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XII Jornadas de Genética e Biotecnologia
II Jornadas Ibéricas de Genética y Biotecnología

Universidade de Trás-os-Montes e Alto Douro

Book of Abstracts

ADNGB
Núcleo de Alunos de Genética e Biotecnologia da AAUTAD

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XII Genetics and Biotechnology Conference/ II Genetics and Biotechnology Iberian Conference

The Genetics and Biotechnology Conference (JGB) of the University of Tras-os-Montes and Alto Douro (UTAD) is an annual scientific event organized jointly by the Nucleus of Students of Genetics and Biotechnology (ADNGB) of UTAD and the Direction of the Course of Genetics and Biotechnology in collaboration with the teaching staff of the Department of Genetics and Biotechnology (DGB). As a result of the scientific-pedagogical partnership established between professors of DGB (UTAD) and of Faculty of Biological and Environmental Sciences of the University of León (UL), Spain, it was considered important to repeat the shared organization of this event between professors and students of the UTAD and UL designating it as XII Genetics and Biotechnology Conference / II Genetics and Biotechnology Iberian Conference (XII JGB / II JIGB). The main objective of the XII JGB /II JIGB is to update knowledge in the area of Genetics and Biotechnology. To this end, the focus of this event is the conferences given by renowned national and international scientists and the thematic workshops that will constitute more practical sessions. The XII JGB /II JIGB will also focus on interaction, exchange of experiences and scientific debates between Portuguese and Spanish students and professors. The best oral and posters presentations will be awarded. The target audience is Portuguese and Spanish students, researchers and university professors from the scientific areas of Biological Sciences and Biotechnology as well as High School teachers from the Biology area. A wide variety of topics will be discussed, in the different areas of Genetics and Biotechnology, such as Plant, Animal, Human, Microbial, Evolutionary, Cancer, Forensic, Ethics, Entrepreneurship, among others.

Committees

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Rector of the University of León - Professor Juan Francisco García Marín
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Professor María Luz Centeno
Professor Penélope García Angulo

Students and members of ABLE:

Luis Getino Alonso
Juan José Vindal Núñes

Program

Thursday 20th February- UTAD

14.30-17.30 h: Workshops

Eurico Lima, Lucinda Vaz Reis - *How to extract, isolate and identify plant-based secondary metabolites with biotechnological and pharmacological relevance?*

Manuela Matos, Marlene Santo, Ana Sofia Soares, Ana Cláudia Coelho - *Molecular Genetics – Genotype vs Phenotype*

Irene Oliveira, Eduardo Pires - *Introduction to Phyton and R programming for applications in Bioinformatics*

Jorge Azevedo - *The history of silkworm production and new technologies*

Carlos Viegas, João Requicha - *Clinical genomics of the periodontal disease*

Friday 21st February- Aula Magna UTAD

8.30-9.30 h: Registration

9.30-10.00 h: Opening Session

10.00-11.00 h: Conference

Nelson Saibo, Professor
ITQB, Lisbon

New insights into different regulatory networks controlling both plant responses to environmental cues and C4 photosynthesis

11.00-11.15 h: Coffee break

11.15-12.00 h: Conference

David Caparrós Ruiz, Professor
Universitat Autònonma de Barcelona, Spain

The role of O-methyltransferases in lignin biosynthesis in maize

12.00-13.00 h: Oral Communications

Balboa Álvaro J. - *Changes in gene expression in lentil subjected to drought stress*

Santos M. - *Foliar application of magnesium and potassium as mitigation strategy of sweet cherry cracking: effects on fruit quality and gene expression*

Santos R. - *How close are Portuguese and African cowpea accessions? An evaluation by SSR markers*

Machado M. T. - *Multivariate data analysis of morphological characterization data in Portuguese maize landrace accessions*

Vázquez A. - *Recycling of citric wastes to obtain products with commercial value*

14.30-15.30 h: Conference

Luísa Vasconcelos, PhD
Instituto Gulbenkian de Ciência, Lisbon

The study of innate behavior using the fruit fly

15.30-16.15 h: Conference

Carmen Marin, Professor
Universidad de Léon, Spain

A newly described function of the TP73 tumour suppressor gene as an architect of epithelial tissue

16.15-16.30 h: Coffee break

16.30-17.00 h: Poster Session

17.00-18.00 h: Oral Communications

Canedo-Ribeiro C. - *Preimplantation genetic testing for aneuploidy (PGT-A) as a useful tool in cattle breeding programmes*

Barros S. - *Simvastatin disrupts zebrafish (Danio rerio) lipid and energy metabolism after long-term exposure*

Bezerra M. - *Dalbavancin as a therapeutic option to treat human osteomyelitis caused by methicillin-resistant Staphylococcus aureus (MRSA) infection*

Soares A.S. - *Indoors Fungal biodiversity - Penicillium and Alternaria*

Meireles D. - *Listeria monocytogenes wall teichoic acid glycosylation promotes surface anchoring of virulence factors, resistance to antimicrobial peptides and susceptibility to antibiotics*

18.00-18.30 h: Round Table with Former Students
(exclusive event for former and current students of 1st, 2nd and 3rd cycles in the field of Genetics and Biotechnology)

20.30 h: Formal Dinner

Saturday 22nd February- Aula Magna UTAD

9.30-10.30 h: Conference

Patrícia Monteiro, Professor
Universidade do Minho

Genetics models of Autism Spectrum Disorders (ASD): the Shank3 gene

10.30-11.15 h: Conference

Olga Amaral, PhD
INSA, Porto

Novel tools in cell and molecular biology: induced pluripotent stem cells in the field of lysosomal storage disorders

11.15-11.30 h: Coffee break

11.30-12.00 h: Conference

Joaquim Sá, Dr.
CGC Genetics / Centro de Genética Clínica, Unilabs, Porto

The usefulness of medical genetics in real clinical practice

12.00-13.00 h: Oral Communications

Lino A. - *qPCR assay for Colletotrichum acutatum detection and quantification*

Silva-Reis R. - *Morphometric analysis of rat parameters during an animal model of colorectal cancer*

Ribeiro I. - *Identification of transcription factors associated with a cancer stem cell phenotype in colon cancer cells*

Lucas D. - *FA-SAT ncRNA depletion induces apoptosis – is it through p53 mediation?*

Lopes M. - *SatDNA involvement in rob(14;21) formation: Assessing the robustness of technique interdependency*

14.30-15.30 h: Conference

Joana Azeredo, Professor
Universidade do Minho

The biotechnological potential of bacteriophages to control infectious diseases

15.30-16.15 h: Conference

Agostinho Antunes, Professor
Universidade do Porto

Understanding Adaptation with Genomics and Bioinformatics: Biotechnological Relevance

16.15-16.30 h: Coffee break

16.30-17.00 h: Musical moment

17.00-17.30 h: Award Ceremony and Closing Session

Speakers

Nelson Saibo, Professor

Nelson Saibo graduated in Biology by the University of Aveiro in 1994 and completed a MSc in Physiology and Biochemistry of Plants by University of Lisbon in 1997. In 2003, he was awarded a PhD in Sciences - Biotechnology by the University of Ghent (Belgium). Since 2004 he has been researcher at ITQB NOVA, first as a post-Doc and after 2007 as auxiliary researcher. In 2013, he became principal investigator and since then he has been the group leader of the Plant Gene Regulation laboratory (http://www.itqb.unl.pt/research/plant-sciences/plant-gene-regulation) and also the head of the Plant Sciences Division at ITQB NOVA. His research addresses the molecular basis of photosynthetic performance (particularly gene regulation) as well as plant responses to adverse environmental conditions and how this information is translated into growth. Nelson has coordinated or been involved in many different research projects, including the EU projects 3to4 (Converting C3 to C4 photosynthesis for sustainable agriculture) and PHOTOBOOST (A holistic approach to improve the photosynthetic performance and productivity of C3 crops under diverse environmental conditions), and published more than 40 international peer-reviewed papers and five book chapters. He is member of the Portuguese Plant Physiology Society´s Direction (since 2008) and ITQB representative at the Portuguese ELIXIR hub. Nelson is a regular lecturer for ITQB NOVA PhD programmes and co-coordinates one MSc and two PhD curricular units. Since 2014, he has been the director of the International PhD programme in Plant Sciences *Plants for Life* (run in collaboration with VIB-PSB, TSL, MPI-MPP, and SLCU).

David Caparrós Ruiz, Professor

In 1996, I obtained the Bachelor degree in Biochemistry (Universitat Autònonma de Barcelona, UAB). In 1997, I moved to France to obtain my master degree (Bases de la Production Végétale, option Biotechnologie et Amélioration des Plantes, Université de Montpellier II, Université de Perpignan and l'Ecole Nationale Supérieure d'Agronomie

(ENSA) de Montpellier, France). Then, I obtained a TMR-Marie Curie Research Training Grant to do my PhD at the CNRS-Université de Perpignan, (France) and I became Doctor in July 2002. After a period of post-doc at the Molecular Department at CID-CSIC (Barcelona) with a grant associated to and European project, in 2003, I was granted with a "Ramon y Cajal" contract (agriculture section) and I joined CRAG. Since then, I started to stablish my own research group and I started to supervise master and PhD students as well as several postdoctoral researchers. Although my main job is as permanent researcher at CRAG, I am also currently Associate Professor at the Department of Biochemistry of the Faculty of Bioscience at the UAB to give courses of Plant Molecular Biology and Biotechnology. In addition, I also participate in the coordination and teaching in the CRAG-UAB-UB interuniversity master "Plant Biology, Genomics and Biotechnology". The main interests of my scientific work has been devoted to study how maize is able to synthesize the lignin polymer. Maize is one of the major crops worldwide and has been predominantly used as a forage crop due to its high nutritional value. In recent years, to substitute the contaminant fossil fuels, maize has been also used as source for the production of bioethanol. Initially, bioethanol has been produced from sugars accumulated in the seeds (first generation biofuel), directly competing with fields dedicated to food and feed. A solution to avoid this contest is the use of the maize stover (lignocellulosic biomass) but its huge amount of cellulose is nowadays discarded due to its interaction with the lignin polymer. These interactions imply that an important percentage of these polysaccharides are not digested by ruminants or extracted for the production of cellulosic bioethanol (secondary generation biofuel), thus, reducing the nutritional and energetic values of this biomass. Our main interests are addressed to understand how the modification of lignin genes affects the content and composition of the lignin and polysaccharides polymers within the maize cell walls. During the last years we have worked with maize mutants, transgenic and different inbred lines. This knowledge is essential for further undertaking new biotechnological approaches leading to new maize lines with improved nutritional and energetic values of the lignocellulosic biomass, thus making its high polysaccharides content available for these industrial proposes. During this time, I have been Principal Investigator of several research projects (national and European), and I have published more than twenty papers in peer-reviewed (first quartile) journals.

Maria Luísa Vasconcelos, PhD

Luisa has developed her graduate studies with Dr. S. Lawrence Zipursky at University of California in Los Angeles and has defended her thesis at the New University of Lisbon. She studied Dscam, a cell surface molecule of the immunoglobulin superfamily that plays an important role in the sculpting of neural circuits. In 2004 she joined the Lab of Dr. Richard Axel at Columbia University. There she identified components of a neural circuit that governs dimorphic sexual behaviour. In 2008 she moved to Instituto Gulbenkian de Ciência to start her group focused in the neuronal circuits that control innate behaviors. In 2012 She joined the Champalimaud Research where many group share the scientific interest in the neural underpinning of behavior.

Carmen Marin, Professor

I am Associate Professor of Cell Biology at the University of Leon and the Principal Investigator (ORCID: 0000-0002-7149-287X) of the research group: Cellular Differentiation and Development of Cell Models at the Instituto of Biomedicine (IBIOMED). I am the author of 40 articles with an accumulated impact factor of 357.33 (JCR-2018), h-index =25, i10-index= 31, and more than 4649 citations. I have a Bachelor degree in Biology from the University of Salamanca, and obtained my PhD in Biomedical Sciences at the MD Anderson Cancer Center, affiliated to the University of Texas, and supported by an NIH fellowship. My PhD work focused on the functional interaction between p53 and Bcl-2 during lymphomagenesis. During my postdoctoral training, I worked at the Dana Farber Cancer Institute (Harvard University) in Dr. William G.. Kaelin laboratory (Nobel Prize in Physiology or Medicine 2019) supported by a postdoctoral NIH Training grant and HHMI funds. My work was essential for the initial description of p73, the first p53-homologe identified. During this training, I authored 6 scientific papers and 1 review, all in top 10% impact factor journals. The relevance of my work is highlighted by the fact that two of

these publications are considered citation classics, with more than 600 citations each one, and another two that score more than 400 citations each. Since my incorporation to the University of Leon in 2005, despite of being in a small institution, the group that I lead has published 13 scientific articles, some of them in top journals like Nature Cell Biology, Cell Death & Differentiation, Cell Death & Disease, among others, and nowadays is considered one of the leading groups in the p73 field worldwide. The group has been funded continuously by competitive, overlapping, national and regional grants. As shown by our publications, we maintain active collaborations with top international groups leaders in our working area. Moreover, the group holds a solid and productive collaboration with biotech companies like the Instituto Biomar, S.A., in order to develop physiologically relevant differentiation model systems for the screening of pharmacological candidate compounds. These projects have been funded by competitive national grants (PETRI, PROFIT, RETOS-COLABORACION). Since my integration at the University of León, I have supervised 7 PhD students and 13 Master theses and I am the co-inventor of a patent. Moreover, all my former trainees are currently working in scientific institutions, biotech companies or have a science related career. Along with my research experience, I try to be active in knowledge transfer, lecturing Cell Biology courses in several bachelor and master programs at the University of León. I also participate in woman empowering programs and contribute to several initiatives for Women and Girls in Science as well as diffusion activities through scientific news agencies and local media.

