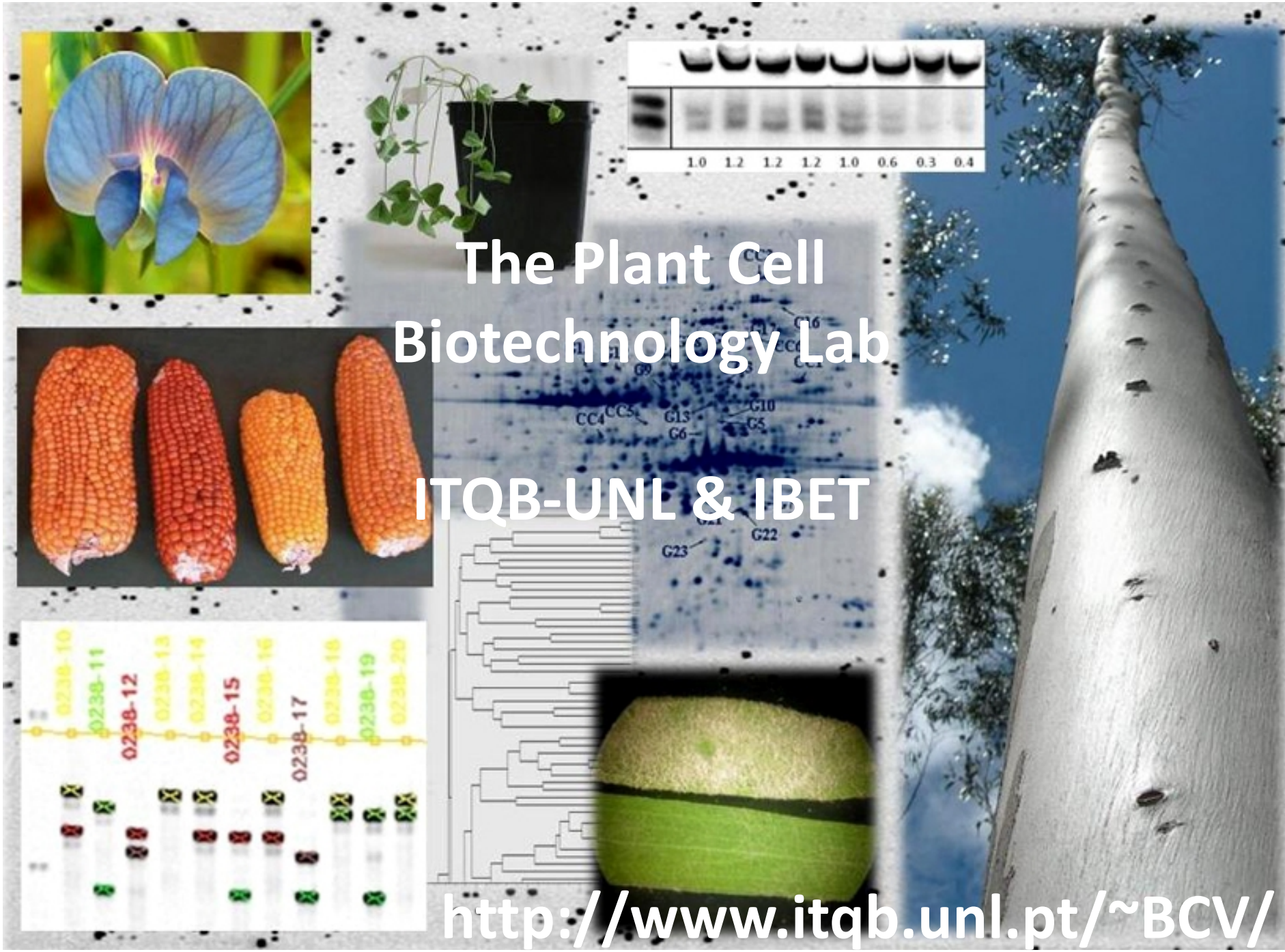


The Plant Cell Biotechnology Lab

ITQB-UNL & IBET

http://www.itqb.unl.pt/~BCV/

Mission
Plant Cell Biotechnology Lab

- Develop molecular strategies to support plant breeding programs, including molecular biodiversity analysis, quantitative genetics and molecular marker-trait associations
- Develop a model to introduce and to study the expression of genes related to water deficit tolerance
- Apply biotechnology to the development of company's strategies with incidence on forest trees
- Apply and develop strategies to produce bio-products (metabolites, enzymes, recombinant proteins) (fading)
- Teach and train researchers and technicians in plant biotechnology and molecular biology
- Develop public awareness and professional certification on plant biotechnology

BCV Structure

Direction
Pedro Fevereiro
(FCUL/ITQB/IBET)

Plant Systematics and Phylogeny
Susana Neves (ITQB)

Eleusine
Bupleurum
Menispermaceae

Environmental Stress in Legumes
Susana Araújo (IICT) Sofia Duque (ITQB)

Medicago truncatula

Plant Quantitative Genetics
Carlota Vaz Patto (ITQB)

Phaseolus vulgaris
Lathyrus sativus

Zea mays

Genomics of forest trees
Jorge Paiva (IICT/IBET)

Eucalyptus globulus
Pinus pinaster
Quercus suber

Genetic diversity of crops
Pedro Fevereiro
Jorge Cunha (INIAV – ex-EVN)

Vitis vinifera
Olea europaea

Nanoparticles and plant cells
Pedro Fevereiro

Quantum Dots

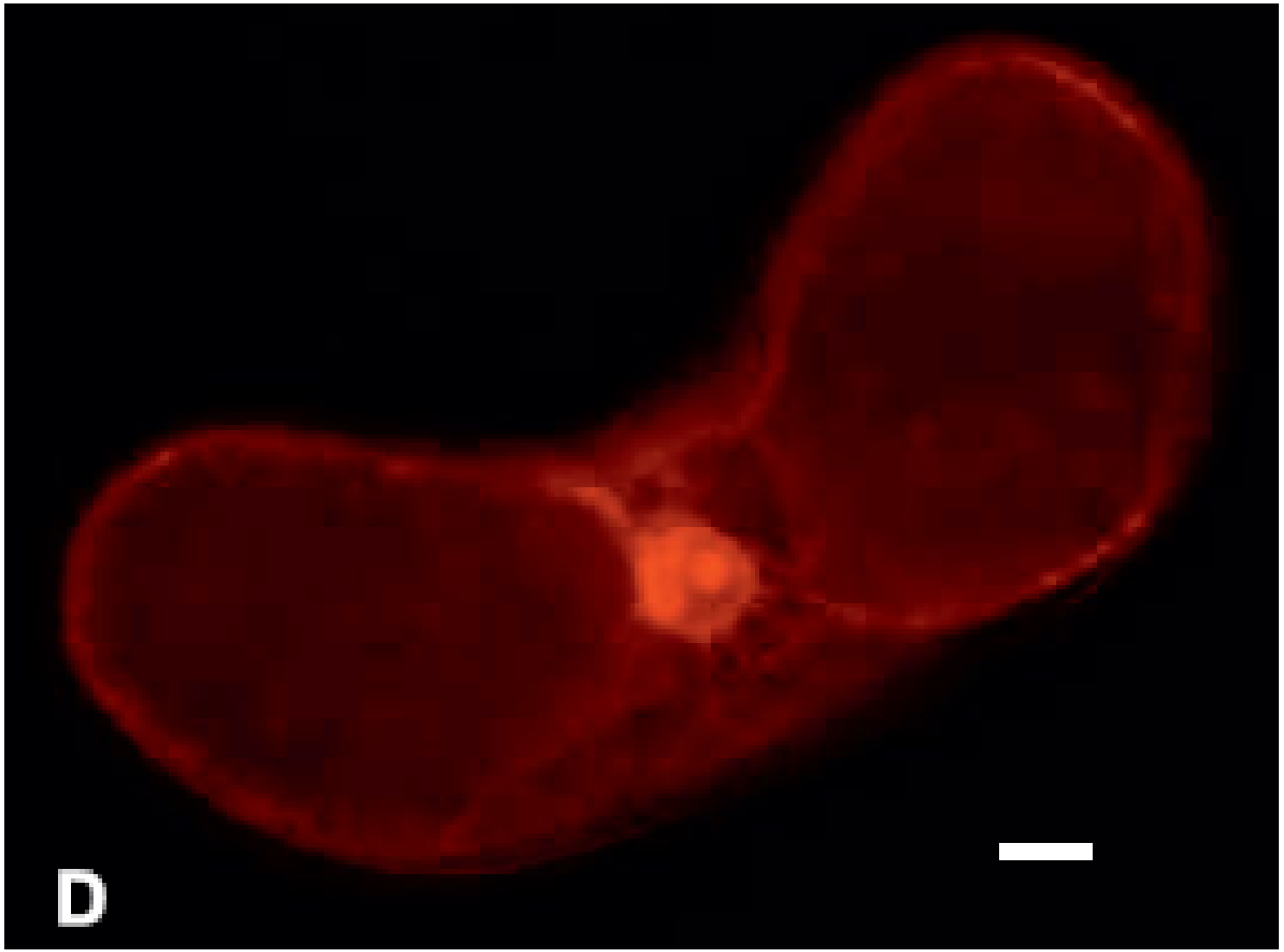
Science Comunication
Rita Caré (CiB)

