a long progamic phase that provides a comprehensive system for comparative studies in development and sexual reproduction in a non-model plant [1]. Studies on the sexual reproduction of cork oak are essential to understand the molecular mechanisms of fertilization and identify the difficulties associated with the production of acorns destined either for nursery production or for animal food. Cell surface proteoglycans such as arabinogalactan proteins (AGPs) and pectins play important roles in cell growth and development. AGPs and pectins belong to a superfamily of highly glycosylated hydroxyproline-rich glycoproteins found in the entire plant kingdom, in almost all plant organs and cell types from root to flowers [2]. At the subcellular level, AGPs can be found in the cell wall, in the apoplast or anchored to the plasma membrane via a GPI anchor attached to the C-terminal domain of the AGP backbone. In reproductive tissues, the expression of AGPs is associated with the sporophyte–gametophyte transition [3]. Pectins are important cell wall polysaccharides, together with AGPs have been reported to play important roles in plant growth and development. Pectins are mainly composed of homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II).

Immunofluorescent localization of AGP and pectin epitopes in female flowers was performed with a set of monoclonal antibodies directed to the carbohydrate moiety of the cell wall polysaccharides, JIM8 and JIM13 recognizing AGPs; and LM5, JIM5 and JIM7 recognizing pectins. The antibody binding to pectin HG epitopes for highly methyl-esterified homogalacturonans labeled all cell walls of the female flower tissues. The labeling obtained with anti-AGP antibodies in female flowers showed a dynamic distribution making AGPs useful as molecular markers for cork oak pistil development.

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Epigenetic mechanisms involved in PWN defense in two pine species with different tolerance: *Pinus yunnanensis* and *Pinus pinaster*

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Pine wilt disease (PWD) caused by the pinewood nematode (*Bursaphelenchus xylophilus*) Nickle and vectored by the cerambycid beetle (*Monochamus* sp.) is causing serious economic damage in worldwide coniferous forests. The Asian species *Pinus yunnanensis* Franch presents tolerance/ resistance against PWN infection, though the molecular and metabolic mechanisms behind such resistance are not known. In the present study, we provide the analysis of the response towards PWD infection in one tolerant species (*Pinus yunnanensis*) and in one highly susceptible species (*Pinus pinaster*) over a time course (0h, 6h/24h, 48h and 7 days) and using the new Ion Proton sequencing technology.

Four cDNA libraries from each species inoculated with *Bursaphelenchus xylophilus* produced between 29.819.330 and 39.004.139 reads after quality control. The comparative transcriptome analysis showed that in *P. yunnanensis*, cell wall-related genes, and genes coding for antifungal proteins, metallothionein-like protein, enzymes involved in terpenes´synthesis, pathogenesis-related protein, histones, histone acetyltransferases, histone deacetylases and histone-lysine N-methyltransferase were higher expressed than in *P. pinaster*. These results suggest that defenses related with tolerance of *P. yunnanensis* are likely to be regulated by epigenetic mechanisms.

On the other hand, results put in evidence that some defense/ oxidative stress mechanisms are also up-regulated in *P. pinaster* involving the expression of genes coding for antimicrobial peptide, probable phospholipid hydroperoxide and thioredoxin. However, these mechanisms are insufficient to stop disease progression.

Altogether these results provide putative biomarkers associated with resistance that can be useful in breeding programs.

Sex identity in *Quercus suber*: a MAD(S) business?