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Patrícia Monteiro, Professor

Doctor Patricia Monteiro is a Research Assistant Professor in the Department of Neuroscience, ICVS/School of Medicine, University of Minho. She holds a BSc/MSc degree in Pharmaceutical Sciences and a PhD degree in Neuroscience. Dr. Monteiro did her Ph.D. training with Professor Guoping Feng at the Massachusetts Institute of Technology (MIT, USA), where she focused on understanding brain circuitry mechanisms underlying autism-spectrum disorders (ASD) and her postdoctoral training with Professor Nuno Sousa at Minho University. She published a study showing reversal of autistic-like behaviors in adult mice by restoring the *Shank3* gene and collaborated in a study showing that particular human mutations in the *SHANK3* gene can lead to distinct neuropsychiatric disorders. Dr. Monteiro has received numerous awards namely an EMBO postdoctoral fellowship and the prestigious Branco Weiss fellowship attributed by the Swiss Federal Institute of Technology Zurich (ETH).

WebProfile:
http://www.icvs.uminho.pt/research-scientists/neurosciences/people/patriciamonteiro

Olga Amaral, PhD

Olga Amaral studied at the University of London, and had short-term fellowships at the University of North Carolina and Mount Sinai School of Medicine. She obtained her Doctorate Degree at the University of Porto in 2000/2001. Her professional beginning was as a junior grantee at CGM Jacinto Magalhaes. She has worked in the field of molecular and cell biology focusing on rare diseases, particularly those of Lysosomal involvement. She was responsible for setting up molecular genetic diagnosis for Lysosomal Storage Diseases in Porto at IBMC, and supervised that area from 1996-2006. Also collaborated and supervised various projects and students in this field. Currently, works at the National Health Institute Ricardo Jorge (INSA, IP) in the Research and Development Unit of the Department of Human Genetics. She is team member of the Research groups S.Alves (INSA) and CECA do ICBAS (UP). She is the Principal Investigator of the Project: Cellular Models for the study of Lysosomal Dysfunction, with financing from FCT Project PTDC/BIM-MEC/4762/2014, in collaboration with the José Bragança from University of Algarve, among others, and supervisor of two ongoing Doctorate students at ICBAS - University of Porto. Her other research interests are the new generation sequencing and rare diseases (Project “Desvendar”). Her main areas of expertise are Lysosomal Storage disorders; Human Genetics; Science literacy; Molecular Biology.

Joaquim Sá, Dr.

Degree in Medicine from the University of Coimbra completed in 2001, General Internship at Hospital de Aveiro completed in 2003, Complementary Internship in Medical Genetics at Centro Hospitalar e Universitário de Coimbra, concluded in 2008. Since then, he has been a geneticist at the Medical Genetics Unit Pediatrics Hospital, at the Prenatal Diagnosis Cente Maternity Bissaya Barreto and at CGC Genetics. He also collaborate in part-time with Hospital do Funchal, for three years, and Centro Hospitalar do Algarve, for seven years. In addition to the assistential activity, he has participated in several research projects that resulted in the co-authorship of fifteen articles indexed in PubMed and, in 2008, in the clinical research prize of the Portuguese Society of Human Genetics. The focus of clinical activity has been prenatal diagnosis and oncogenetics. Still, in the near future, it intends to study the interpretation of variants and notions of bioethics.

Joana Azeredo, Professor

Biological Engineering of the University of Minho and develops her research activity at the Centre of Biological Engineering, where she is a member of the direction board, leading the Biofilm Science and Technology research Group and the Bacteriophage Biotechnology Group. Her current research focuses on interaction of bacteriophages with biofilms and development of bacteriophage based biotechnological applications for detection and control pathogens. Joana Azeredo was graduated in 1994 as Biological Engineer at the University of Minho and obtained her PhD in Chemical and Biological Engineering also at the University of Minho (1998). She developed pioneer studies in collaboration of Ian Sutherland (Univ. Edinburgh) on the characterization of the interaction of bacteriophages with biofilms and demonstrated the efficacy of bacteriophages against biofilms. She and her team have isolated and sequenced several new bacteriophages giving an important

contribution to phage taxonomy and have demonstrated in vivo and in vitro the efficacy of bacteriophages and derived enzymes in controlling several human pathogens. She has supervised 16 PhD students and lead 2 European and 7 National and Regional projects. She regularly serves as international expert project reviewer for national and international funding agencies and as peer-reviewer for international scientific journals. She has extensive publishing and editorial experience, both as an author (+200 international peer reviewed papers), member of editorial boards and editor of thematic issues and books. Her work has received +6000 citations and her current h-index is 45 (Scopus, October 2019).

Agostinho Pereira, Professor

Prof. Agostinho Antunes earned his Ph.D. in Genetics and Evolution (2002) at the University of Porto (Portugal), in collaboration with the INRA (Paris, France) and the Washington University (St. Louis, USA). He undertook Post-Doctoral work at the Laboratory of Genomic Diversity (LGD), National Cancer Institute, National Institute of Health, Frederick, Maryland, USA (2002-2004), and he continued affiliated with LGD as a Visiting Scientist until 2011. He became a Researcher (Computational Biochemistry) at the REQUIMTE, University of Porto, in 2005, then moved in 2007 to the CIIMAR (Interdisciplinary Centre of Marine and Environmental Research), University of Porto, and in 2013 became the Head of the Evolutionary Genomics and Bioinformatics Group. Since 2011 he has been also Professor at the Department of Biology from the University of Porto and Director of the Environmental Monitoring Centre – CMIA, in Matosinhos Municipality, Porto, Portugal. His major research interests include understanding the evolutionary significance of genomics in natural adaptation, diversification and speciation, and its conservation relevance, notably by integrating genomics, bioinformatics and biotechnology to describe overall diversity patterns from microorganisms to animals.

Conferences

New insights into different regulatory networks controlling plant responses to environmental cues and C4 photosynthesis

Borba A.R.[1], Gorska A.[1], Cordeiro A.[1], Andrade L.[1], Carvalho P.[1], Lourenço T. [1], Serra T.[1], Gouveia P.[1], Peterhaensel C.[2], Langdale J.[3], Wigge P.[4], Oliveira M.M.[1], Hibberd J.M.[5], Saibo N.J.M.[1*]

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Keywords: Transcription factors, *cis*-elements, rice, maize, abiotic stress, plant productivity, photosynthetic efficiency

One of the greatest scientific challenges in the coming decades is to enhance crop productivity. In our lab, we aim at identifying regulatory networks that ultimately can be selected by advanced breeding programs. One of our research lines regards the study of the rice responses to adverse environmental conditions, such as high salinity, low temperature, and drought. We have identified and functionally characterized a number of rice transcription factors and other regulators involved in abiotic stress responses and plant development. Lately, we have been particularly interested in deciphering the role of the rice Phytochrome Interacting Factors (PIFs) mediating the rice responses to different environmental cues. Our second line of research aims at identifying photosynthesis regulators that ultimately can be used to boost photosynthesis and crop yield. It is well known that C4 photosynthesis is much more efficient than C3 photosynthesis and many efforts have been developed to implement the C4 metabolism in C3 crops. However, among others, the little knowledge regarding the regulation of the C4 photosynthesis has impaired this challenge. Our lab has contributed to better understand the molecular mechanisms underpinning the regulation of the C4 photosynthesis. Since the C4 metabolism is highly dependent on the compartmentation between mesophyll and bundle sheath cells, it is fundamental to unveil the molecular networks regulating the differential accumulation of key photosynthetic proteins in these specialized cells. Using different approaches, we have identified and characterized a number of transcription factors and *cis*-acting regulatory elements involved in the cell-specific gene expression essential for the C4 metabolism. I will present and discuss our last results and future perspectives.

Acknowledgments: This work was supported by EU (GA nº: 289582) and FCT (UID/Multi/04551/2013, PTDC/BIA-BCM/099836/2008, and PTDC/BIA-FBT/31070/2017). ARB, AG, AC, AL, PC, TL, TS, and NJMS were funded by FC; TS and PG by EU (GA nº: 289582).

The role of *O*-methyltransferases in lignin biosynthesis in maize

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Keywords: Cell wall polysaccharides; Lignin; Maize; Methyltransferases; Tricin

Caffeoyl coenzyme A 3-*O*-methyltransferase (CCoAOMT) and caffeic acid-*O*-methyltransferase (COMT) are key enzymes in the biosynthesis of coniferyl and sinapyl alcohols, the precursors of guaiacyl (G) and syringyl (S) lignin subunits. The function of these enzymes was characterized in single and double mutant maize plants. In this work, we determined that the *comt* (*brown-midrib 3*) mutant plants display a reduction of the flavonolignin unit derived from tricin (a dimethylated flavone), demonstrating that COMT is a key enzyme involved in the synthesis of this compound. In contrast, the *ccoaomt1* mutants display a wild-type amount of tricin, suggesting that CCoAOMT1 is not essential for the synthesis of this compound. Based on our data, we suggest that CCoAOMT1 is involved in lignin biosynthesis at least in midribs. The phenotype of *ccoaomt1* mutant plants displays no alterations, and their lignin content and composition remain unchanged. On the other hand, the *ccoaomt1 comt* double mutant displays phenotypic and lignin alterations similar to those already described for the *comt* mutant. Although stems from the three mutants display a similar increase of hemicelluloses, the effect on cell wall degradability varies, the cell walls of *ccoaomt1* being the most degradable. This suggests that the positive effect of lignin reduction on cell wall degradability of *comt* and *ccoaomt1 comt* double mutants is counteracted by changes occurring in lignin composition, such as the decreased S/G ratio. In addition, the role of the flavonolignin unit derived from tricin in cell wall degradability will be also discussed.

Acknowledgments: This work was supported by the Spanish Ministry of Economy and Competitiveness and from the Severo Ochoa Programme for Centres of Excellence in R&D; the CONSOLIDER-INGENIO Programme from the Spanish Ministerio de Ciencia e Innovación and by CERCA Programme / Generalitat de Catalunya and the Suport a Grups de Recerca Programme from the Autonomous Government of Catalonia.

The study of innate behavior using the fruit fly: How flies progress from courtship to copulation attempt

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Keywords: Drosophila, behaviour, neuronal circuits, courtship behaviour, genetic manipulation

Our laboratory studies how the activity in defined neuronal circuits elicits behaviour. Sexual behaviours represent a robust set of innate responses. In *Drosophila* courtship behaviour is initiated by the male and involves orientation towards the female, following the female, tapping, wing vibration, licking, attempted copulation and, if the female is receptive, copulation. A virgin female initially runs from the male, but if receptive, she slows and positions herself to facilitate copulation. Once the female has copulated she will become unreceptive to a male's advances. Female receptivity is thought to be dependent on the wing vibration of the male, the pheromone profile of the male and whether the female has mated previously. Genetic studies have elucidated how *Drosophila* male courtship behaviour is specified and its circuit components are being dissected at a surprising speed. The circuit of female behaviour on the other has been largely uncharacterized. Here we will report our study of ovipositor extrusion a female behaviour performed exclusively during courtship. We describe how we identified a pair of descending neurons (DNp13) controlling ovipositor extrusion. We used the genetic access to these neurons to activate and inactivate DNp13 during courtship to determine the role of ovipositor extrusion in courtship. We found that ovipositor extrusion prompts the male to attempt copulation.

Acknowledgements: Funded by FCT project PTDC/MED-NEU/30105/2017

A newly described function of the TP73 tumour suppressor gene as an architect of epithelial tissue

Maeso-Alonso L[1]., Fuertes-Álvarez S[1], Martín-López M[1], Villoch-Fernández J.[1], Marques MM[2] & Marín MC*[1]

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Keywords: p73, tumour suppressor genes, cytoarchitecture, ciliogenesis, Planar Cell Polarity, morphogenesis

Tissue structure and the coordinated behaviours across epithelial sheets, which set up the basis of morphogenesis and tissual architecture, depend on the correct formation of intercellular junctions and the concomitant establishment of cellular polarity. Coordinated cellular polarity is required for the development and homeostasis of all metazoans. In fact, the loss of normal tissue architecture is a key criterion to identify and categorize disease states. In this regards, disruption of cell polarity is a hallmark of cancer. The *TP73* gene, like the other member of the p53 family of tumour suppressors, is known to regulate many processes like apoptosis, cell differentiation, cell metabolism or stem cell self-renewal. However, the *Trp73* null mice (p73KO) showed phenotypes, like gastrointestinal and cranial haemorrhages, rhinitis or central nervous system defects, that could not be explained by these known functions. Our group proposed that p73 carry out an essential function in the establishment of the tissue architecture and that the disruption of these p73-regulated mechanisms underlie the observed defects of the p73KO mice. To address this, we analysed the effect of p73-deficiency in different *in vitro* and *in vivo* models. First, we observed that in induced pluripotent stem cells, the lack of p73 lead to iPSC-clones with an impaired epithelial phenotype which correlated with altered stemness. Later on, a study of the development of the mice ependymal layer revealed a novel TAp73 function as an essential regulator of the cellular cytoskeleton dynamics, been required for ependymal cell ciliogenesis and planar cell polarity establishment. Finally, in a completely different setting, the vascular endothelium, the analysis of the endothelial cell-cell interactions revealed that p73-deficiency leads to a defective endothelial network formation with affected endothelial permeability. Altogether this data supports p73 role of as an epithelial architect, proposing this tissue organization functions as a non-canonical tumour suppressor activity.