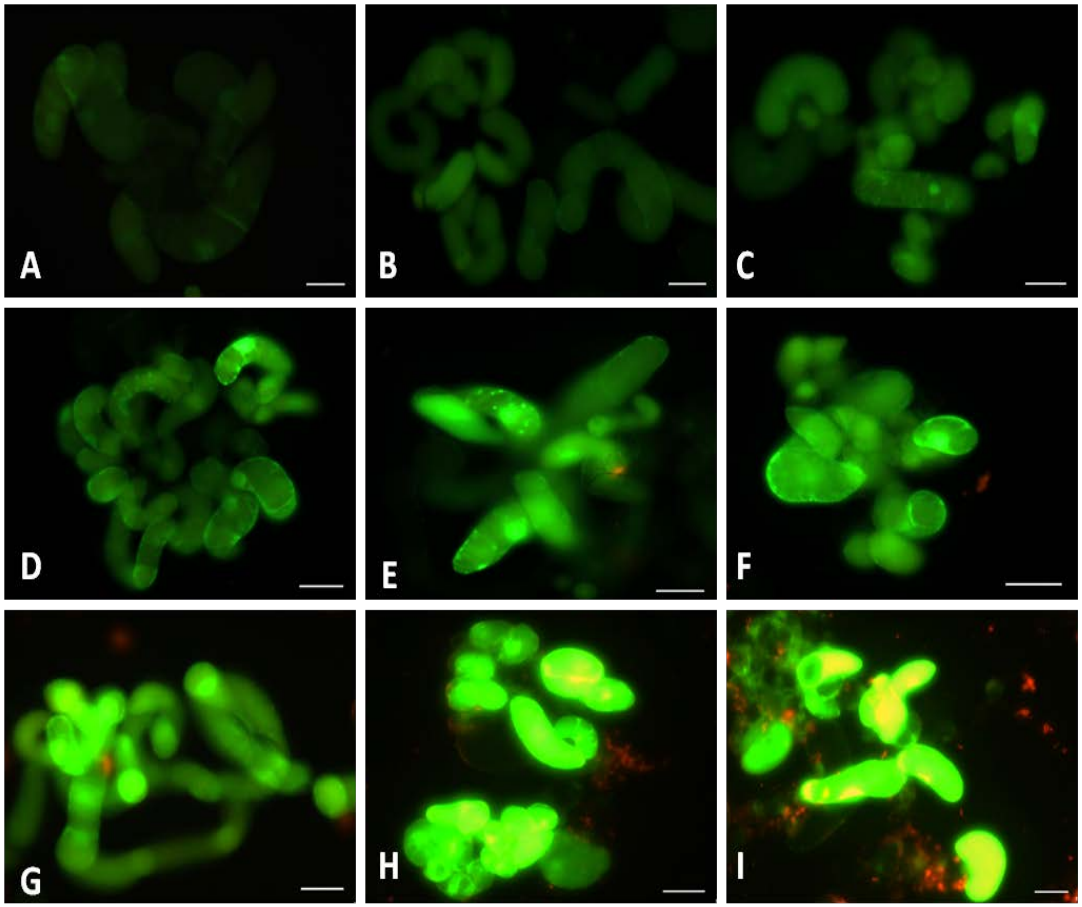
Agrobiotecnology

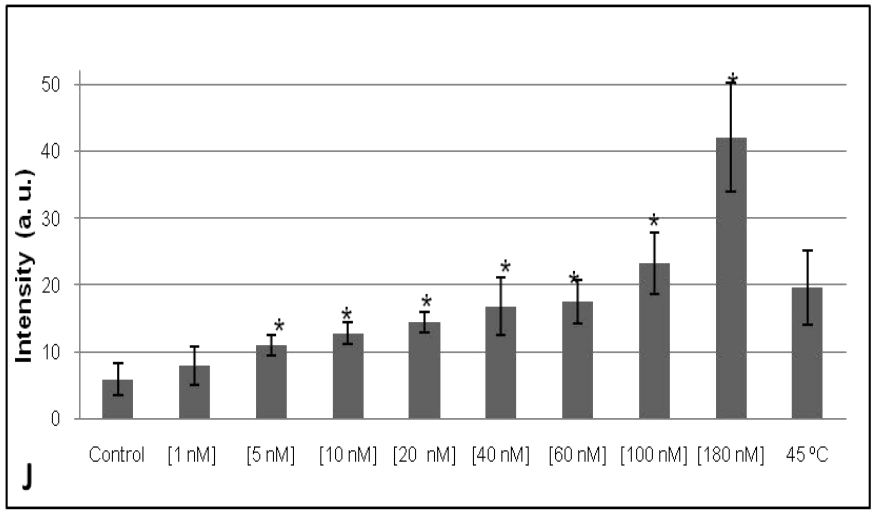

24 Members
INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA
UNIVERSIDADE NOVA DE LISBOA

Group Leader	**Pedro Fevereiro**
Auxiliary Researcher	**Carlota Vaz Patto**
Invited Researchers – IICT	**Jorge Paiva**
	Susana Araújo
Postdocs	**Ana Maria Ferreira**
	Jorge Cunha
	Sofia Duque
	Susana Neves
PhD students	**Cátia Nunes**
	Diana Branco
	Mara Alves
	Matilde Cordeiro
	Nuno Almeida
	Pedro Mendes Moreira
	Víctor Carocha
Master student	**Leila Estaki**
Research fellows	**Ana Catarina Afonso**
	Clara Graça
	Jorge Oliveira
	José Salvado
	Marco Dinis
	Maria Assunção
	Priscila Pereira
	Rita Morgado
	Susana Pera
	Susana Leitão

The impact of CdSe/ZnS Quantum Dots in cells of Medicago sativa in suspension culture