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Angiosperms exhibit a variety of sexual systems that range from hermaphroditism to separate sexual structures in different (dioecy) or in the same individual (monoecy). Albeit in minority, monoecious and dioceious species provide an excellent system to study the specific determinants that underlie male and female flower development. *Quercus suber* is one of the most ecological and socio-economic important forest species in Portugal, being the dominant tree of the oak woodlands. This monoecious wind-pollinated species has a protandrous system and several seasons of flowering. Staminate flowers occur in early spring and autumn, whereas pistillate flowering buds usually appear only in spring. Despite its overall importance, very little is known regarding the genetic mechanisms involved in cork oak sexual identity. Non-normalized libraries of different developmental stages of male and female flowers were generated using 454 GS-FLX Titanium massive parallel pyrosequencing technology. In order to identify genes involved in flower development, the amino acid sequences of MADS genes homologous to the regulatory floral homeotic genes (ABCDE model) *APETALA1* (*AP1*), *APETALA3* (*AP3*) *PISTILLATA* (*PI*), *SEPALLATA1/2/3* (*SEP*), *AGAMOUS* (*AG*), *SHATTERPROOF* (*SHP*) were obtained and their phylogenetic relationships were inferred, confirming the presence of potential orthologues in the Cork Oak EST database. The temporal expression of these genes was analysed using qRT-PCR analysis. Interestingly, A-class transcripts (QsAP1) were more abundant in female flowers, whereas B-class genes were predominantly expressed in male flowers throughout their development. Interestingly, *QsPI* was unique to the male samples. Yeast-two-hybrid analyses showed that QsPI and QsAP3 are able to interact in agreement to what was observed in other species. According to the ABCDE model, the expression of SEPALLATA-like genes was similar in both types of flowers. Future analysis regarding the *QsPI* gene will be performed in order to unveil its sex-specific type of regulation.

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Validation of novel and conserved pre-miRNAs in Maritime pine and Scots pine by quantitative real-time PCR

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The relatively simple structure of pine wood, mainly composed by tracheids, constitutes a surplus for molecular studies related to xylogenesis. The regulation of the molecular mechanisms underlying wood formation could be unraveled by the discovery of novel and conserved microRNAs (miRNAs) in wood forming tissues of *Pinus pinaster* Ait. (Maritime pine) and *Pinus sylvestris* L. (Scots pine). miRNAs are a major players in the post-regulation network of gene expression. Pre-microRNAs (pre-miRNAs) mediate products during the miRNA transcription and can provide hints of miRNA gene expression regulation. This study consisted in the validation of pre-miRNAs by quantitative real-time PCR (qRT-PCR) based on small-RNAs sequencing data and degradome libraries analyses obtained in differentiating xylem and phloem of Maritime pine and Scots pine in order to unravel their expression profiling and specificity per tissue or species. The results demonstrated a glance of pre-miRNAs expression in both pine species and wood forming tissues as well as the relationship between the expression of the precursor and mature forms of miRNAs. This study also revealed the applicability of qRT-PCR for the expression profiling of pre-miRNAs in a time and cost effective manner.

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Controlled pollination in cork oak (*Quercus suber* L.) to support its genome sequencing project

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Controlled crosses to obtain full-sib progenies to use in genetic mapping are critical at highly
heterozygotic species as is the case of Cork oak (Quercus suber L.). Mating design was based on four female parents and ten male parents. For controlled pollination it is crucial to choose trees offering high probabilities on female flowering. Cork oak is a species of complex and rather unpredictable reproductive behavior between years and between trees and female flowering is more irregular than male flowering. The selection of female parents was performed only at the controlled and permanent plot at Quinta da Serra, to benefit from the long run population genetics studies performed, where the reproductive behavior at the level of flowering/fruiting ability and flowering phenology of 24 trees is being studied since 1992. The pre-selection was focused on 10 trees known to have good to high flowering capacity and intermediate or late flowering phenology, in order to allow the use of pollen from male parents with unknown phenology. Final selection for female parents chose trees QS1, QS4, QS13, QS20. To minimize the chances of having a close genetic relationship between the 10 male parent trees were chosen at considerable geographic distance. The tree selected for genome sequencing (HL8) is one of the male parents.

Main results

Tree QS1 and tree QS20 -almost no female flowering; tree QS13 -very heavy flowering; tree QS4 -heavy flowering. Pollen germination tests results are also presented. By December 2014 about 400 acorns from 18 families are in germination at Viveiro de Santo sidro, at Pegões, Portugal. After appropriated traceability of putative full-sib plants the controlled progenies will be used to construct a genetic map that will support the national cork oak genome sequencing project.

Key words

*Quercus sube*r, controlled pollination, flowering phenology, flowering capacity

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