Genetics models of Autism Spectrum Disorders (ASD): the *Shank3* gene

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Keywords: Autism spectrum disorders (ASD), *Shank3*, electrophysiology, behavioural neuroscience, auditory sensory processing

Autism spectrum disorders (ASD) are a group of debilitating developmental disorders that include a wide range, "a spectrum", of symptoms, skills, and levels of disability. Symptoms typically emerge in the first two years of life and include repetitive behaviours, limited interests, and difficulty in communicating and interacting with others. ASD patients also show clinical features suggestive of abnormal processing of auditory information and other sensory information that might interfere with their ability to socially interact with others. Currently, there is no known single cause for ASD and no effective treatments available. Large-scale genomic studies have supported an association between cases of ASD and mutations in the SH3 and multiple ankyrin repeat domains protein 3 gene (*SHANK3*), which encodes a postsynaptic scaffolding protein that is present at glutamatergic synapses and enriched in the postsynaptic density fraction (PSD). Based on this evidence, we have previously generated a mutant mouse line (*InsG3680*) that harbours an ASD patient-linked single guanine nucleotide (*G*) insertion at cDNA position 3680, leading to a frameshift and downstream stop codon. Our data shows that the ASD-linked *InsG3680* mutation results in an almost complete loss of SHANK3 protein expression, reduced spine density and synaptic transmission deficits. *InsG3680* mice manifest profound repetitive self-injurious grooming, sensory motor gating deficits in auditory PPI test and impaired juvenile social interaction, coinciding with the early onset of ASD symptoms. We are now using a multidisciplinary approach combining genetics, optogenetics, electrophysiology and behavioural neuroscience to dissect auditory sensory processing in *InsG3680* mice and to identify potential new targets for the development of effective ASD treatments.

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Novel tools in cell and molecular biology: induced pluripotent stem cells in the field of lysosomal storage disorders

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Keywords: Human Genetics; induced pluripotent stem cells; lysosomal disorders

The lysosome is the center of a group of rare inherited diseases commonly referred to as Lysosomal Storage Disorders (LSDs). LSDs have been long studied; presently, the main objective is to characterize their pathophysiology contributing to their understanding and treatment. Our main commitment is to help providing the insight required for the development of more specific therapies. Existing therapies for LSDs constitute a large financial burden, having a significant impact on health systems and family resources. Therefore, in order to progress with new therapies, one needs to first develop effective and economically viable models for testing specific therapeutic approaches. With the advent of induced pluripotent stem cells (iPSCs) it became possible to establish cellular models for several diseases. In our lab we recently started generating iPSCs from a few lysosomal disorders. This new tool allows easier access to disease specific cells, with the advantage of preserving the original genotype of the donor cells. The development of precise cellular models, in a non-fully dedicated lab, is a long process. The procedure requires several checkpoints involving specific techniques. The process will be briefly described and the applications of iPSCs will be discussed. With specific disease models, we hope to contribute to the increase of choices in terms of availability of material for developing new therapeutic interventions.

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The usefulness of medical genetics in real clinical practice

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Keywords: genetic counselling, massively parallel genomic sequencing, clinical practice

The Portuguese clinical practice setting in medical genetics will be presented, followed by an explanation of the process of genetic counselling, from day to day appointments in mendelian and common disorders, chromosome abnormalities, dysmorphology and genetics syndromes, carrier testing and genetic prediction, prenatal diagnosis to a wider picture with some notions of screening and society related challenges. Finally, we will discuss the integration of massively parallel genomic sequencing into clinical practice.

The biotechnological potential of bacteriophages to control infectious diseases

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Keywords: bacteriophages, endolysins, depolymerases, phage therapy

Bacteriophages, viruses that infect bacteria are the most numerous and fascinating entities on earth. As natural bacteria predators they have an important role in the biosphere controlling prokaryotic biomass and contributing to their diversity. On the other hand, their ability to infect, take over cell metabolism and to kill bacteria makes them attractive antibacterial agents. This concept is not new, it was developed in the early 20th century as a unique antibacterial weapon at the time and was called phage therapy. The awareness about antibiotic resistance has revival phage therapy and currently it is being performed already in some European countries under the concept of the "magisterial preparation". Phage therapy, however, has some limitations that need to be taken into consideration for an effective therapy. The exploitation of the biotechnological potential of bacteriophages is not limited to phage therapy. Phages' DNAs encode a vast number of proteins for the successful infection of a bacterial host. These proteins might as well be explored to develop effective antibacterial drugs. Lysins, for example, are peptidoglycan degrading enzymes displayed at the phage tips to help the penetration of the bacteriophage's DNA /(exolysins) or produced at the end of the lytic cycle (endolysin) for the release of phage progeny. These enzymes can be applied externally to degrade bacterial cell walls of gram positive cells. Another example are depolymerases that "shave" the capsular polysaccharide also to help bacteriophage infection. Capsular bacteria treated with depolymerases are less virulent and become susceptible to serum killing. During this talk I will present the phage therapy concept and explain the advantages and limitations of this therapeutic option and the pathway to develop an effective phage cocktail. I will also discuss the antibacterial potential of bacteriophage's encoded enzymes by presenting some of the latest results obtained by my research group that clearly demonstrates the unlimited biotechnological potential held by bacteriophages and derived enzymes.

Understanding Adaptation with Genomics and Bioinformatics: Biotechnological Relevance…

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Keywords: Adaptation, bioinformatics, biotechnology, genomics, molecular diversification

Deciphering genetic disease and health, species evolution and the diversification of phenotypic traits, can be largely advanced with whole genome sequencing projects. Here, recent results from our group retrieved from comparative genomic/proteomic and bioinformatic analyses of varied animal species will insightfully illustrate cases of adaptive successes to thrive into diverse ecological conditions. The findings pinpoint unique molecular products of critical relevance in species evolution, diversification and conservation, but also highlight genomic novelties with relevance in biomedical research and biotechnology.

Oral Communications

Changes in gene expression in lentil subjected to drought stress

O1

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Keywords: qPCR, drought stress, lentil, differential gene expression

Lentil is a traditional pulse of the Mediterranean, which is usually subjected to drought stress, one of the main causes that reduces the productivity of the crops. The severity of this abiotic stress is going to increase in near future due to the climate changes caused by the global warming. The knowledge of the genetic mechanisms that confers drought tolerance is critical if we want develop new cultivars more tolerant. The present work has two main goals, on one hand, the development of a simple test to determine the level of drought tolerance between lentil cultivars. For this, two methods have been tested with several cultivars of *Lens culinaris* and some wild species. One method based in the evaluation of seed germination in stress condition caused by the addition of PEG, and other that evaluate the impact in seedlings growing in an hydroponic medium to periodic exposition of roots to the air. Secondly, the analysis of changes in expression for 8 genes previously described as partially responsible of drought tolerance in other species. For this, the drought stress was produced only in *L. culinaris* cv Alpo and *L. odemensis* by a method with field-like conditions. In short, the seeds were germinated in Petri dishes and after a week they were transferred to Hoagland's nutrient solution with aeration, where they remained 4 days until the stress treatment started. During 6 days and 4 hours/day, the plants were completely exposed to air. After the treatment, total RNA was extracted from leaves and roots, and with that the cDNA was obtained and several qPCRs were carried out. The results showed similar gene expression changes in Alpo leaves and roots, however in *L. odemensis* the main expression changes were observed in the roots. The relationship of these observations with the level of drought tolerance is discussed.

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O2

Foliar application of magnesium and potassium as mitigation strategy of sweet cherry cracking: effects on fruit quality and gene expression

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Keywords: bioactive properties, cherry cracking, fruit quality, gene expression, magnesium, potassium, *Prunus avium* L., sweet cherry.

The cherry is one of the fruits most appreciated by consumers and economically important due to its nutritional value and bioactive properties, with benefits for human health. However, the cherry cracking has a strong implication in the quality and profitability of cherry production, decreasing its commercial value. To study this disorder, the nutrients potassium (K) and magnesium (Mg) were applied at foliar level in sweet cherry trees (Cv. Burlat) in a plantation located in Resende region, trying to reduce cherry cracking and increase cherry quality. This study intended to analyse parameters related to fruit quality, total phenolics and flavonoids content as well as the antioxidant activity. The cracking index (CI) and quantification of cuticular waxes was also analysed. Moreover, fruits from all treatments were collected at the red stage, total RNA was extracted from fruit exocarp and then the cDNA synthesis was performed. A quantitative analysis of genes potentially involved in cherry cracking was done and a housekeeping gene was used as control. Total phenolic content of the cherries showed to be higher when Mg was applied. Likewise, the flavonoids content was higher in Mg treated cherries. The antioxidant activity was correlated with the content of total phenolics and flavonoids, being higher when Mg was applied. The results also revealed differences in gene expression among different treatments. Moreover, a higher expression of *PaCer1* and *Paβ-Gal* (involved in cuticle deposition and modifications of cell wall) leads to a higher CI and lower waxes content. On the other hand, when *PaLTPG1* (involved in waxes transport) has more expression, the CI is lower and waxes content is higher.

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How close are Portuguese and African cowpea accessions? An evaluation by SSR markers

O3

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Keywords: Microsatellites, *Vigna unguiculata* (L.) Walp., Population structure, Genetic resources, Landraces

Cowpea (*Vigna unguiculata* (L.) Walp.) is a warm-season grain legume, originated from Africa, where it is largely produced and constitutes an important source of proteins. Although its production in Europe is limited, cowpea has a large set of attributes that make it an interesting crop in the context of climate change and food security. Cowpea has a high adaptability to heat and drought and is used in rotation with cereal crops to enhance soil fertility through fixation of the atmospheric nitrogen. Genetic tools, such as simple sequence repeats (SSRs), allow a better understanding of the genetic diversity, a better management of genebanks and provide information for plant breeders. The objective of this study was to determine the genetic diversity and relationships in cowpea accessions originated from Portugal and Africa using SSR markers. In this study, a set of five SSR markers was used to evaluate the genetic diversity of eight Portuguese and twelve African cowpea accessions. A total of 38 alleles were detected, varying from five (VM37) to eleven (VuUGM71) *per locus*. The accessions were grouped into three main clusters with a wide genetic dissimilitude. The largest cluster (I) was divided into two sub-groups: one mainly constituted by seven Portuguese accessions and one from Ghana, and another entirely constituted by African accessions. Cluster II comprised only two accessions from Africa. The third cluster separates the Nigerian accessions from the other African germplasm and, interestingly, one Portuguese accession clustered also in this group, corroborating previous studies with SNP marker. This study revealed a narrow genetic basis of the Portuguese cowpea germplasm. Nevertheless, a Portuguese genotype revealed a close genetic relationship with the Nigerian accessions which gives insights on the dispersion of the cowpea cultivated in Portugal.

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Multivariate data analysis of morphological characterization data in Portuguese maize landrace accessions

O4

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Keywords: Maize, BPGV, Germplasm, Climate Change, Multivariate data analysis, Morphological traits, Agro-ecological groups.

Maize (*Zea Mays L.*) is the most widely cultivated cereal crop in the world, achieving the status of a staple crop worldwide. It has diverse applications such as human food, animal feed, high-fructose corn syrup and even as a thickening agent. The database of the Portuguese Genebank (BPGV), GRIN-GLOBAL (Germplasm Resource Information Network) stores over 2000 accessions of Portuguese maize landraces resulting from diversity collection missions in the national territory. Considering the growing threat of climate change and the susceptibility of maize to abiotic threats, particularly during the flowering and grain-filling stage, it becomes increasingly urgent to screen maize germplasm collections for drought and heat stress resistant genotypes. To achieve this end, a core sample of 566 accessions representing all the Portuguese districts, including the Madeira and Açores archipelagos, was established and exploratory data analysis was performed on several years of characterization data. Multivariate data analysis was performed on highly heritable morphological traits representing vegetative, phenological and yield components. Hierarchical clustering was performed on 7 principal components resulting from principal component analysis (PCA), which represented over 70% of total sample variation, resulting in several main clusters. These clusters were described according to both the quantitative traits used in the PCA and supplementary qualitative traits, and a first proposal for the Portuguese maize landraces agro-ecological groups based on morphological traits, origin and evolution is presented. This is the first step in identifying abiotic stress resistance within the Portuguese maize collection and proposing promising genotypes within interesting agro-ecological groups.

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Recycling of citric wastes to obtain products with commercial value

O5

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Keywords: essential oil, extract, pectins, TLC, albedo, flavedo

During the processing of citrics in order to obtain juices, wastes are generated. The peels can be used to get products with commercial value. Essential oils can be obtained from the flavedo. These are a type of terpenoid secondary metabolites widely used in cosmetics, food and pharmacy. They have a high market value, moving about $5 billion a year. Due to the method used for extraction, essential oil or extract can be obtained, with different purity and commercial value. Because of this, it is important to be able to distinguish them in order to avoid fraud. On the other hand, pectins can be obtained from albedo, which are highly commercially demanded heteropolysaccharides for their applications in the food industry as gelling agents and in the biomedical industry for the manufacture of medicines. The provenance of pectins and extraction factors, such as temperature and time, affect the quality of pectin and its physicochemical properties, such as equivalent weight and percentage of methoxylation. Therefore, the optimization of extraction conditions is essential to obtain maximum production. The objectives of this study were to design fast and economical protocols to obtain and characterize the products and compare them with their commercial counterparts. Two approaches were made to this end: (1) The essential oils and extracts were extracted from the flavedo of orange peels, and organoleptic, solubility and density tests and a thin-layer chromatography were used to compare their quality and composition using commercial samples as controls; (2) for pectins, acidic water extraction was performed from the albedo of orange wastes. The equivalent weight and degree of esterification were subsequently measured using volumetric techniques. After this study we could conclude that the proposed methods are simple, fast and totally valid to characterize and distinguish both the orange essential oils and pectins at the routine level.