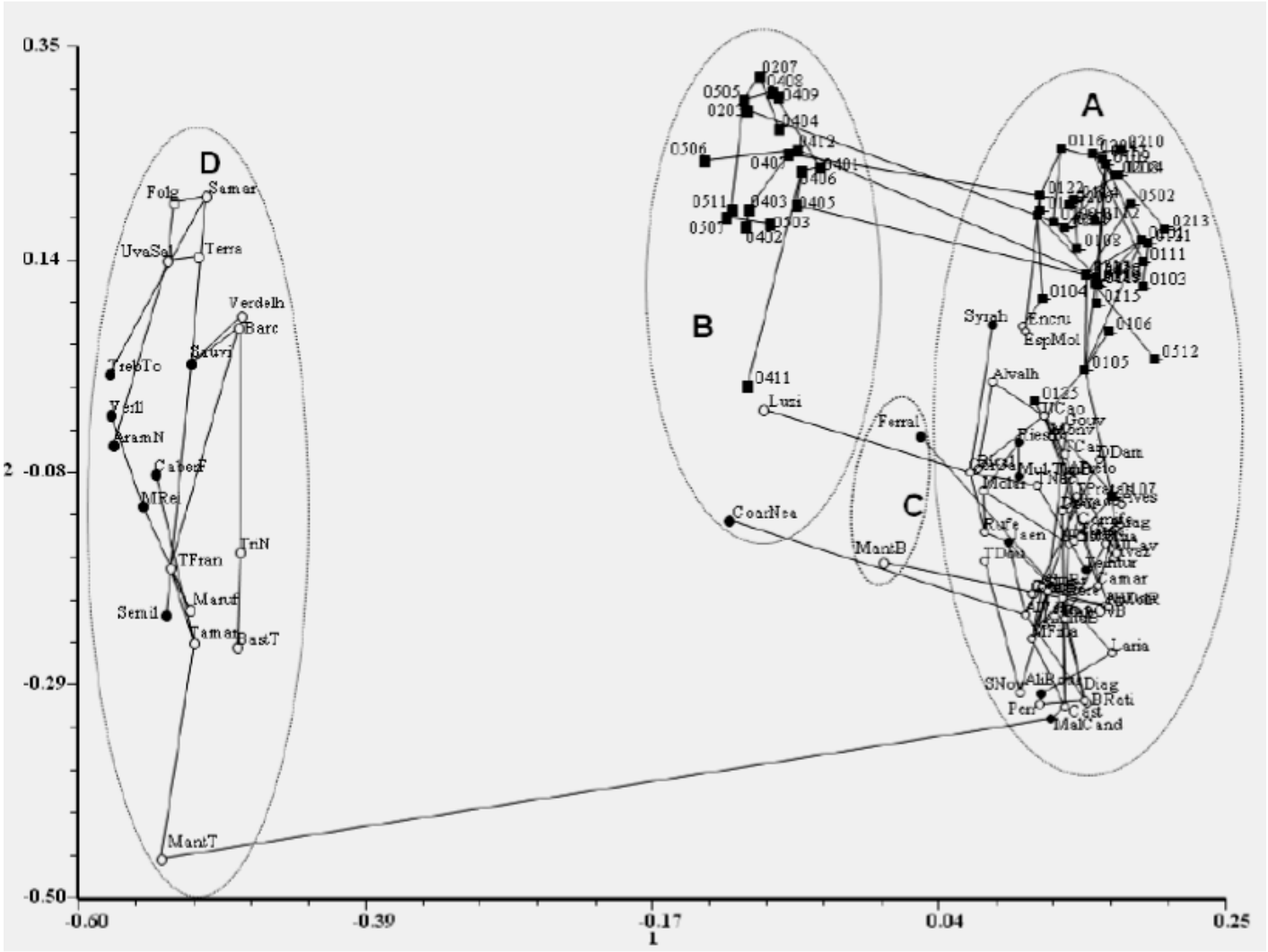






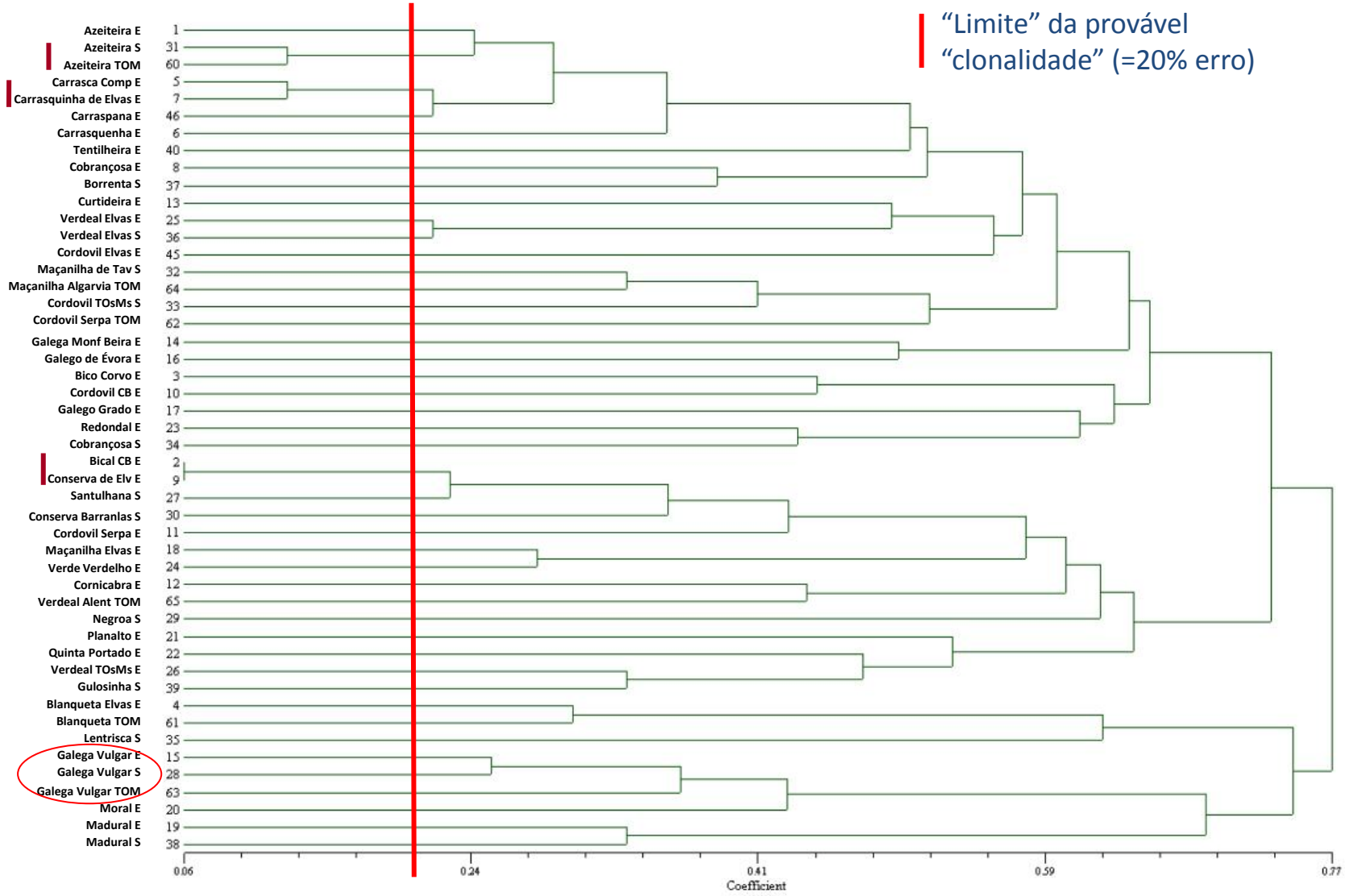
Oxidative stress dose response

Genetic relations between wild and cultivated vines



PCA of nuc & chlor SSRs - Portuguese wild vines and Portuguese and foreign cultivated accessions. Minimum spanning tree superimposed. Wild vines – numbers; Portuguese accessions – open circles; foreign accession – closed circles. A, B, C and D - Chlorotypes.

Molecular Diversity of Olive Cultivars (Elvas, Santarém, Mirandela)



Plant Quantitative Genetics
Carlota Vaz Patto

AIM:

Genes/QTL identification-location-effect estimation on Portuguese germplasm of national interest, development of tools to increase efficiency and selection speed

Current focus I

Cereals: Maize (*Zea mays*)

Bread making ability
Organoleptic, nutritional quality
Ear fasciation

1. PTDC/AGR-ALI/099285/2008 (ITQB + ESAC + INIAV + UFLA, Brazil): “Exploiting antioxidants, flavours and aromas diversity on “broa” bread maize breeding”
2. FP7-SOLIBAM - KBBE-2009-1-2-04 (INRA + 22 teams, 13 countries): ”Strategies for Organic and Low-input Integrated Breeding and Management”

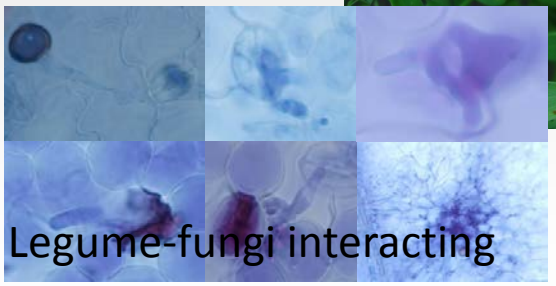
Current focus II

Legumes: Grass pea/Chikling pea (*Lathyrus sativus*, *L. cicera*)

Durable disease resistance
Powdery mildew,
Rust,
Ascochyta....
Drought resistance


Grass pea


Rust fungi infection


Legume-fungi interacting

1. PTDC/AGR-GPL/103285/2008 (ITQB + CSIC, Spain): "Exploiting transcriptional variation to identify genes underlying quantitative resistance to major grain legume pathogens"

Current focus III

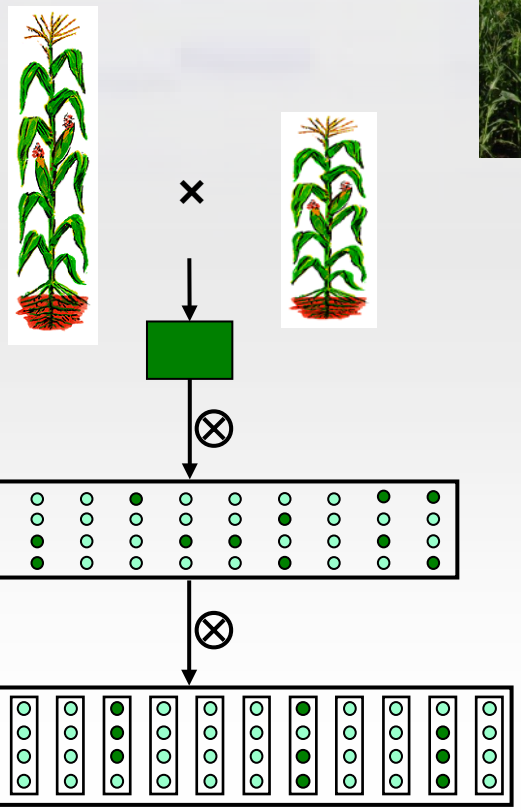
Legumes: Common bean (*Phaseolus vulgaris*)

Organoleptic, nutritional and processing Quality
Durable disease resistance
Drought resistance

1. PTDC/AGR-TEC/3555/2012 (ITQB + INIAV + IICT + CSIC, Spain): "Exploiting BEan GEnetics for food Quality and Attractiveness innovation (BEGEQA)"
2. FP7-MEDILEG- ARIMNet EU call 2011 (CSIC+ 12 teams, 8 countries): "Breeding, agronomic and biotechnological approaches for reintegration and revalorization of legumes in Mediterranean agriculture"
3. FP7-SOLIBAM - KBBE-2009-1-2-04 (INRA+21 teams, 13 countries)

Field

Generation	Procedure
Parents	A × B
F1	⊗
F2	⊗
F2:3	

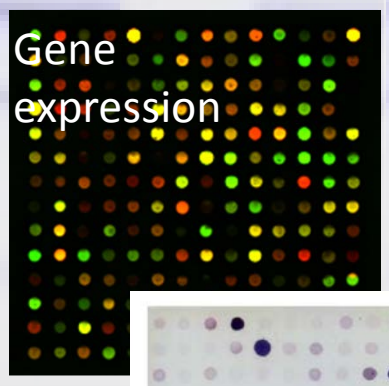


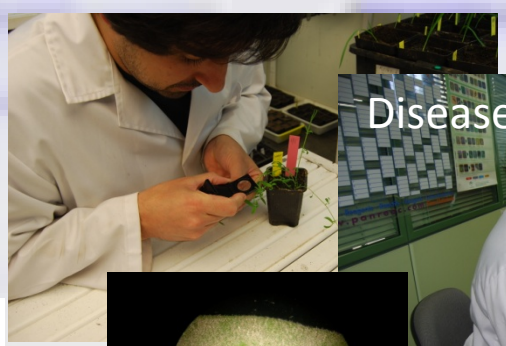
Alternative populations: Backcross1, Recombinant Inbred Lines, Double Haploids (adapted from Ed Buckler)

Laboratory

Quantitative trait measurement

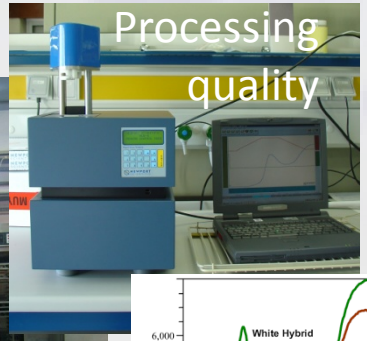
Gene expression



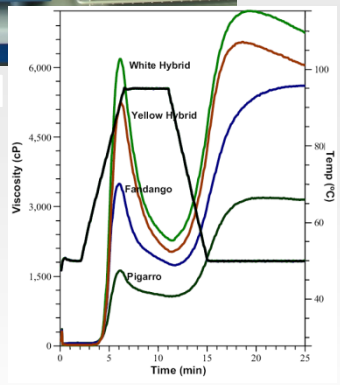


Disease resistance

Processing quality

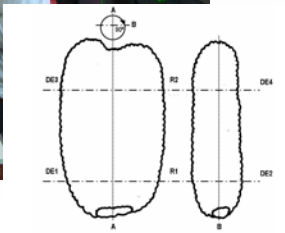


Nutritional and Organoleptic quality



Agronomic traits

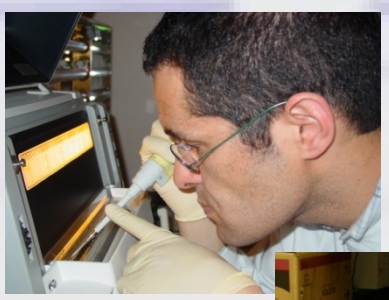
Ear fasciation



Drought resistance

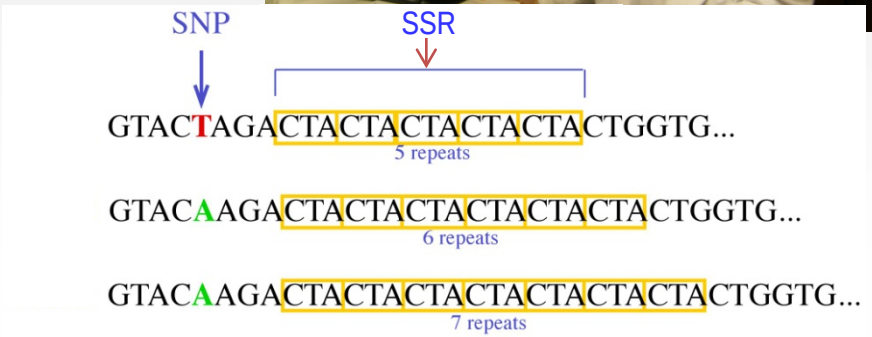
Laboratory

Molecular Markers screening



Molecular markers visualise organisms composition at DNA level, and flag DNA sequence differences

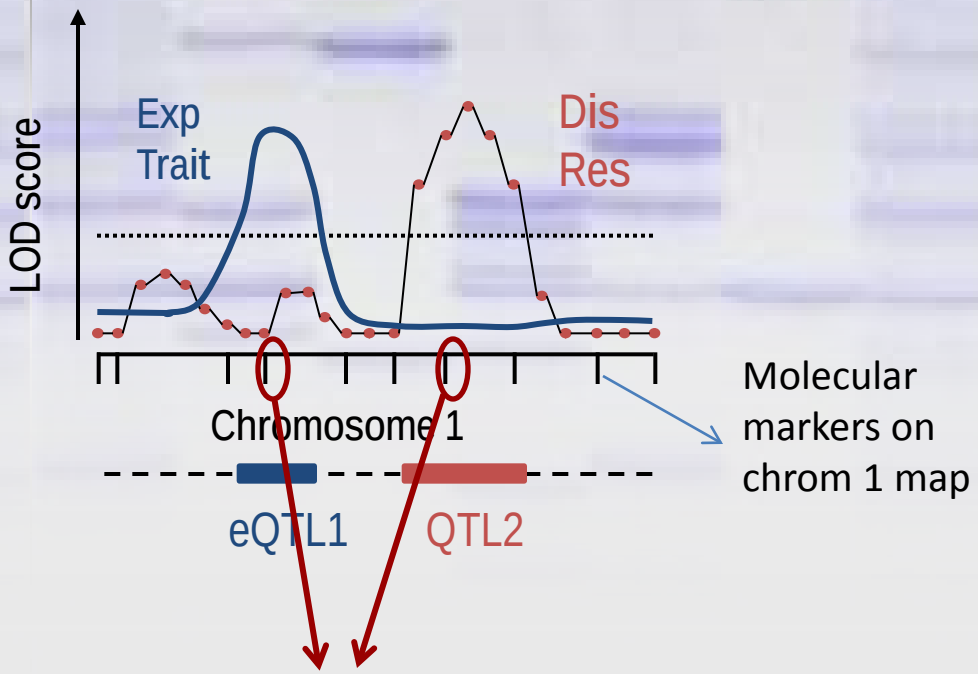
#	Marker 1	2	3	4	5	..	M	Disease Resistance	Expres. Trait
1	B	B	H	H	A	..	A	25	1.5
2	H	A	H	A	A	..	H	75	0.8
3	B	B	H	H	H	..	A	89	0.5
4	H	H	B	B	B	..	H	30	1.3
5	H	B	H	H	A	..	B	90	1.7
.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.
N	A	H	H	H	A	..	A	55	0.6



Quantitative traits to study

Office

QTL **detected** if significantly clear that observed association not from random process (LOD stats), size of allelic **effect** and **interaction** estimation