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Preimplantation genetic testing for aneuploidy (PGT-A) as a useful tool in cattle breeding programmes

O6

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Keywords: embryology, bovine, chromosomal abnormalities, selective breeding

Single Nucleotide Polymorphism (SNP) data obtained at the embryonic stage is used in cattle breeding to calculate the genetic value of an animal before a pregnancy is established, leading to improved selective breeding. However, the cost of the procedure can be high due to the number of recipients not getting pregnant after receiving a SNP typed embryo. Aneuploidies are the most common cause of embryo development arrest, so it might be beneficial to screen out aneuploid embryos by employing preimplantation genetic testing for aneuploidy (PGT-A). Here, we applied a novel PGT-A algorithm, which employs the same SNP information used to calculate genetic merit, to obtain ploidy diagnoses. We performed a retrospective analysis in n=1129 bovine embryos of transferable quality produced by the company Boviteq (Saint-Hyacinthe, Canada). Embryo morphology (good or excellent) affected pregnancy rates, which were 59.9% for excellent embryos and 44.9% for good embryos (general linear model, P=.035). Nevertheless, live birth rates were not improved by morphology-based selection (40.1% vs 39.1%, P=.88). In contrast, pregnancy rates were significantly higher in euploid rather than aneuploid embryos (60,9% vs 16,1%, P=1.3E-10) as were live birth rates (50.9% vs 12.5%, P=4.7E-8). The proportion of aneuploid embryos in this sample was 13.8% (156/1129); 25.3% (41/162) of chromosomal abnormalities had paternal origin and 74.7% (121/162) maternal origin. The incidence of monosomy appeared higher than that of trisomy, however the difference was not significant (58% vs 42%, chi-square, P=.1241). Our results indicate that embryos classified as euploid by PGT-A have better developmental competence and their selection for transfer could reduce for breeders the economic losses associated with pregnancy loss. At present, a bigger database is being analysed to elucidate the effects of specific chromosomal abnormalities.

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Simvastatin disrupts zebrafish (*Danio rerio*) lipid and energy metabolism after long-term exposure

O7

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Keywords: Zebrafish, Simvastatin, Chronic exposure, Low-level exposures, Liver, Brain

Simvastatin (SIM), a hypocholesterolaemic compound, is among the most prescribed pharmaceuticals for cardiovascular disease prevention worldwide. Several studies have shown that acute exposure to SIM causes multiple adverse effects in aquatic organisms. However, uncertainties still remain regarding the chronic effects of SIM in aquatic ecosystems. Therefore, the present study aimed to investigate the effects of SIM in the model freshwater teleost zebrafish (*Danio rerio*) following a chronic exposure (90 days) to environmentally relevant concentrations ranging from 8 ng/L to 1000 ng/L. This study used a multi-parameter approach integrating distinct ecologically-relevant endpoints, i.e. survival, growth, reproduction and embryonic development, with biochemical markers (cholesterol and triglycerides). The results showed that SIM was able to affect embryo development, levels of biochemical markers, and modulated the transcription of key genes involved in the lipid and energy metabolisms of brain and liver tissues. Taken together, these findings expand our understanding of statin effects in teleosts, demonstrating significant impacts at environmentally relevant concentrations and highlight the importance of addressing the effects of chemicals under chronic low-level concentrations.

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O8

Dalbavancin as a therapeutic option to treat human osteomyelitis caused by methicillin-resistant Staphylococcus aureus (MRSA) infection

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Keywords: MRSA; Antibiotic Resistance; Osteomyelitis; PCR; Dalbavancin.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen associated with high mortality which can induce infections in several tissues of the human body being the most common cause of acute and chronic hematogenous osteomyelitis in adults and children. Thus, this study aimed to characterize antibiotic resistance of 41 MRSA strains isolated from human osteomyelitis and test dalbavancin efficiency against these strains. The antimicrobial susceptibility of the isolates was tested by Kirby-Bauer disk diffusion method and genotypic characterization was performed by PCR using specific primers and conditions. Minimum inhibitory concentrations (MIC) of dalbavancin were determined by broth microdilution method using 13 strains. Forty-one MRSA isolates were recovered from osteomyelitis. All MRSA showed resistance to cefoxitin and oxacillin. Resistance to penicillin (n=40), ciprofloxacin (n=38), erythromycin (n=32), tobramycin (=5), kanamycin (n=4), gentamicin (n=3), clindamycin (n=3), fusidic acid (n=3) and tetracycline (n=2) was also detected in this study. Resistance to aminoglycosides was encoded by the *aph*(3')-IIIa (n=4), *aac*(6')-Ie-*aph*(2'')-Ia (n=5) and *ant*(4')-Ia (n=1) genes. Isolates presenting resistance to macrolides and lincosamides harbored the *erm*C (n=28), *erm*B (n=5), *erm*A (n=9) and *msr*(A/B) (n=2) genes. MICs of dalbavancin ranged from 0.2 (n=3) to 0.4 (n=10) µg/ml. Giving these results, dalbavancin represents an alternative to conventional antibiotics in the treatment of deep-tissue infections, such as osteomyelitis.

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Indoors Fungal biodiversity - *Penicillium* and *Alternaria*

O9

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Keywords: Biodiversity, Houses, *Penicillium, Alternaria*

The objective of this study was to isolate and identify fungi from different places in houses and materials from different sources, such as bed pillows of daily use and in the dust of the house. This screening has performed through routine mycological techniques for fungal isolation. 10 samples were collected in pillows and in the dust of the house in different rooms. To made the analysis in the pillows, the surface of the pillowcase was rubbed for 1 minute, using a toothbrush according to the modified Mackenzie technique. For the analysis of the house dust, the entire surface of the television screen was rubbed, using a swab moistened with sterile distilled water, followed by inoculation in specific media. The samples were transferred to Petri dishes with the culture medium Potato Dextrose Agar and the microscopic identification was carried out using the Lactophenol with Cotton Blue technique for staining the filamentous fungi and identification of the genera. In the samples collected on the pillows, 2 filamentous fungal genera were isolated in 10 rooms: *Penicillium* and *Alternaria*. *Penicillium spp.* was the highest occurrence in all samples, having been isolated in 7/10 samples. *Alternaria* spp. was isolated in 2/10 samples. From the samples obtained in the television dust, a total of 33 fungal isolates and 3 different filamentous fungal genera were obtained, *Penicillium* spp. prevailed, followed by the genus *Fusarium*, and with less occurrence the genus *Alternaria*. The results obtained suggest that in some houses the inhabitants are in contact with fungal agents.

O10

Listeria monocytogenes wall teichoic acid glycosylation promotes surface anchoring of virulence factors, resistance to antimicrobial peptides and susceptibility to antibiotics

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Keywords: *Listeria monocytogenes*, wall teichoic acids glycosylations, antimicrobial peptides, antibiotics, virulence factors

Gram-positive (Gram+) bacteria are major human pathogens associated with increasing clinical infections due to the acquisition of new virulence properties and/or antimicrobial resistance. *Listeria monocytogenes (Lm)* is a Gram+ foodborne pathogen and a recurrent problem of public health and food industry. Its pathogenicity is provided by several virulence proteins, most of them associated to the cell wall. The Gram+ cell wall is densely decorated with glycopolymers such as wall teichoic acids (WTAs) with key roles in bacterial physiology and virulence, including protection against the activity of antimicrobial peptides (AMPs) and antibiotics. In this work, we demonstrated that specific *Lm* WTA-glycosylations are not only crucial to maintain levels of cell surface associated virulence factors, but also to promote resistance to AMPs and antibiotics. Moreover, we observed a synergetic effect conferred by the absence of several WTA-glycosylations. Our data suggest that WTA-glycosylations delay the crossing of antimicrobials through the bacteria cell wall, possibly by mis-localizing cell wall associated proteins or by decreasing cell wall permeability. Altogether, our data reveal the potential of WTA-glycosylations as targets for innovative anti-virulence drugs that will simultaneously disarm and sensitize Gram+ pathogens.

qPCR assay for *Colletotrichum acutatum* detection and quantification

O11

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Keywords: *Olea europaea L.*, pathogen detection, qPCR, standard curve

Anthracnose disease, caused by *Colletotrichum acutatum*, affects mostly olive drupes and is responsible for significant yield loss, and poor fruit and olive oil quality. The disease is difficult to identify at an early stage, *i.e*., before symptoms' appearance. Therefore, the existence of reliable and cost-effective pathogen detection methods is required. The aim of this study is to develop a real-time polymerase chain reaction (qPCR) assay for *C. acutatum* detection and quantification, based on the establishment of a standard curve. A *C. acutatum* specific DNA sequence of ~390 bp was cloned into a pUC19 vector, and 10 positive clones were validated by Sanger sequencing using M13 universal primers. The standard curve was designed based on SYBR® Green dye, using known Log^{10} transformed plasmid DNA concentration (10-fold) serial dilution. The designed assay exhibited high performance, with average values for PCR efficiency of 95.1%, slope of -3.445 and R^2 value of 0.996, which are within the acceptance criteria for standard curve design. The standard curve obtained will be applied for *C. acutatum* quantification in olive drupe and olive oil samples. This assay may help to monitor the infection progression in olive drupes in the field and to detect the presence of the pathogen in olive oils.

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Morphometric analysis of rat parameters during an animal model of colorectal cancer

O12

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Keywords: Chemical carcinogenesis, 1,2-Dimethylhydrazine, humane endpoints, morphometric parameters

Colorectal cancer is one of the leading causes of death by cancer worldwide. Animal models have been used in experimental research to find new solutions for old biomedical questions. This research aimed to make a contribution to characterize the rat model of colorectal cancer. Twelve male Wistar rats obtained from Charles River were randomly divided into two groups: control group and induced group. All ethical issues were considered, following the guidelines of the Portuguese *Direção Geral de Alimentação e Veterinária* (approval number 010535). Animals from the induced group received a weekly intraperitoneal injection of N, N'-Dimethylhydrazine (DMH), for seven consecutive weeks. All rats were monitored for signs of distress, weight loss, and food and water consumption. Abdominal ultrasound examinations were performed before the first DMH administration and the animals' sacrifice. Thirteen weeks later, all surviving animals were sacrificed, organs and blood were collected. Animals from control group showed a higher mean body weight. The mean food consumption of group II was lower in the weeks of the administration ($p<0.05$). The mean relative weight of soleus was lower in induced animals when compared to the control animals ($p<0.005$), which may suggest the development of anorexia. Although there were no significant differences in colon's weight, induced animals had shorter colon. The microhematocrit was not different between groups. The ultrasound examination showed a high vascularized abdominal mass in induced animals. Some induced animals had a swollen abdomen which difficult the ultrasound examination. The animals showed little changes in their biological parameters, suggesting that the disease was at an early stage. Histological analysis of animals' organs will provide a better perception of the colorectal cancer induction.

Identification of transcription factors associated with a cancer stem cell phenotype in colon cancer cells

O13

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Keywords: Colorectal cancer, Cancer Stem Cells, SOX2, SORE6

Colorectal cancer remains a serious health concern, being the third most common and the second leading cause of cancer related deaths. The efficacy of therapeutic solutions is limited due to drug resistance. Emerging evidence has shown that a subpopulation of self-renewing cells - Cancer Stem Cells (CSCs) is capable of initiating and sustaining tumorigenesis. CSCs possess properties that make them clinically relevant since they are responsible for cancer therapy failure and disease recurrence. In colorectal cancer, SOX2 transcription factor (TF) is expressed in about 20% of the tumors and is associated with worse patient survival and worse response to chemotherapy. We suggest that SOX2 is one of the TFs involved in CSCs reprogramming so it has been used as a starting point to identify critical signaling and transcriptional regulatory network in intestinal CSCs. We have implemented a strategy proposed by Tang et al (2015) to sort cancer cells based on the activity of SOX2/OCT4 transcription factors. For that, we transduced SW480 colon cancer cell line with a lentivirus containing a Green Fluorescent Protein (GFP) under the control of a promoter containing SOX2/OCT4-responsive elements (SORE6) and sorted the cells by Fluorescence-Activated Cell Sorting, according to GFP expression. We have established two sub-populations, one that is highly enriched in GFP-expressing cells and exhibits features of CSCs - with both significantly increased resistance to chemotherapeutic drugs and higher capacity to form colonospheres - and the other one highly depleted in GFP-expressing cells. OCT4 is not expressed in both cell sub-populations and the expression of SOX2 is comparable between them. Therefore, we are currently trying to identify the TFs associated with SOX2 activity and the observed CSCs phenotype in colorectal cancer cells.

FA-SAT ncRNA depletion induces apoptosis – is it through p53 mediation?

O14

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Keywords: FA-SAT; non-coding RNA; PKM2; p53; apoptosis.

FA-SAT is the major satellite DNA (satDNA) sequence of cat (*Felis catus*) and it is composed by a monomer, repeated tandemly more than 100000 times. In cat´s genome, this sequence is located, predominantly, at the telomeres (but also at the centromeres and interspersed). Recently, it was found that FA-SAT is highly conserved in genomes of many Bilateria species and that this satDNA is transcribed into non-coding RNAs (ncRNAs). It was reported that this ncRNA interacts with PKM2 protein, playing important roles in cell proliferation. Also, the disruption of the *FA-SAT* ncRNA/PKM2 complex results in apoptosis (being also observed an increase of p53 protein in HeLa cells). In order to better understand the FA-SAT ncRNA role in cells, the transcription profile of FA-SAT in different human cancer cell lines was accessed. Also, the p53 involvement in apoptosis phenotype in FA-SAT silencing was scrutinized. In this study, three cancer cell lines were used, HeLa, A549 (p53 wild-type) and H1299 (p53 null), being the FA-SAT transcriptional profile analysed. FA-SAT transcripts were observed, for the first time, in the nucleus of all these cell lines, but differences in their appearance were found. In order to ascertain the involvement of p53 in the apoptosis phenotype, FA-SAT silencing experiments were carried out, using a customized antinsense LNA GapmeR in A549 and H1299 cells. Then, it was possible to establish a correspondence between silencing of *FA-SAT* and the decrease of PKM2 expression and its protein nuclear location, as it happens in cat and HeLa cells. The FA-SAT silencing phenotype previously observed in HeLa cells – apoptosis – was also detected in p53 null and p53 wild-type cells. This leaded us to hypothesize that this process can occur dependent or independently of p53 mediation.