Candidate Resistance Genes

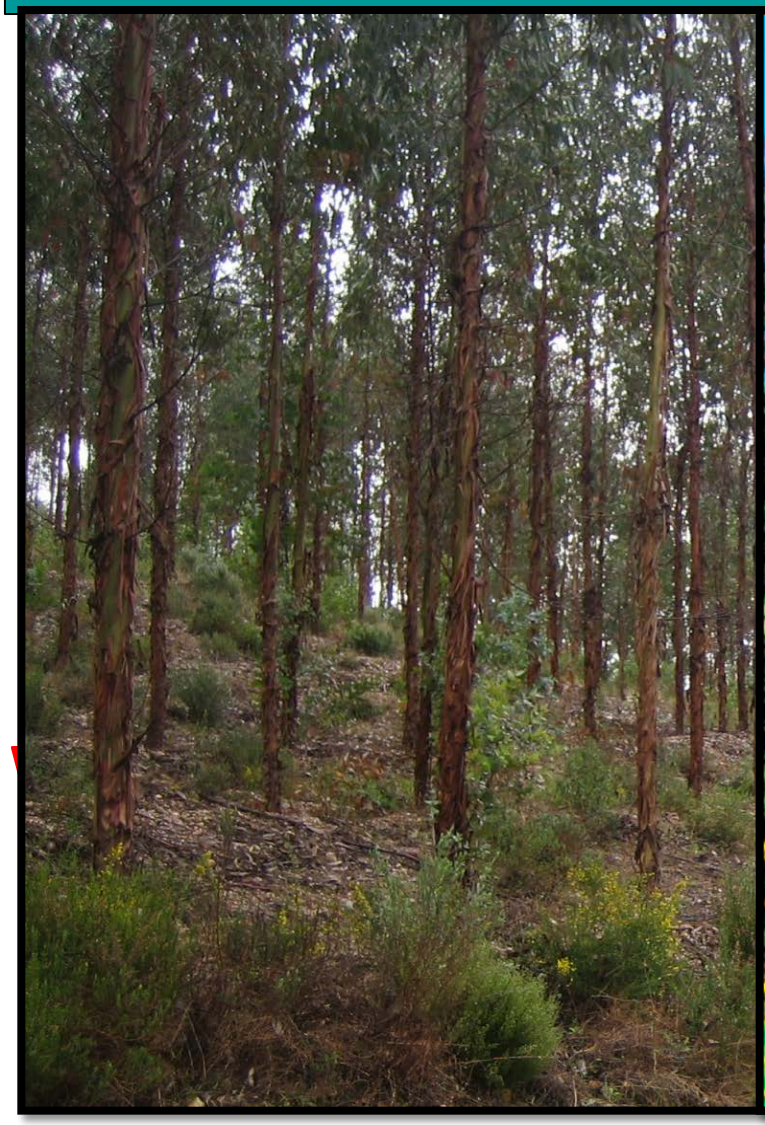
(structural or regulatory)

"Harvest" the genes underlying quantitative traits, dissecting its complex genetics and retriving information on its regulatory networks.

Design specific markers **as tools** for plant selection at DNA level.

Genomics of wood formation

Eucalyptus globulus



Identify the major genetic factors underpinning the physicochemical properties of plant cell walls

Selection of candidate genes involved in transcriptional and post transcriptional regulation of wood formation in eucalypts and poplar

High-throughput NIR spectroscopic methods for wood property measurements

Structural and functional architecture of wood quality in *Eucalyptus* and *Populus*

microEGo - Did you ask for something small? The microRNAs power in a Eucalyptus tension world!

1) **“Which miRNA are expressed during *E.globulus* xylogenesis?”**

Identify and characterize *E. globulus* miRNAs and target-genes putatively involved in the regulation mechanisms of wood formation

2) **“How the expressed miRNA modulate *E.globulus* xylogenesis?”**

Use several complementary validation strategies to confirm the putative role of some of these miRNA providing insights on the post-transcriptional regulation of their target-genes.

Long term aim:

to control *E. globulus* wood quality by modulation of xylogenesis

Task 1 –Tension wood induction and sampling

Bending of trees so that the stem was positioned at an angle of about 45° for 4 weeks, 3 weeks, 2weeks, 1 weed and 1 day

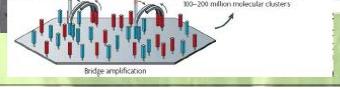
Points kinetics
1 week (1W)
2 weeks (2W)
3 weeks (3W)
4 weeks (4W)

3 genotypes
3 ramets/genotypes

Tissues collected:
Tension wood (T)
Opposite wood (O)
Non-bent trees (Control)

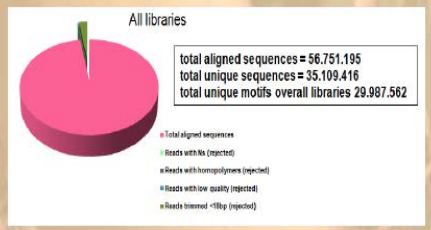
Task 3 - Production and sequencing of transcript libraries

9 small RNA libraries constructed and sequenced (4 time points of bending, tension vs opposite vs vertical wood)



Task 2 - Anatomical and chemical caracterization of xylem samples

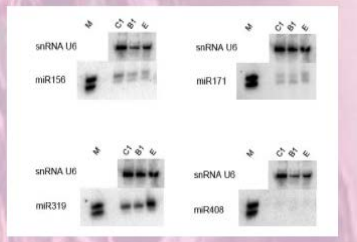
Task 4 - Prediction of miRNA and their targets

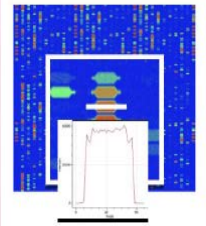


Cravela

Task 6 - Functional analysis of *E. globulus* miRNA and their cleavage target genes

Task 5 - Validation of predicted miRNA and targets





µParaflo® microfluidic biochip

Main partners

P. Fevereiro

Jorge Pinto Paiva

J. Grima-Pettenati

Ana T. Freitas

J.C. Rodrigues

Luis Leal

Funding

PLANT-KBBE

Other collaborations

A. Fonseca

G. Pappas

T. Dalmay

J.C. Leplé

L. Harvengt

J.M. Gion

M. Fladung

P. Schonicke

F. Gallardo & F. Canton

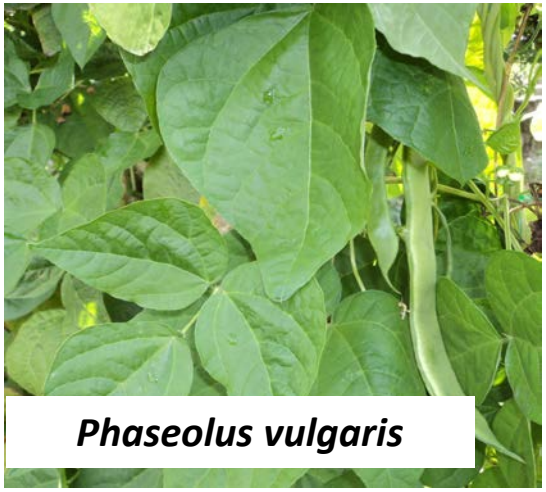
I. Allona

G.L. Scollo

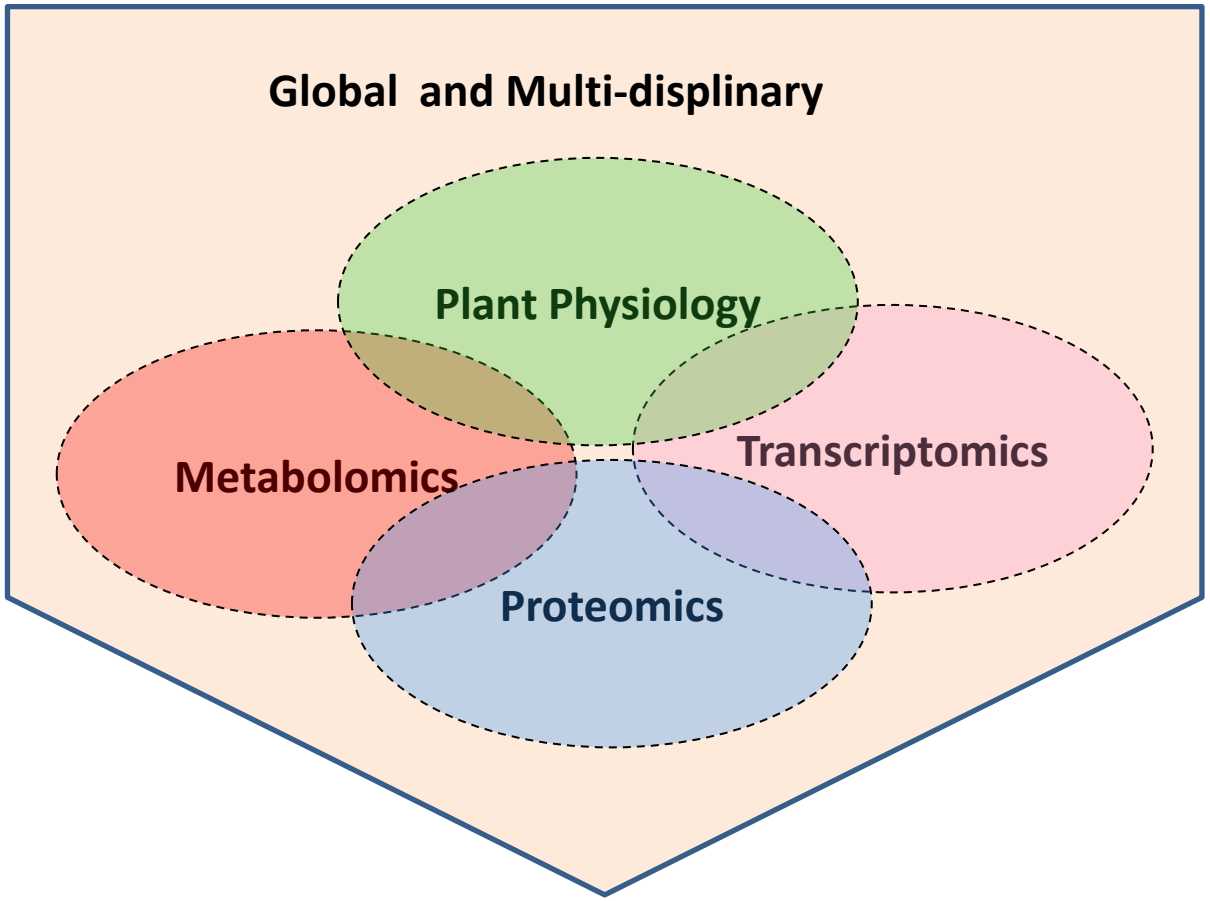
H. Sixto-Blanco

Understanding the molecular mechanisms underlying Legume adaptation to water deficit

Medicago truncatula



Phaseolus vulgaris



Candidate genes and regulatory mechanisms *involved in water deficit responses*

Manipulating the *M. truncatula* trehalose metabolism

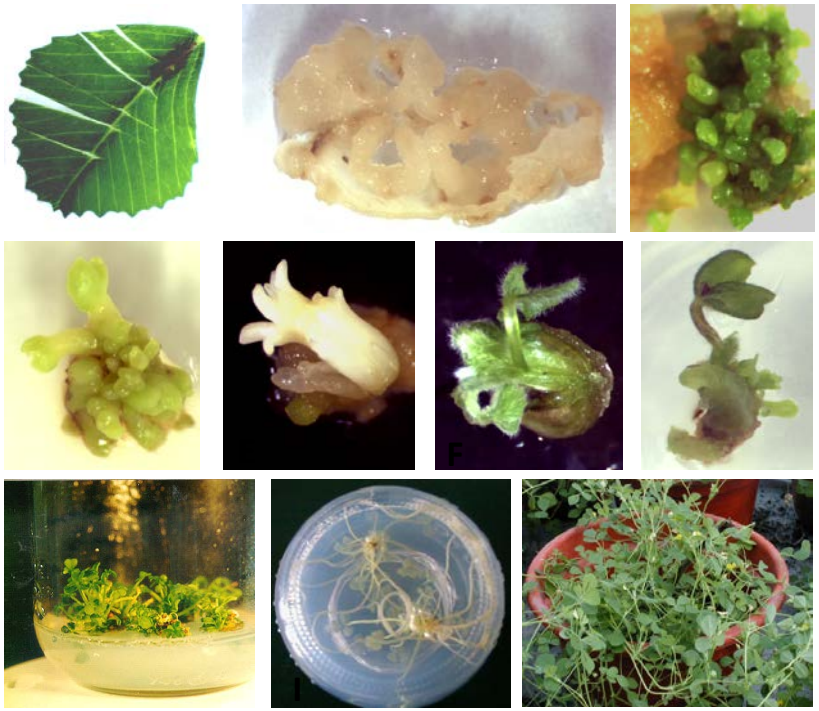
Glucose 6-P + UDP-glucose → Trehalose 6-P → Trehalose

Trehalose-6-Phosphate synthase 1 (TPS1)

Trehalose-6-Phosphate Phosphatase (TPP)

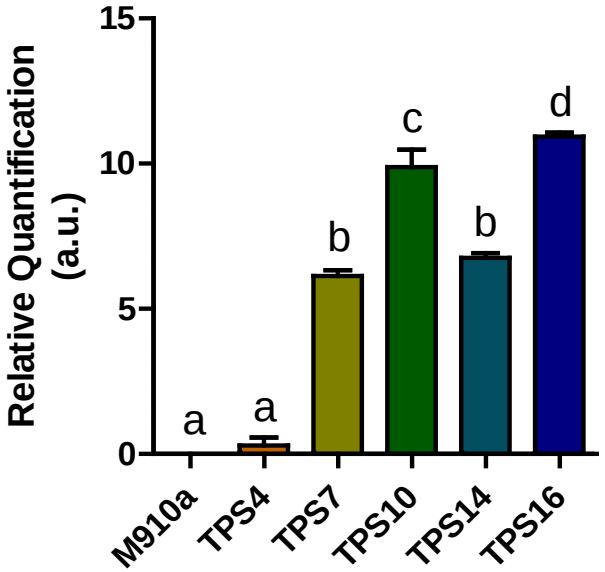
The role of trehalose metabolism in plant WD responses is still uncertain

Constitutive expression AtTPS1 – A. thaliana



***Transformation of M. truncatula* –main steps**

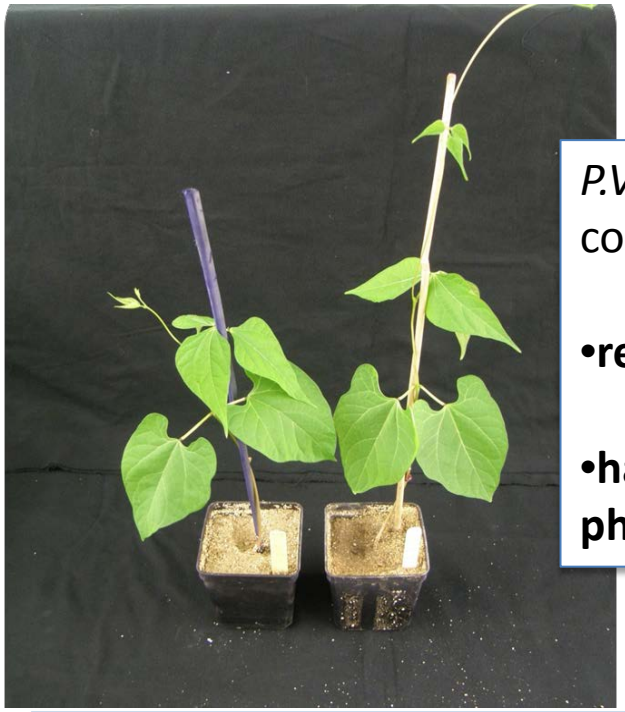
AtTPS1 expression



1) Physiological evaluation AtTPS transgenics
2) Comparision using Omics platforms

Deciphering the molecular mechanisms underlying better grain filling in *Phaseolus vulgaris* under WD

Common bean is one of the most important pulses, namelly in Africa and South America countries



P.Vulgaris lines with contrasting

- **responses toward WD**
- **hability to remobilize photsynthates to grain**

Expected outputs

1) **impact of WD on grain filling**
2) **transcriptome, proteome and metabolome profiles across the different grain developmen stages**
3) **Candidate genes for improved grain filling under WD**

On going projects

2011-2014 - PTDC/AGR-GPL/110244/2009 - Deciphering grain filling mechanisms in *Phaseolus vulgaris L*. under water deficit – PI: Susana Araújo – (IICT- 189 000 €)

2010-2013 - PTDC/AGR-GPL/099866/2008 - Integration of transcriptomic, proteomic and metabolomics profiles to understand the role of T6P in the water deficit response and recovery in *Medicago truncatula*. PI. Susana Araújo (ITQB -189 392 €)

FP7- ARIMNet - MEDILEG - Breeding, agronomic and biotechnological approaches for reintegration and revalorization of legumes in Mediterranean agriculture. Agricultural Research In the Mediterranean Network

Collaborations

IICT
Instituto de Investigação Científica Tropical

BioFIG
Center for Biodiversity, Functional and Integrative Genomics

ITQB
INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA /UNL
Knowledge Creation

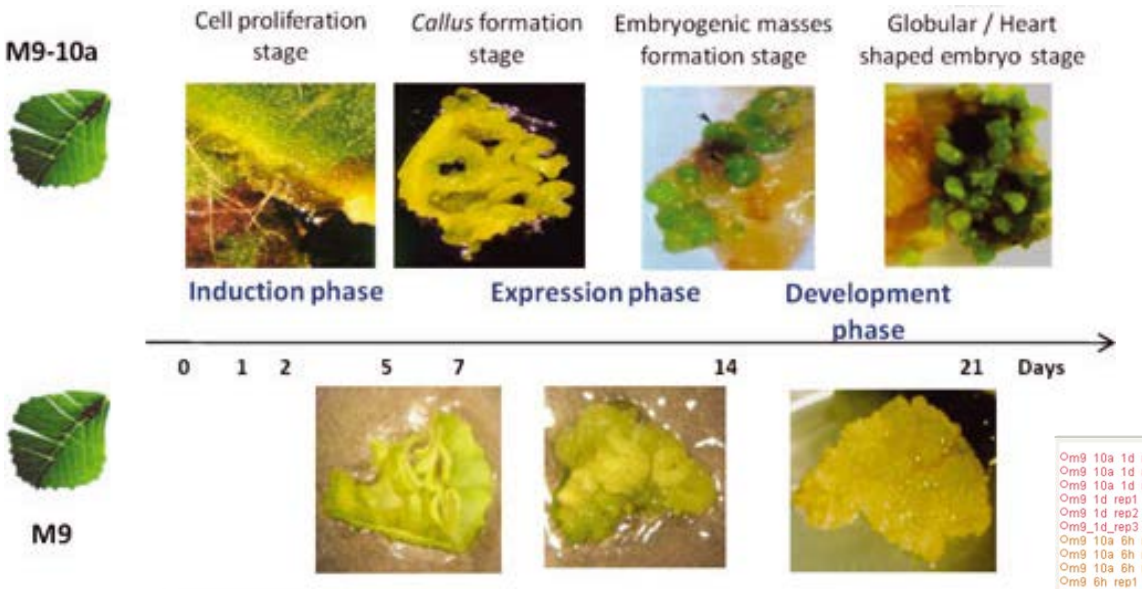
Max-Planck-Institut für Molekulare Pflanzenphysiologie