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SatDNA involvement in rob(14;21) formation: Assessing the robustness of technique interdependency

O15

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Keywords: Satellite DNA; Robertsonian translocations; Physical mapping; *In silico* mapping; ROB mechanism.

Satellite DNA (SatDNA) sequences are an essential element of (peri)centromeres, being feasibly associated with sequence recombination between non-homologous acrocentric chromosomes, and thus potentially informative in the context of Robertsonian translocations (ROBs). Given the striking association of rob(14;21) with Down syndrome, the need of more intensive studies on related satellites sequences in chromosomes 14 and 21 is imperative. In this work, (peri)centromeric SatDNAs were physically and *in silico* mapped, in order to analyze their presence and organization. Physical maps for chromosomes 14, 21 and der(14;21) allowed us to infer about the etiological and mechanistic origin of ROBs and the possible chain of events leading to this rearrangement. Physical mapping information was compared with *in silico* analysis, leading to the recognition of a substantial number of assembly gaps in the human reference genome. The present study also demonstrates the disproportional representation of satellite families in general comparatively to αSat (greatly represented in HSA14 and HSA21). Consequently, it is possible to recognize the conserved utility of physically mapping satellite probes to achieve accurate maps for pericentromeric/short-arm regions of acrocentric chromosomes. In order to properly address these genomic regions with recent long-read technologies, sequential mapping steps must be followed. Besides providing a full gathering of current genomic data, this work delivers a clear mapping basis for sequencing approaches.

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Poster communications

Spanish cowpea (*Vigna unguiculata* L. Walp.) landraces' genetic diversity

P1

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Keywords: *Vigna unguiculata* L., molecular markers, SSRs, genetic resources, fingerprint

Cowpea (*Vigna unguiculata* L. Walp.) has its centre of diversity in Africa. This species belongs to the Fabaceae family and as all grain legumes, it has the ability to fix the atmospheric nitrogen and so its cultivation contributes to the sustainability of the environment. In addition to its ability to grow on low fertility soils, cowpea has a high resistance and adaptation to high temperatures and low water regimes. The high protein content and nutritional value make this a valuable plant food with economic importance. Despite this, Europe is one of the continents with the greatest deficit in the production of cowpea. To better characterize and evaluate the cowpea landraces, an analysis of their genetic variability is essential and allows the use of germplasm in plant breeding programs. Molecular characterization of genetic resources is advantageous since it permits the detection of changes in DNA sequences that would not be detectable through morphological characterization. In this study, 32 Spanish cowpea landraces (21 of the cultigroup unguiculata and 11 of the cultigroup sesquipedalis) from 22 different provinces were characterized by a set of five SSR *loci* in order to evaluate their genetic variability and the phylogenetic relationships. The five *loci* revealed to be multiallelic and a total of 24 alleles and 21 different genotypes were detected. This analysis showed a considerable degree of polymorphism and diversity within the studied Spanish cowpea landraces. Nevertheless, clustering of the accessions revealed a few individuals of the cultigroup sesquipedalis with a close phylogenetic relationship with those of the cultigroup unguiculata.

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P2

Multidisciplinary characterisation of progenies of bread wheat plants biofortified with Iron and/or Zinc

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Keywords*:* Biochemistry; Cytogenetics; nutripriming; stress memory; yield-related components

Seed priming with micronutrients (nutripriming) has various biochemical and agronomic advantages and is commonly used in bread wheat biofortification. Nutripriming is performed in each generation, and until the accomplishment of the present work, the transmission of the biochemical and agronomic benefits to the resulting progeny was unknown. In this work, we studied the first seed generation (S1) of bread wheat cv. 'Jordão' plants that resulted from nutripriming with 4 $mg.L^{-1}$ and/or 8 $mg.L^{-1}$ of Fe and/or Zn, using as control the S1 seeds of untreated plants. In the parental (S0) generation it was previously observed cytotoxicity, nucleolar stress, but also a high total soluble proteins content in whole wheat flour. These results allowed us to question if the cytotoxicity-related stress memory and/or the high protein content was transmitted to the S1. The progenies' flour was characterised biochemically (free amino acids, sugars, total starch and crude protein). Root-tips were used for cytogenetic analysis, and seven production components were evaluated in the adult plants. In the S1 progenies were identified 16 free amino acids and five soluble sugars by HPLC, which increased relative to the control. The same was noticed for glucose and crude protein. The latter being significantly higher in the S1 offspring of seeds primed with 4 $mg.L^{-1}$ or 8 $mg.L^{-1}$ of Fe + Zn, being coincident with the S0 results. The cytogenetic data revealed cytotoxicity attenuation relative to the S0. The production components of the S1 surpassed those of the S0 plants and the control. Our data showed that the nutripriming benefits were transmitted to the S1 progenies without the need for repeating the treatment.

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Differential response of infraspecific *taxa* of *Pinus nigra* to PEG-induced osmotic stress assayed by cytogenetic and alkaline comet-assay analyses

P3

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Keywords: Alkaline Comet-assay, European Black Pine, mitotic cell cycle, seed osmopriming

In the 1980s, the Portuguese *Pinus nigra* stands were classified as belonging to the subspecies *laricio*, *salzmanni* and *nigra*. A recent molecular characterisation performed to the allochthonous Portuguese *P. nigra* populations evidenced their high genetic similarity with foreign samples belonging to varieties *corsicana* and *calabrica* of subsp. *laricio.* In addition to the hard natural regeneration of *P. nigra*, the early mortality of pine seedlings under water stress is highly frequent. Since more severe and persistent drought episodes are forecasted to Europe, the selection of pine *taxa* more tolerant to water stress is required. In this work, seeds of the *P. nigra* infraspecific *taxa* previously assigned to Portugal were osmoprimed with 10% and 20% polyethylene glycol (PEG) solutions to mimic water stress, and to study its impacts in mitosis and nuclear DNA. Hydroprimed seeds and resulting seedlings were used as controls. Globally, the PEG-osmopriming induced mitotic cell cycle anomalies in the root-tips and DNA damage in the young seedlings. The negative impacts were more pronounced in var. *corsicana* treated with 20% PEG, being lethal to these seedlings. This work evidenced that the osmotic stress imposed to the pine seeds generated mitotic cell cycle irregularities probably due to the occurrence of DNA damage that persisted through the seedlings development since it was observed in the young needles. Since the impacts of osmotic stress were less pronounced in subsp. *nigra* var. *austriaca* and subsp. *laricio* var. *calabrica*, these *taxa* may have higher water stress tolerance. This information will be valuable for the definition of national and international afforestation/reforestation strategies.

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Seed priming of bread wheat with copper sulphate: impacts in germination and mitotic cell cycle

P4

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Keywords: Cytogenetics, mitosis, nutripriming, *Triticum aestivum* L. em. Thell

The amount of copper (Cu) in soil depends on its physical-chemical properties and anthropogenic activities, resulting in its deficit or excess. In some stages of the plant's life cycle, higher amounts of Cu may be required. In some countries, seed priming with copper sulphate ($CuSO_4.5H_2O$) has been tested in different crops. Germination and mitotic cell cycle analyses can be useful to address the proper dosage of $CuSO_4.5H_2O$ to be used in seed priming. Here, we aimed to analyse the effects of seed priming with $CuSO_4.5H_2O$ solutions with three concentrations (100, 300 and 600 ppm) in the germination and mitotic cell cycle of the bread wheat cv. 'Jordão'. Hydroprimed and unprimed seeds were used as controls. Our results evidenced that seed priming performed with dosages higher than 100 ppm of $CuSO_4.5H_2O$ affected the germination relative to the controls negatively. We observed a significant increase ($p < 0.05$) of the mitotic index (MI) in the Cu-treated seeds relative to the controls. The Cu treatments also increased the percentage of dividing cells with anomalies (%DCA) that was significantly higher ($p < 0.05$) than the controls in seeds treated with 600 ppm. Different types of cell cycle and chromosomal anomalies were observed. Most of the normal and irregular dividing cells were arrested in prophase or metaphase. Therefore, the 100 ppm of $CuSO_4.5H_2O$ treatment was considered the most suited for seed priming of bread wheat cv. 'Jordão' since it presented: the highest percentage of germination (75%); a reasonable MI (68.8%); 3.16% of DCA; and a higher average number of normal dividing cells in the different mitotic phases.

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Cowpea root RNA extraction – protocols comparison

P5

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Keywords: *Vigna unguiculata* (L.) Walp., abiotic stress tolerance, drought, root.

Root system has an important role on plant physiology and ecosystem functioning. The modification of the root system architecture may contribute to improvements of desirable traits such as drought tolerance and yield. Cowpea (*Vigna unguiculata* L. Walp has high tolerance to drought stress and high temperatures, being important to understand the mechanisms and pathways that confer these tolerances. Effective, reliable and high-quality RNA extraction is an indispensable procedure in any functional molecular biology research. The main objective of this study was to select the most suitable protocol for cowpea root RNA extraction. Four RNA isolation protocols - one phenol-based protocol and three commercial kits – were tested for to evaluate the quantity and quality of RNA obtained from cowpea roots. RNA integrity and DNA contamination were assessed in an agarose gel electrophoresis, while RNA concentration and quality was estimated using A_{260}/A_{280} ratio through a spectrophotometer. All protocols yielded sufficient RNA concentration for good results in a real-time reverse transcription-PCR (RT-PCR) for cowpea roots samples. However, the *NucleoSpin RNA II* (Macherey-Nagel) allowed to obtain the highest yield (1 ng/µl), a very good ratio A_{260}/A_{280} (≈2.1) and, without genomic DNA contamination. The phenol-based protocol revealed to be the less suitable due its high genomic DNA contamination and lower RNA yield. This procedure is the first step for the development of new approaches to understand and comprehend the mechanisms involved on drought tolerance.

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Much more than beer: Hops residues as effective antimicrobial alternative

P6

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Keywords: *Humulus lupulus, Aspergillus fumigatus, Pseudomonas syringae, flower, residues, antimicrobial.*

Hops *(Humulus lupulus)* is a Cannabaceae family plant with a wide use in beer industry due to its bitter nature and antimicrobial activity given by molecules present in the female flower (FL). This last property has a great potential in both pharmaceutical and agrochemical industry, which compete directly with the beer industry for this resource and consequently rising its prize. For this reason, in the present study, the agricultural flower residue (RFL) was analysed to determine its possible antimicrobial properties. In order to verify this, a bioassay was carried out using mediums prepared with RFL and FL (control) at different concentrations. Then, its antimicrobial effect against *Pseudomonas syringae* bacteria and *Aspergillus fumigatus* fungi was analysed. Results showed similar growth inhibition effects between FL and RFL for both microorganisms, being slightly higher for the latter, indicating that RFL has a high bactericide and fungicide potential. Thereby, these results open doors to the study of antimicrobial components present at RFL, which could be used in different fields besides beer production, avoiding industrial competition for hops flower and reducing the amount of agricultural residues.

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Effect of growth regulators on *Jasione montana* L. in vitro culture

P7

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Keywords: Auxin, benzylaminopurine, cytokinin, *Jasione montana* L., micropropagation

Jasione, a genus with small flowers belongs to Campanulaceae family, are herbaceous, annual, biannual or perennial plants; form a rosette of leaves during winter and produce upright stems in spring, ending in a blue flower inflorescence. This plant is distributed throughout Europe, North Africa and Southeast Asia, with its center of diversity in the Iberian Peninsula. It is widely distributed in our country with a greater incidence in the North and central Portugal. Micropropagation is one of the measures suggested for preserving endangered species. The main purpose of this technic is to obtain a considerable number of individuals to reduce the loss of natural populations. The main objective of this study was to understand the effect of cytokinin benzylaminopurine - BAP, simple or with naphthalene acetic acid – NAA, on *Jasione montana* L. in vitro culture. Thus, it was used complete Murashige and Skoog (MS) medium, with 25g of sucrose and 8g of agar, both on its own and supplemented with different concentrations of growth factors, BAP (1mg/L and 2mg/L) and NAA (0,2mg/L). Throughout four weeks, the length and number of shoots, were evaluated and registered. The results show that the number and length of shoots is higher in the medium supplemented with 1mg/L of BAP and 0,2mg/L of NAA, with an average of 6,5 shoots per explant with 70,2 mm.

Effects of a free amino acids-based biostimulant in the mitotic cycle of *Prunus dulcis*

P8

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Keywords: Almond tree, chromosomal anomalies, Plant Cytogenetics

Prunus dulcis has high economic and nutritional importance. Biostimulants are interesting for sustainable agriculture, improving the nutritional value, product quality and abiotic stress tolerance. This work evaluated the effects of foliar application of a free amino acids-based biostimulant in the mitotic cycle of almond trees at NE Portugal. Leaf samples of untreated trees were used as control. Treated trees were sampled in July and August 2019 after two and three foliar biostimulant applications, respectively. Sampled leaves were fixed, enzymatically digested and used for cell suspensions. Chromosomal spreads were prepared by dropping. After silver nitrate staining, interphase and dividing cells were scored, and chromosomal anomalies identified. The mitotic index (MI) revealed significant differences (p<0.05) for the effects treatment (T), sampling date (S) and TxS. The MI increased significantly (p<0.05) in the treated samples being significantly higher (p<0.05) in July. Despite the high average air temperature in August, the samples of this month showed a significant (p<0.05) lower percentage of dividing cells with anomalies (%DCA) relatively to the control. In both treated and control plants, the dividing cells were mostly in prophase, indicating cell cycle arrest. Our preliminary data suggest that the amino acids-based biostimulant improved the MI, decreased the %DCA and activated cell cycle checkpoints under unfavourable environmental conditions, with more pronounced effects after a higher number of foliar applications.