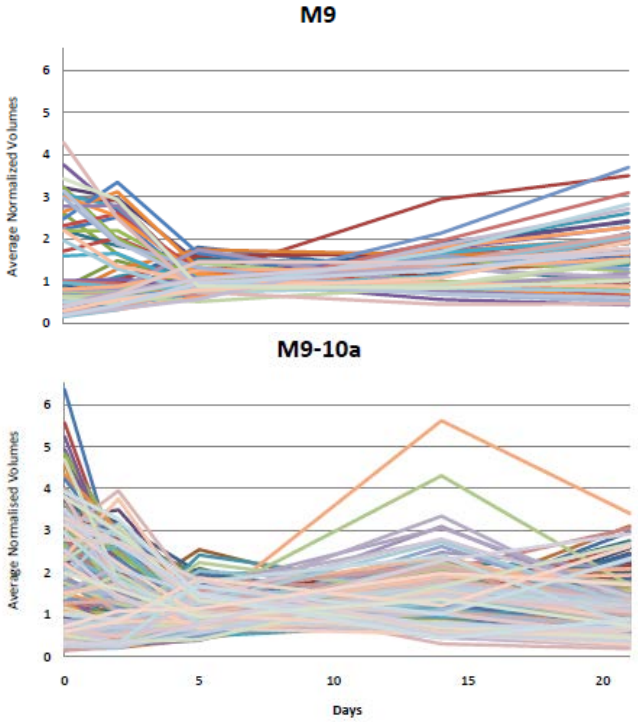
CIAT
Partners in Research Cultivating the Future

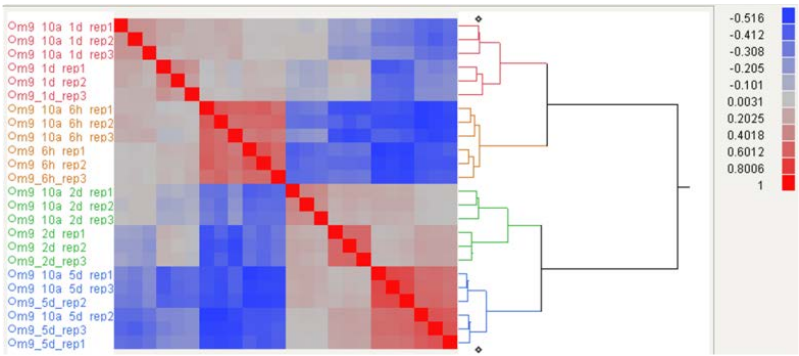
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DE LISBOA

Somatic embryogenesis in *Medicago truncatula*

Comparative molecular analysis of the response to embryogenic induction of one highly embryogenic *M. truncatula* line (M9-10a) and its non embryogenic ancestor line (M9).







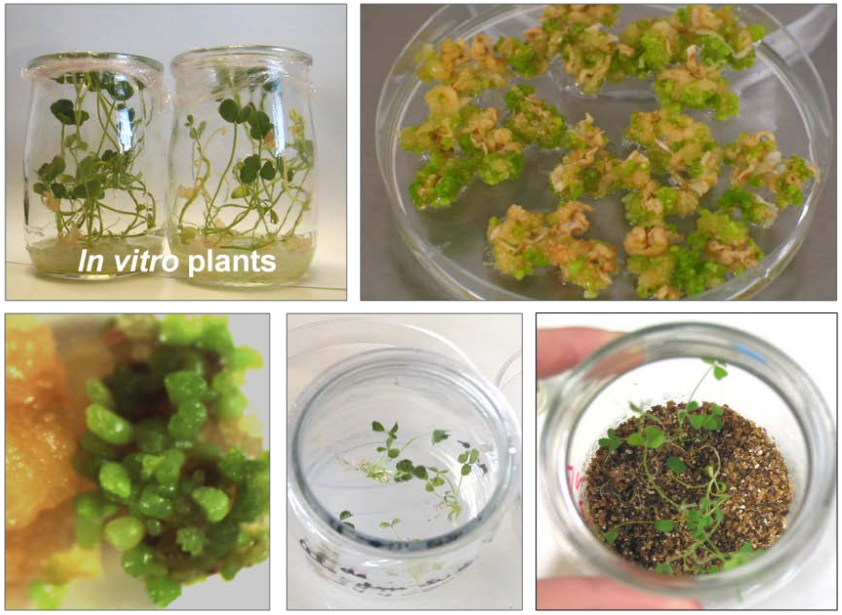
- 136 differentially expressed proteins
- 218 differentially expressed genes
- 42 differentially expressed miRNA

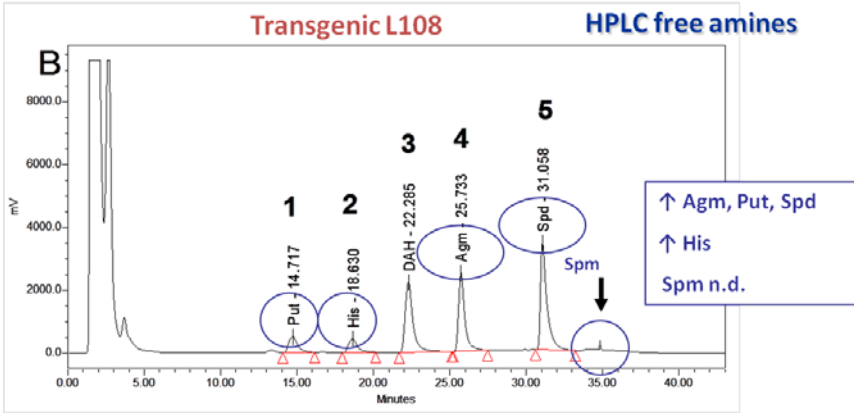
Participants: Sofia Duque (ITQB/UNL), André Almeida (IICT/MNE)
Collaborations: Vagner Benedito (USA-WVU), Tamas Dalmay (UEA-BIO)
Companies: FERTIPRADO, LDA (POR)

Engineering polyamine accumulation to improve abiotic stress resistance in *M. truncatula*

- Over expression of the arginine decarboxylase gene (*adc)* from *Avena sativa*
- Evaluation of polyamine levels in transgenic plants (RT-HPLC)
- Physiological evaluation of transgenic plants under water deficit and high salt conditions

Agrobacterium-mediated transformation





- 22 independent transformed lines
- 6 lines with aot*Adc* single *locus* insertion
- 2 homozygous lines with altered PAs levels
- Physiological evaluation of homozygous transgenic plants under way

Participants: Sofia Duque (ITQB/UNL)
Collaborations: Miguel Gomez (UG-Spain); Susana Araújo (IICT/ITQB); Anabela Silva and Jorge Silva (FCUL; BioFIG)

Susana S. Neves

Bupleurum APIACEAE

Molecular phylogeny and Systematics

- **180-190 species**
- herbs and shrubs
- Worldwide : N Hemisphere
- medicinal uses

Menispermaceae

Molecular phylogeny and fruit evolution

" The moonseed family "

~70 genera ~500 species

- mostly **vines or lianas**
- Worldwide : mainly **Tropical**
- ethnobotanical and medicinal uses

Bupleurum

Collaborations

Menispermaceae

ROYAL BOTANIC GARDEN EDINBURGH

THE NEW YORK BOTANICAL GARDEN

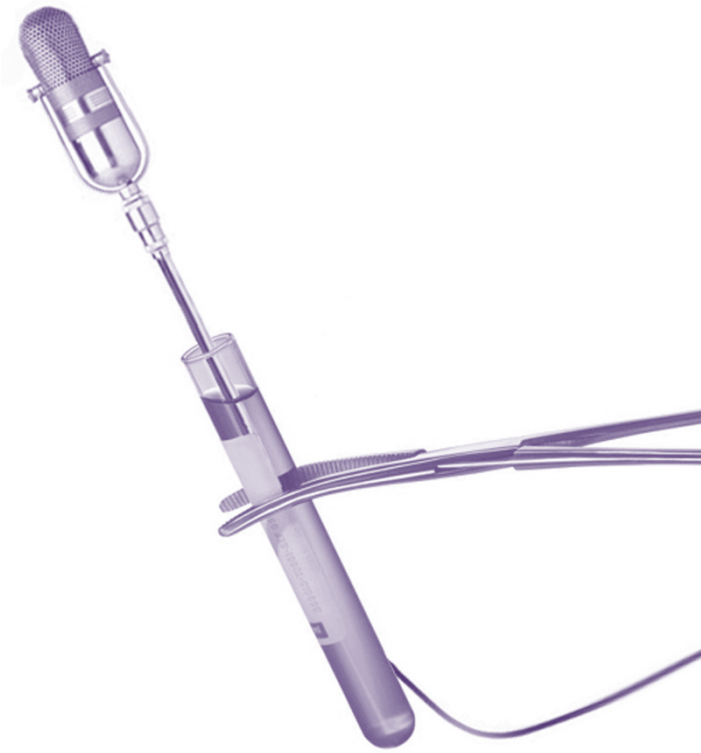
MISSOURI BOTANICAL GARDEN

UNIVERSITY OF JOHANNESBURG

UWM UNIVERSITY of WISCONSIN MILWAUKEE

CiB
Centro de Informação de Biotecnologia
Centre for Biotechnology Information

www.cibpt.org
blog - cibpt.wordpress.com



CiB - Centro de Informação de Biotecnologia

Centre for Biotechnology Information

CiB is…

- … a Portuguese private non profit association created in October 2002 supported by different entities: companies, industries, public and private institutions and even associated sole proprietorships.