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Cytogenomic evidence of mitosis progression and increased antioxidant capacity in kaolin-treated grapevines under summer stress

P9

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Keywords: Cytogenetics, genes encoding antioxidant enzymes, kaolin, mitosis, *Vitis vinifera* L.

Summer stress (SS) combines water deficit, high temperature and solar irradiance, and ultimately, generates oxidative stress. Foliar application of kaolin (KL) in grapevine decreases leaf temperature reducing SS impacts, but its cytogenomic effects are unknown. In this work, we evaluated the leaf mitosis and gene expression of transcripts involved in heat stress response, mitosis regulation and synthesis of antioxidant enzymes, in grapevines of Touriga Franca (TF) and Touriga Nacional (TN) varieties under SS at the 'Douro Superior' (NW Portugal). Leaves were sampled in KL-treated and non-treated (control) plants, through the summers of 2016 and 2017, and fixed for chromosome spreads preparation and mitosis analysis. Fresh frozen leaves were used for RNA extraction and quantitative real-time PCR (qPCR) to profile the genes expression. Normal and irregular mitotic cells were detected in the two varieties, treatments and years. Normal mitotic cells were mostly in prophase, suggesting cell cycle arrest, whereas the irregular ones were arrested in metaphase/anaphase. KL-treated plants showed down-regulation of the *HSP17.9A* gene, confirming the leaf temperature decrease. KL-treated TN plants sampled in July 2017 showed up-regulation of the *VvCYCA3* gene suggesting mitosis progression, and down-regulation of the *VvICK5* gene that halts the cell division. All genes encoding for antioxidant enzymes were up-regulated in KL-treated plants, even in the hottest summer (2016). Overall, KL mitigated the SS impacts by allowing the mitosis progression and boosting the antioxidant capacity in both varieties, despite being more effective in TN and the year of 2017.

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Nucleolar activity analysis in triticale upon seed treatment with micronutrients

P10

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Keywords: Triticale; seed priming; Iron; Zinc; nucleolar activity; cytotoxicity.

Low concentration of micronutrients like Iron (Fe) and Zinc (Zn) in soil often compromise plant growth and development. Seed priming with micronutrients (nutripriming) can overcome this issue. However, suitable nutripriming dosages for each species/cultivar should be established, once the excess of Fe and/or Zn induces cytotoxicity and nucleolar activity disturbance. This work constituted the first study of the effects of nutripriming with 8 mg/L of Fe and/or Zn in the nucleolar activity of triticale (AABBRR, 2n=6x=42). Fixed root tips from triticale cv. 'Douro' seeds primed with distilled water (hydropriming, control), 8 mg/L of Fe and/or Zn were used for chromosome spreads. After silver nitrate staining, the nucleoli number per interphase, the nucleolar morphology and area per treatment were evaluated. The nucleoli number ranged from one to four. Per treatment, the average nucleolar area decreased significantly ($p < 0.001$) with the increase of the nucleoli number. The average nucleolar area differed significantly ($p < 0.001$) among treatments, and the highest values were presented in the 8 mg/L Fe treatment. All treatments showed irregular interphases with silver-stained particles in nucleoplasm, irregularly shaped nucleoli and nucleolar disruption. This work allowed us to conclude that all nutripriming treatments affected the nucleolar activity in triticale 'Douro' being the 8 mg/L Fe + 8 mg/L Zn the one that induced less nucleolar stress.

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Effects of seed priming with Iron and/or Zinc in the mitotic cycle of triticale

P11

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Keywords: Cytogenetics, micronutrients, mitotic anomalies, seed priming, triticale

The deficit of Iron (Fe) and Zinc (Zn) in soils and human diet is a worldwide concern. Biofortification by seeds imbibition in micronutrient-rich solutions can be used to overcome this problem. However, high micronutrient concentrations can induce cytotoxicity. Suitable dosages of Fe and/or Zn to be used in triticale seed priming were never tested. In this study, we investigated how seed priming with 8 mg L^{-1} of Fe and/or Zn affects the regularity of the mitotic cell cycle of triticale cv. 'Douro' (2n =6x=42; AABBRR). Hydroprimed seeds were used as control. Root-tips were collected, immediately fixed and stained with acetic carmine 2% for chromosome spreads preparation. Interphase, normal and irregular mitotic cells were scored during the observation of the chromosome spreads on the optical microscope. The data were statistically analysed for a significance level of 95%. Overall, relative to control, the mitotic index and percentage of dividing cells with anomalies showed no significant differences ($p > 0.05$). The 8 mg L^{-1} Fe treatment presented a significantly higher ($p < 0.05$) number of irregularities in different mitotic phases. Contrarily, priming with 8 mg L^{-1} Fe + 8 mg L^{-1} Zn showed the lowest average number of irregular mitotic cells. The most common anomalies were chromatin stickiness and disturbance in chromosomal orientation. In conclusion, seed priming performed with Fe and Zn seems to attenuate the cytotoxicity induced by each micronutrient alone, constituting an alternative approach for triticale's biofortification.

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Analysis of enzimatic dregadability and amiloplast's morphology for caracterization of coeliac's suitable flours

P12

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Keywords: Starch, α-amylase, amylose, amylopectin, microscopy.

The gluten-free food industry is expected to increase its volume up to 5% annually between 2015 and 2021. Possible contamination with flours unsuitable for coeliacs (such as wheat) would particularly carry high risks for consumers suffering from the disease, as well as serious legal problems for the company in which it occurs. This work focuses on applying a simple and economical method for flour's contamination detection. Samples from a factory that often uses potato flour on its coeliac's suitable products are employed. The method started by differentiating amyloplasts of different flours by staining with lugol and observing them under optical microscope. This is based on the fact that amyloplasts from different species are clearly distinct. Once several flours collected at the factory were identified, two potato flours were selected: (1) commercial and (2) potato washes coming from the industry. In order to better characterize both flours, they underwent through a degradability test on plate with α-amylase. It was found that both derive from potatoes. Despite this, they do not have the same enzymatic degradability. It is assumed that they differ in their amylose/amylopectin ratio, which may make them suitable for different industrial uses.

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Natural variation of hazelnut allergenicity: a proteomic approach

P13

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Keywords: Hazelnut allergy, Hazelnut-allergic patients' sera, Proteomics, Immunoblot, Nutrition

Hazelnuts (*Corylus avellana* L.) have an important role in human nutrition and health. Hazelnut proteome is of great interest because some proteins can elicit allergic reactions that range in severity from mild to life-threatening. Due to hazelnut varietal diversity, variety-dependent differences in the IgE-binding properties may be suspected, which could allow therapeutic strategies based on the use of hypoallergenic varieties to induce desensitization. In a proteomic approach, we aimed to evaluate the allergenic potential of a genetically diverse set of hazelnuts (n=13 varieties). IgE-reactivity was similar for all varieties using sera from seven allergic individuals. The predominant IgE-reactive proteins were Cor a 9 (100%) and Cor a 1.04 (60%), with the former being the most frequently identified by a 2-DE-based proteomic approach. Therefore, it seems that the conventional exclusion diet will hold its ground for the time being.

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Semiquantitative analysis of genes involved in sweet cherry cracking under pre-harvest application of calcium and seaweed

P14

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Keywords: calcium, cherry cracking, Cv. Burlat, gene expression, *Prunus avium* L., seaweed.

Portugal has a high potential in fruit production, particularly, sweet cherry, due to its edaphoclimatic conditions. Despite the high commercial value of the cherry, the cracking is a factor that devalues the price of the fruit, leading to high losses at economical level. In order to understand this mechanism and solve this problem, several studies have been developed, namely in terms of gene expression, to evaluate the effect of the genes potentially involved in the cherry cracking. Consumers prefer fruits with good appearance, size, firmness and flavor; therefore, it's important to find solutions to this problem in order to improve fruit quality, by reducing the commercial losses. The *Prunus avium* L. cultivar Burlat from an orchard located in Resende region was selected for this study, being applied calcium and seaweed based biostimulant at high and low as mitigation strategy of sweet cherry cracking. In order to understand how the applied compounds influence sweet cherry cracking at molecular level, fruits with and without cracks were collected at the green/red stage from all treatments, total RNA was extracted from fruit exocarp and then the cDNA synthesis was performed. A semiquantitative analysis genes potentially involved in cherry cracking was done and a housekeeping gene was used as control. The results revealed differences in gene expression for the different treatments and among fruit with and without crack while the housekeeping gene maintained their expression.

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Disclosing the Genetic Relationship among Sugarcane Genotypes Based on High Resolution Melting Analysis

P15

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Keywords: Sugarcane, Genetic Diversity, SSR markers, High-Resolution Melting, Designation of origin

Sugarcane (*Saccharum officinarum* L.) has been widely used as a raw material for Rum, “white gold”, production in Madeira Island. Modern sugarcane cultivars are derived essentially from interspecific hybridisation involving different *Saccharum* spp. The *Saccharum* genus is complex and uncharacterized genetically. In Madeira’s sugar cane mill, six varieties (“Branca”, “Canica”, “Roxa”, “Violeta”, “Verde” and “Amarela”) are used for sugarcane rum and sugarcane honey production. However, the accurate varietal identification is unclear. High resolution melting analysis is a specific and sensitive approach, based on the real-time measure of double stranded DNA denaturation at a high resolution. The aim of this study was to combine the knowledge recently obtained on sugarcane genetic diversity, using nine SSR markers from the International Sugarcane Microsatellite Consortium (ISMC), with HRM analysis to develop several HRM assays that will help to uncover the Madeira’s sugarcane varietal variability. Sugarcane samples were collected from different regions of the Madeira Island. The SSR-HRM assays were designed to target *SMC336BS*, *SMC863CG*, *SMC248CG*, *SMC477CG* and *SMC1039CG loci*. Based on HRM assays four distinct profiles were obtained. The *locus SMC336BS* generated a specific melting curve profile that clustered varieties according to their SSR repeats. The results obtained reveal that SSR-HRM is a reliable method to define sugarcane variety, and that it can be used as a tool for sugarcane certification and may be for a Protected Denomination of Origin recognition.

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Evaluation of the genotoxicity of titanium dioxide nanoparticles – an innovative approach in the comet assay using HepG2 cells

P16

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Keywords: nanoparticles, titanium dioxide, genetic toxicology, DNA damage, comet assay, *in vitro*, human cells

Titanium dioxide nanoparticles (TiO_2-NPs) addition to, mainly, food products, cosmetics, paints, electronic devices and construction materials, leads to improved product characteristics. In food industry, for example, TiO_2-NPs are used to add texture, whiteness and opacity to food, being classified as a colourant (E171). However, according to some genotoxicological studies, mainly in the *in vitro* comet assay, TiO_2-NPs showed genotoxicity. Nonetheless, the scientific community is divided regarding this matter since many inconsistencies on test conditions are found. Thus, we performed the *in vitro* comet assay for evaluating the genotoxic potential of TiO_2-NPs in HepG2 cells. Anatase TiO_2-NPs dissolved in PBS were assessed. HepG2 cells were maintained at 37 ºC and 5% CO_2/95% air until the assay. HepG2 cells and agarose were set on slides as gels and exposed, during 20 min at 37 ºC, to 1.0, 1.5 or 2.0 mg/mL of TiO_2-NPs. Negative and positive (H_2O_2 at 25 µM) controls were considered. Eight gels were prepared per condition, with half being subjected to an enzymatic treatment with formamidopyrimidine-DNA glycosylase (FPG). The genetic damage index (GDI), GDI + net enzyme-sensitive sites (NSS) and NSS were calculated. Results showed no genotoxicity induced by TiO_2-NPs, in any tested concentration, for each one of the parameters evaluated. For GDI, H_2O_2 demonstrated his capacity for inducing DNA strand breaks, with significant ($p < 0.0001$) differences relative to the other conditions. Therefore, anatase TiO_2-NPs did not induce DNA strand breaks in HepG2 cells, for the tested conditions, even when using FPG for the detection of DNA oxidative damages. Comparisons to studies evaluating anatase TiO_2-NPs in the comet assay must be done prudently, since the treatment method used in our study is pioneering for this nanomaterial. Further studies on TiO_2-NPs genotoxicity are needed, especially *in vivo* ones.

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The potential involvement of transposable elements in spinal muscular atrophy, a neuromuscular disease

P17

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Keywords: Transposable elements, Alu, Spinal Muscular Atrophy, Neuromuscular disease

Transposable elements (TEs) are interspersed repetitive DNA sequences with the ability to mobilize in the genome, either using “cut and paste” or “copy and paste” mechanisms. Mammalian genomes are dominated by TEs, which can reach copy numbers in the hundreds of thousands and comprising up to 45% of the human genome. Transposable elements can be classified in DNA transposons and retrotransposons (non-LTR and LTRs), being the human genome dominated by LINEs and SINEs (amongst these especially Alu sequences) retrotransposons, LTR retrotransposons, and DNA transposons. It is believed that TEs had and continue to have significant impacts on mammalian genome evolution and in gene regulation. The recent development of improved tools for evaluating TE derived sequences in genomic studies has enabled an increasing attention to the contribution of TEs to human development and disease. Alu elements and other transposable elements promote non-allelic homologous recombination and contribute towards genomic instability. TEs also seem to be ready-to-use regulatory sequences and they may affect various post-transcriptional steps. Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disease mainly caused by mutations, especially deletions, in the survival motor neuron (*SMN1*) gene. SMA is characterized by loss of lower motor neurons in the spinal cord and brainstem nuclei, leading to progressive muscle weakness and atrophy. It affects approximately 1/6,000 to 1/10,000 individuals and is the most common inherited cause of childhood mortality. Curiously, SMA determining gene, SMN1 is highly enriched in Alu sequences, even in the promotor region of the gene. In this way and due to the characteristics of TEs, several questions arise: Are these sequences involved in SMA onset and progression? How does this occur? How do TEs orchestrate in the regular functioning of the human genome?