- Promoting communication of Biotechnology in Portuguese.

- Communicating with different audiences to explain biotechnological applications in everyday life.

- Participating in public debates & political hearings, development of legislation, regulation and standards on biotechnology issues

Highlight Activities - 2012

- **Workshop – Talking with Media – Communicating AgriBiotechnology – CiB & ITQB**
- **Seminar - AgriBiotechnology in Portugal – CIB, ISA & USA Embassy**
- **Talking with people: "Tertúlia Setubalense" – Transgenic Plants – CiB & Culture Club of Setubal**
- **Meetings with Decision Makers/Deputies of Political Parties and Political Commissions (European Affairs, Agriculture and Environment) of Portuguese Parliament**
- **Contest for High schools – BioNanotech & Medicine**
- **2nd Meeting – Biotech and Agriculture – CiB & ESACoimbra**
- **2nd Forest Genomics Meeting: Transgenic Forest Trees: time to harvest? – Cost Action - IICT, IBET, ITQB, INIAV, CiB e LRSV**
- **Communication through website, blog, facebook, twitter and press releases to Media**

Relevant papers last 3 years

- **Nunes C.**, Primavesi L.F., Patel M.K., Martinez-Barajas E., Powers S.J., Sagar R., **Fevereiro P.S.**, Davis B.G., Paul M.J. **(2013)** Inhibition of SnRK1 by metabolites: tissue-dependent effects and cooperative inhibition by glucose 1-phosphate in combination with trehalose 6-phosphate. *Plant Physiology and Biochemistry* 63: 89-98.
- Almeida A.M., Parreira J.S., Santos R., **Duque A.S.**, Francisco R., Tomé D.F.A., Ricardo C.P., Coelho A.V., **Fevereiro P. (2012)** A proteomics study of the induction of somatic embryogenesis in*Medicago truncatula* using 2DE and MALDI-TOF/TOF. *Physiologia Plantarum* 146: 236–249.
- **Cardoso S.**, Lau W., Eiras Dias J., **Fevereiro P.**, Maniatis N. **(2012)** A candidate-gene association study for berry colour and anthocyanin content in *Vitis vinifera* L. *PLoS ONE* 7(9): e46021.
- Pires A.S., Rosa S., Castanheira S., **Fevereiro P.**, Abranches R. **(2012)** Expression of a recombinant human erythropoietin in suspension cell cultures of *Arabidopsis*, tobacco and *Medicago*. *Plant Cell, Tissue and Organ Culture* 110: 171-181.
- **Capitão C.**, **Paiva J.A.P.**, Santos D.M., **Fevereiro P. (2011)** *Medicago truncatula*, water deficit modulates the transcript accumulation of components of small RNA pathways. *BMC Plant Biology*11:79.
- Fondevilla S., **Almeida N.F.**, Satovic Z, Rubiales D., **Vaz Patto M.C.**, Cubero J.I., Torres A.M. **(2011)** Identification of common genomic regions controlling resistance to *Mycosphaerella pinodes*, earliness and architectural traits in different pea genetic backgrounds. *Euphytica* 182: 43-52.
- **Neves S.S. (2011)** *Eleusine*. In: C. Kole, ed. *Wild Crop Relatives: Genomic and Breeding Resources*, *Millets and Grasses*. pp. 113-133. Springer, Berlin and Heidelberg.
- **Paiva J.A.P.**, Prat E., Vautrin S., Santos M.D., San-Clemente H., Brommonschenkel S., Fonseca P.G.S., Grattapaglia D., Song X., Ammiraju J.S.S., Kudrna D., Wing R.A., Freitas A.T., Bergès H., Grima-Pettenati J. **(2011)** Advancing *Eucalyptus* genomics: identification and sequencing of lignin biosynthesis genes from deep-coverage BAC libraries. *BMC Genomics* 12:137.
- Ricardo C.P.P., Martins I., Francisco R., Sergeant K., Pinheiro C., Campos A., Renaut J., **Fevereiro P. (2011)** Proteins associated with cork formation in *Quercus suber* L. stem tissues. *Journal of Proteomics* 74: 1266-1278.
- **Santos A.R.**, Miguel A.S., **Tomaz L.**, Malhó R., Maycock C., **Vaz Patto M.C.**, **Fevereiro P.**, Oliva A. **(2010)** The impact of CdSe/ZnS Quantum Dots in cells of *Medicago sativa* in suspension culture.*Journal of Nanobiotechnology* 8:24.

http://www.itqb.unl.pt/~BCV/papers.htm

Citation Report Author=(Fevereiro)

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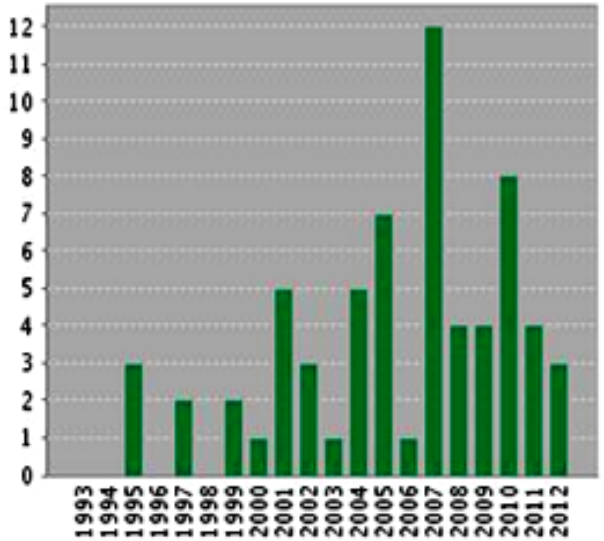

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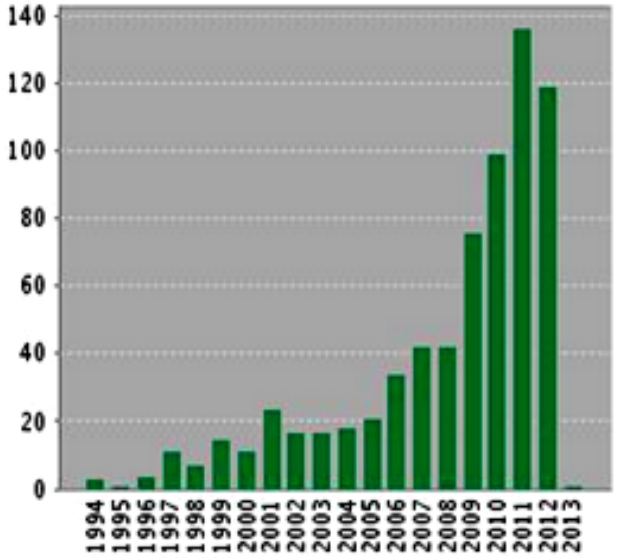

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Pedro Fevereiro

- **Auditor Científico** dos Exames Nacionais do Ensino Secundário de Biologia e Geologia (componente de Biologia) 2009 -
- **Presidente do Centro de Informação de Biotecnologia** 2004 -
- **Membro do Conselho de Leitura "Ciência e Técnica Vitivinícola”** 2009 **-**
- **Membro do Conselho Nacional da Ordem dos Biólogos** 2002 -
- **Presidente da Associação de Pais da Escola Básica António Rebelo de Andrade** 2011 -

- **Past**

- **Primeiro Bastonário da Ordem dos Biólogos** 1999-2002
- **Membro do Conselho Nacional de Ética para as Ciência da Vida** 2003-2010
- **Presidente do Colégio de Biotecnologia da Ordem dos Biólogos** 2004 –2009