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Fungal diversity in teas, tisanes and infusions - is it dangerous to drink tea?

P18

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Keywords: *Penicillium*, Teas, Herbal teas, Infusions, Health.

Plants over have played a fundamental role in traditional medicine, due to their medicinal properties. Various drinks have been developed from the use of different parts of a plant (stems, roots, leaves and flowers), for example, teas, infusions and herbal teas. Tea is considered the second most consumed drink by humans after water. This work aimed at a brief review of the development of tea production and consumption and the distinction between the concepts of tea, herbal teas and infusions that are often terms that are difficult to distinguish by society. As well as, to evaluate the fungal diversity in different samples of teas, tisanes and infusions, through their isolation and identification. In a total of 15 samples of teas, tisanes and infusions obtained from different commercial surfaces, the fungi were isolated after the preparation of these samples according to the instructions on the packaging. Inoculation was performed with swab in a specific medium for mycological culture. The macroscopic and microscopic identification of the fungi was carried out; the latter being carried out using the Lactophenol with Cotton Blue technique. Of the 15 samples analyzed, 13 showed fungal growth, in which only *Penicillium* was identified. This study made it possible to increase knowledge about the diversity of fungal genera found in teas, herbal teas and infusions. Fungi in favorable conditions have a tendency to rapid proliferation, which after ingestion can be harmful to health.

P19

Evaluation of three different methods for the detection of biofilm formation in *Pseudomonas aeruginosa* from clinical isolates

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Keywords: Biofilm formation, *Pseudomonas aeruginosa,* Congo Red method; Tube method and Microtiter method

Pseudomonas aeruginosa is one of the leading causes of hospital-acquired infections, also known as nosocomial infections. Bacterial biofilms have been implicated in 80% of bacterial infections in immunocompromised humans involve biofilm-associated microorganism and are recognized to colonization in biotic and abiotic superficies. The objective of this work was to study the biofilm production since *P. aeruginosa* is a bacterium with a greater ability to form biofilm. The biofilm production from different clinical specimens was evaluated by three distinct methods: Congo Red method, Tube method and Microtiter method. Thirty-two *P. aeruginosa* isolates and one *Pseudomonas* spp., recovered from Medical Center of Trás-os-Montes e Alto Douro (CHTMAD) were characterized for the quantification of biofilm production evaluated by Congo Red, Tube and Microtiter method. Strains were classified as none, moderate or high biofilm producers. In the current study, the Congo Red method showed low positivity (6.0 %), Tube method showed a moderate biofilm forming isolates (75.7%), and Microtiter method showed high biofilm formation (100%). When compared to other methods, Microtiter method was found to be the most effective method in the detection of biofilm production. The lack of positivity in the Congo Red method may be related to the deficiency expression of the gene *pel,* responsible for the absent production of the glucose-rich extracellular matrix. The hardiness of biofilms and their resistance to antibiotics has led to a development problem in health-care settings, *P. aeruginosa* has turn one of the most relevant model organisms for the study of biofilm-associated infections.

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Detection of *Francisella* spp. in wild animal lymph nodes and lungs by PCR and histopathological techniques

P20

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Keywords: *Francisella* spp.; wild animals; PCR; histopathology

Among the emerging infectious diseases, of zoonotic origin, tularemia, or rabbit fever, requires special attention. The disease is caused by various strains of *Francisella tularensis*, a bacterium used as a biological weapon since antiquity, and belonging to the group of high danger in terms of infectiousness, along with agents such as *Bacillus anthracis* and *Yersinia pestis*. The most frequent manifestation of this disease involves symptoms that include fever, myalgia, dyspnoea and cough, among others. In addition to the unspecified clinical picture, its largely unknown natural occurrence and the high number of known reservoirs and vectors, are factors that reinforce the need for epidemiological studies in these groups of animals. In this study, a screening of *Francisella* spp. was performed on 130 tissues of 79 wild animals from Beira Interior Sul de Portugal, using the polymerase chain reaction (PCR) and histopathology techniques. The results pointed to potential cases of infection by this bacterium in 20 of the animals analysed (25.3%) and in 18 of the studied tissues (13.8%). In both screenings, the organs most affected were the mesenteric lymph nodes and lungs. In conclusion, this study demonstrated the presence of the potential agent of tularemia in economically important species, which may pose a risk to public health in terms of contaminated products. Finally, the locations of occurrence provide a higher risk of incubation of the agent for future zoonotic outbreaks.

Five years in the detection of *Talaromyces marneffei* in Portugal. It's time to think about!

P21

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Keywords: *Talaromyces marneffei*; Occurrence; Portugal; microbiology; PCR, ISSRs

Talaromyces marneffei causes a disease called penicilliosis. The majority of the penicilliosis casas are disseminated infections, clinically resembling histoplasmosis, cryptococcosis, and tuberculosis. This fungus can cause fatal systemic mycosis in both immunocompetent and immunocompromised patients. The infection responds to antifungal treatment, but untreated cases, especially in immunocompromised patients, are usually fatal. *Talaromyces marneffei* is also characterized by its geographic distribution since it is endemic in Southeast Asia and Southern China. In countries where the infection is endemic, the protocol for immunocompromised individuals includes screening of the pathogen and therapeutic protocols. Over the past 5 years, the Medical Mycology Research Team in UTAD, constituted by researchers from the Medical Microbiology Laboratory and the Genetics and Microbiology Laboratories, in collaboration with Polytechnic Institute of Castelo Branco, isolated and detected by microbiological, PCR and sequencing methods *Talaromyces marneffei* in samples from different species. *Talaromyces marneffei* has been identified in indoor air samples and objects of daily use, in nasal mucosa of humans and animals, and in wild animals such as the weasels, egyptian mongooses and hares. These results proved that the microbiological techniques associated with molecular techniques are sufficient to detect the agent, and show that this fungus is present in Europe and specifically in Portugal, and that it is time to give it due importance in public health.

The use of Next Generation Sequencing to unveil the problem of antibiotic resistance in *Enterococcus* spp.

P22

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Keywords: *Enterococcus* spp.; Processed Meat; Antibiotic Resistance; Next Generation Sequencing

The intensive use of antibiotics contributes to the development and spread of resistant bacteria. Enterococci are commensal bacteria of the intestinal microbiota and are becoming increasingly reported as an opportunistic pathogen because of their ability to survive in a wide diversity of conditions. After mass spectrometry identification of 21 enterococci isolated from processed meat, the susceptibility test was performed with 12 antibiotics according to EUCAST standards. All isolates were multiresistant and the 19 VREs showed high rates of resistance to teicoplanin, erythromycin and ampicillin. The whole genome sequence of strains was determined by *de novo* assembly of 2x150-bp paired-end reads generated with Illumina sequencing technology using multiple assemblers. All *E. faecium* and *E. durans* isolates were *vanA*-positive and one *E. gallinarum* isolate harboured both *vanC* and *vanA* genes. The presence of *erm*(B) gene confirmed the resistance to erythromycin and the presence of *aac*(6')-*Ii* and *ant*(9)-*Ia* genes confirmed the resistance to aminoglycoside antibiotics. These results confirm the presence of VREs in food and highlights its potential in the spread of vancomycin resistant bacteria in nature. We also must pay attention to the analysis of VREs in food of animal origin and the use of antimicrobials in veterinary and livestock medicine.

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Refinement of oral route administration in C57BL/6J and FVB/n mice – a gelatine flavours trial

P23

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Keywords: C57BL/6J mice, FVB/n mice, refinement, animal welfare.

Oral administration by the intragastric gavage technique may have technical complications if not done properly or following repeated dosing. This study aimed to refine oral administration techniques, improving, thus, animal welfare through the use of gelatine pellets in two different mouse strains: C57BL/6J and FVB/n. After ORBEA approval, 7-week-old male C57BL/6J mice (n=35) and 8-week-old male FVB/n mice (n=35) were equally divided into five groups (n=7/group): Group I, Control; Group II, Gelatine with neutral flavour; Group III, Gelatine with strawberry flavour; Group IV, Gelatine with lemon flavour; and Group V, Gelatine with ground diet incorporated. The body weight variation and time of ingestion of the total pellet were registered daily in a total of 8 days. Along the study, for both strains, the body mass of each group did not suffer any significant variation. Comparing the ingestion times of FVB/n mice with that of C57BL/6J mice, the time that FVB/n mice took was significantly higher for neutral, strawberry and ground diet flavour. For lemon flavour, except for day 1 and 2, the differences were not statistically significant between mouse strains. In conclusion, these results show that there is a difference between the two mouse strains used in this study, where the C57BL/6J mice are more likely to eat gelatine, also there is a tendency towards lower preference for lemon flavour regardless of mouse strains.

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Screening of EGFR mutations in non-small cell lung cancer patients in liquid biopsies

P24

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Keywords: Non-small cell lung cancer, EGFR, mutations, liquid biopsies, cell-free DNA, Next-Generation Sequencing.

Lung cancer is the most common type of cancer worldwide, representing the non-small cell lung cancer (NSCLC) about 80-85%. Specific EGFR mutations have been used as predictive biomarkers for the application of EGFR-targeted therapies. Tumour biopsies (usually formalin fixed and paraffin embedded-FFPE) are analysed by PCR and Sanger sequencing, presenting several disadvantages. With the aim of overcoming these difficulties, it is our goal to develop a simple, certified and analytically sensitive test for mutation detection, associated with different therapeutic responses in liquid biopsies of NSCLC patients. This system will consist of an innovative range of reagents that will allow the cell-free DNA (cfDNA) extraction in liquid biopsies, amplification of EGFR mutations by multiplex PCR and its detection by Next-Generation Sequencing (NGS). All this data will come out in a simple output that can be used in clinical context.

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Proliferation index in chemically and hormonally induced prostate cancer rat model: the role of physical exercise

P25

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Keywords: Prostate, Cancer, Animal models, Physical exercise, Proliferation index

An active lifestyle is associated with reduced risk of prostate cancer development. This study aimed to evaluate the role of physical exercise on proliferation index in a rat model of chemically and hormonally induced prostate cancer. Fifty-five male Wistar Unilever rats (*Rattus norvegicus*) with 12 weeks of age were randomly divided into four groups: control (n=10), induced (n=15), control exercised (n=10) and induced exercised group (n=20). The animals of exercised groups started the exercise program in a treadmill at the age of 8 weeks (Treadmill Control LE 8710, Harvard Apparatus, USA) for 28 weeks (5 days/week). Lesions were induced at 12 weeks of rat's age with flutamide (50 mg/kg, TCI Chemicals), N-methyl-N-nitrosourea (30 mg/kg, Isopac®, Sigma Chemical Co.) and testosterone propionate implants. Animals were sacrificed at 61 weeks of age. Data were analysed using SPSS 25. Animals experiments were approved by *DGAV* (no. 021326). Animals from induced sedentary group showed 85.7% dysplasia, 64.3% PIN and 64.3% microinvasive carcinomas of the dorsolateral prostate. Animals from induced exercised group showed a decrease in the number of lesions: 70% dysplasia, 58.8% PIN and 58.8% microinvasive carcinomas ($p>0.05$). Ki-67 index was higher in animals from induced exercised groups when compared with induced sedentary in all identified lesions ($p>0.05$). Our results suggest that the exercise decrease the severity of prostate cancer induced lesions although it has increased proliferation index. Other markers should be used to analyse the effect of exercise on prostate lesions development.

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P26

Detection of virulence genes in clinical isolates of *Pseudomonas aeruginosa*: a contribution in pathogenesis

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Keywords: *Pseudomonas aeruginosa*, virulence genes, pathogenesis

Pseudomonas aeruginosa is a leading nosocomial pathogen worldwide. The extensive clinical use of antibiotics represents a challenge for antimicrobial therapy, due to exhibition of intrinsic resistance to a variety of antimicrobial agents such as most β-lactams. This pathogen has a great versatility due to a complex interplay between antimicrobial resistance and virulence factors. The pathogenesis of *P. aeruginosa* is not correlated to a single virulence factor, but association with a diversity of different virulence factors, which contribute to the bacterial invasion, adhesion, resistance and toxicity. The objective was to evaluate the virulence mechanisms in *P. aeruginosa* clinical isolates. Thirty-two *P. aeruginosa* isolates, recovered from various human clinical samples from Medical Center of Trás-os-Montes e Alto Douro (CHTMAD), were characterized by the following analyses: susceptibility against 12 antimicrobial agents by disk-diffusion method (EUCAST 2019) and were screened for the prevalence of different virulence genes of *P. aeruginosa*, such as *tox*A, *alg*D, *plc*H, *plc*N and *pil*A by PCR assay. Thirty *P. aeruginosa* isolates have a multi-resistant phenotype, resistance to at least three different classes of antibiotics. The most commonly detected virulence genes were *plc*H (78.1%), *tox*A (65.6%) and *alg*D (43.8%). The virulence genes *plc*N (12.5%) and *pil*A (21.9%) demonstrate a low prevalence in the clinical isolates. *P. aeruginosa* possess a range of virulence factors that they contribute to different levels of intrinsic virulence and pathogenicity. Our results showed a moderate prevalence of virulence factors (42.5%) and this may depend on several causes including nature of place, immune status of patients, degree of contamination, source of infections, type and virulence of the strains.

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Varroa Destructor in Portugal: Resistance to Pyrethroids Status

P27

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Keywords: *Varroa destructor*; Pyrethroids resistance; *Apis mellifera*; Portugal

The drastic and abrupt loss of honeybee colonies around the world has long unsettled the beekeeping community. The phenomenon is far from being solved, but there is relative consensus regarding the detrimental effect the mite Varroa destructor exerts over A. mellifera populations. In Portugal, the parasite was first recorded in the late 80s and since then, the beekeepers' method of choice for controlling the mite are synthetic acaricides, most notably, the pyrethroids tau-fluvalinate and flumethrin. However, its intensive use has led to a resistance, currently associated with mutations at position 925 of the voltage-gated sodium channel, and consequently a reduction of its efficacy against the Varroa mite. Although reports alert to resistance in several countries, little is known about the current situation in mainland Portugal. In order to unveil the varroa resistance status to pyrethroids, in Portuguese beehives, a total of 119 V. destructor mites were collected between April and August of 2019 across several localities and PCR-RFLP resistance assessment was performed. The study found 40% of the sampled mites exhibiting the mutation correlated with resistance. However, due to heterogenous sampling throughout the country, our results may not reflect the actual proportion of mites tolerant to the active ingredient. Nonetheless, these findings stress the need for a current characterization of the acaricides being primarily used and description of how the treatments are been applied (number, month and duration). Only then, the risk of resistance to acaricides can be successfully assessed.

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Evaluation of physiological parameters in FVB/n mice supplemented with an anthocyanin-rich elderberry extract

P28

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Keywords: natural colorants, *in vivo*, toxicity, *Sambucus nigra* L., *Mus musculus*

Black elder (*Sambucus nigra* L.) is a widespread species in most countries. The juice obtained from the berries of the black elder contains many phenolic compounds, such as anthocyanins, which are known for their great colorant capacity and health benefits. The aim of this work was to evaluate the effects of anthocyanin-rich elderberry extract (EE) supplementation on mice. The anthocyanin profile and quantification were performed by HPLC-DAD-ESI/MS. Twenty-four FVB/n 8 weeks-old female mice, divided into four experimental groups, were exposed to different EE concentrations: Group (G)-I (control), G-II (12 mg/mL), G-III (24 mg/mL) and G-IV (48 mg/mL), dissolved in drinking water for 28 consecutive days, G-I only received tap water. Animals were sacrificed by a ketamine/xylazine overdose, blood and organs were collected. The analysis of the extracted juice revealed cyanidin-3-*O*-sambubioside-5-*O*-glucoside and cyanidin-3-*O*-sambubioside as the main anthocyanin compounds. Animals from G-II (p=0.012) lost body weight, whereas animals from G-III and IV did not show any significant differences when compared to the control group. The average weekly food and drink consumption was significantly greater in G-II and G-I, respectively (p<0.05). Relative organ weight of the heart was significantly bigger in G-II when compared to G-I (p=0.11), G-III (p=0.02) and G-IV (p=0.01), probably due to the presence of residual blood following cardiac puncture. Additionally, the left kidney's was significantly bigger in G-I than G-II (p=0.13) and G-III (p=0.13). Further studies are necessary to understand the impact of EE in liver and kidney physiology.

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Coronavirus 2019-nCoV

P29

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Keywords: 2019-nCoV, coronavirus, Wuhan, SARS-CoV, MERS-CoV, specific PCR diagnostic test.

On December 31st 2019, a new virus, 2019-nCoV, was identified as the cause of a respiratory infection in Wuhan, China. The Chinese government and local researchers managed to sequence the 2019-nCoV in a record time. On January 11st 2020 the genetic sequences of the virus were released to the public so that anyone could analyze them.
Thanks to a phylogenetic analysis, it was established that 2019-nCov belongs to the family of the positive single-stranded RNA coronavirus. Coronaviruses are a large family of viruses that can cause respiratory diseases in both humans and animals. Rarely, animal coronaviruses can mutate and infect men and subsequently spread from one person to another, such as what occurred with Severe Acute Respiratory Syndrome (SARS), that first appeared in November 2002 in China; and Respiratory Syndrome Middle East coronavirus (MERS), that was first identified in 2012 in Saudi Arabia. The 2019-nCoV sequence shows similarities with the coronaviruses found in bats, but they are genetically distinct from other coronaviruses as SARS-CoV and MERS-CoV. 39 coronavirus species have been recorded. Species like 2019-nCoV have been discovered recently, as several particular strains had not been previously identified in humans. There is little information about their transmission, possible reservoirs, severity of the infection and their clinical impact. There are currently no approved treatments. However, several of the symptoms can be treated. Having the Wuhan coronavirus (2019-nCoV) genetic sequences has allowed the development of a specific PCR diagnostic test. This has made the sweeping of symptomatic people in international airports possible; as an attempt to prevent its transmission worldwide.

The role of angiogenesis in Canine Cutaneous Histiocytoma spontaneous regression

P30

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Keywords: angiogenesis, CD31, histiocytoma, canine, Langerhans cells

Canine Cutaneous Histiocytoma (CCH) is a benign tumor of the dog's skin that, in most cases, undergoes on spontaneous regression. It can occur anywhere on the body of the dog, resulting from the neoplastic proliferation of dendritic cells of the epidermis, called Langerhans cells. It is classified as a tumor of round cells, being reported as the most frequent cutaneous neoplasm in young dogs. Its ethology, both at the level of genetic and molecular events, as well as its progression and development, is still an open door in veterinary clinical research. CD31, also known as platelet endothelial cell adhesion molecule (PECAM-1), is an endothelial cell marker that allows the identification of blood vessels. In immunohistochemical tests, CD31 is used to verify the presence of endothelial cells in histological sections, being an important tool in the analysis of tumor angiogenesis. In order to clarify the role of angiogenesis in CCH regression, the number of neovases was evaluated by CD31 immunoreaction in 50 tumours, categorized into 4 histological groups according to a regression scale. Microvessel density was lower in groups 1 and 2 CCH, and higher in groups 3 and 4. Thus, tumors in groups 1 and 2 have few vessels suggesting that angiogenesis is compromised in early phases of CCH development. Our data reinforce previous studies related to the immunohistochemical expression of the VEGF angiogenic factor and its receptor in canine cutaneous histiocytoma, which suggests that angiogenesis could be a switch that determines CCH regression.

Immunoexpression of TGFβ-1 in dogs and cat's lung tumours

P31

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Keywords: TGFβ-1, lung tumours, dog, cat, immunohistochemistry

The transforming growth factor beta superfamily is composed of a large group of proteins. The TGFβ-1 is a multifunctional cytokine that participate in several physiological cell functions as growth, proliferation, differentiation and apoptosis. TGFβ-1 also play an important role in the carcinogenesis of different tumors, including human lung cancer. However, its role in the development of animal lung tumours has not been established. To investigate the expression of TGF-β1 protein in canine and feline lung cancer, immunohistochemistry was carried out in 28 cases of lung tumours (13 dogs and 5 cats) and 10 cases of non-tumoural lung tissues (7 dogs and 3 cats). Tumors were classified according WHO classification. TGFβ-1 was evaluated semiquantitatively in extension and intensity. A score of immunoreactivity was determined by the product of extension and intensity, which when lower or equal to 4 translated a low immunoreactivity for TGFβ-1 and when or greater than 4 translated a high immunoreactivity for TGFβ-1. Of the 18 tumors, 11 showed high immunoreactivity, and 7 showed a low immunoreactivity. In non-tumoural lung samples, 2 presents a high immunoreactivity and 2 a low immunoexpression. These differences between tumour and normal lung were statically significant (p=0,043). However, none of histological features evaluated (mitotic index, nuclear pleomorphism, necrosis and differentiation) were associated with TGFβ-1 reaction. Results from the present study allow to conclude that TGF-B might play a role in carcinogenesis of dog and cat lung tumours.

Bioterrorism

P32

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Keywords: Bioterrorism, anthrax, botulinum toxin, ebola, danger.

The intentional use of biological, chemical, nuclear or radiological agents to cause disease, death or environmental damage is known as bioterrorism. Although the definition could sound up-to-date, the use of biological weapons in warfare comes from the Middle Ages, when the bodies of soldiers with plague were thrown into besieged castles or walled cities to spread the disease. The range of agents that could potentially be used as weapons is wide. However, only a few of these agents fulfil all the requirements, making them ideal for that purpose. Based in these requirements, bioterrorism agents are classified in three categories, called A, B and C, being category A the most dangerous. Among all the bioterrorism agents, we highlight several notorious biological weapons, such as the anthrax agent, botulinum toxin and ebola virus, and analyse the reasons why they are so dangerous. In addition, we review the difficult response against those agents in the United States and the European Union, knowing that the release of those agents in laboratories could cause workers deaths.

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Importance of the determination of d-dimers in disseminated intravascular coagulation and thromboembolic diseases in dogs

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Keywords: D-Dimer, CID, Dog, Thromboembolic disease

Some disorders, such as: intravascular hemolysis, viremia, parasitic infections, septicemia, obstetric complications, massive tissue injury, neoplasia, venoms, toxins, hepatic disease, pancreatitis, and other conditions (gastric dilatation-volvulus) are associated with a pathophysiologically intermediary mechanism of disease called Disseminated intravascular coagulation (DIC). This disease's pathogenesis involves the activation of coagulation and fibrinolysis accompanied by the generation of thrombin and plasmin. When plasmin is in its pathological state it splits soluble fibrin and fibrinogen, resulting in Fibrin Degradation Product fragments X, Y, D and E. Persistent cleavage of X-oligomers generates fragment E bound noncovalently to two D fragments (D-dimer). Both human and veterinary patients are commonly afflicted by thrombos that form in the arterial and/or venous system, which can be deadly, especially when in combination with thromboembolic disease. D-dimer is the only laboratory marker that has proved to be clinicaly usefull in the discernment of early embolism in humans and dogs, being the most reliable test in patients with confirmed DIC. The crosslinking of C-chains from D domains of 2 adjacent fibrin monomers, mediated by FXIIIa, produces the neoantigen D-dimer epitope. Monoclonal antibodies against this neoantigen recognize them within X-oligomers and are used to measure D-dimer concentrations in ELISA and latex-agglutination and immunoturbidometric assays. The applicability for testing human D-dimers in dogs has been reported. Semiquantitative methods of latex agglutination and human antibody based immunoturbidimetric tests have been successfully tested on dogs. In this study, we evaluated the D-dimers concentration by a semi-quantitative Latex agglutination method (D-Di test, Stago®) in canine citrated plasma samples. After routine centrifugation 200ul of fresh plasma were analised within 1 hour post collection according to the manufacturers instructions. This procedure allows DIC and thromboembolic disease diagnostic in dogs.

Imagiological aspects of urine sediment in companion, exotic and wild animals: preliminary data for the elaboration of an online veterinary urine atlas

P34

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Keywords: Urinary sediment; Hematuria; Leukocyturia; Crystalluria

Urinalysis is a very useful method for assessing and diagnosing diseases of the urinary tract, but it can also reflect the presence of some systemic diseases. Urinalysis is a simple, low-cost exam being considered a routine exam. A complete Urinalysis includes the evaluation of urine physical characteristics (color, turbidity and density) by macroscopic evaluation; evaluation of biochemical parameters (pH, nitrites, blood, glucose, ketone bodies, bilirubin, urobilinogen and proteins) assessed by reactive urine strips and microscopic examination of urinary sediment where it is possible to assess the presence, among others, of erythrocytes, leukocytes, epithelial cells, cylinders, crystals, microorganisms, parasites and sperm. In this work, microscopic aspects of diverse elements present in the urinary sediment will be demonstrated. Additionally, the graphic organization of an online atlas under preparation, will also be revealed. Urine samples from different animal species were collected and analyzed between October 1, 2019 and December 31, 2019. For this study, urine samples from 129 animals (62 dogs; 55 cats; 2 guinea pigs; 6 hedgehogs, 3 rabbits and 1 macaw bird) were analyzed. Of the dogs 45% had Hematuria, 30% Leukocyturia and 25% Crystalluria. In cats 59% had Hematuria, 18% Leukocyturia and 23% Crystalluria. Regarding the other animals, 1 in each species, presented Crystalluria. The images were collected at the Clinical Pathology Laboratory of HVUTAD and are part of the collection that is being organized with a view to preparing an Online Urine Atlas. The realization of a study of this nature it is very useful because, with regard to exotic and wild species, the available imaging data in the literature are scarce. The elaboration of a Veterinary Urine Online Atlas, besides impact for clinicians and students, will also allow knowledge dissemination in the scope of an open science.

Blood cell morphology in veterinary species: preliminary data for the elaboration of an online veterinary hematology atlas

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Keywords: Erythrocytes; Leukocytes; morphological changes

Blood cells morphology in companion animals (dog and cat), ruminants, horses, exotic and wild animals can vary. The knowledge of morphological characteristics in each species is fundamental in order recognize morphological changes associated with disease states. The erythrocytes in mammals are generally very similar and may vary in size and in the presence of a greater or lesser area of central pallor. They appear as rounds cells, anucleated with an intense eosinophilic staining. In birds and reptiles, erythrocytes appear as oval cells and with the presence of a nucleus. Leukocytes due to their morphological characteristics are divided into two large groups according to their nuclear characteristics and the presence or absence of cytoplasmic granules: Granulocytes (neutrophils, eosinophils and basophils), have segmented nuclei and granules in their cytoplasm; Agranulocytes (lymphocytes and monocytes) present nuclei without constriction and are devoid of cytoplasmic granules. In the most common domestic species, normal cell morphology is quite similar for all cell types except for eosinophils and basophils that vary by species. In the exotic and wild species, the variability can be extended to other cell types. In this work, several aspects of normal blood cell morphology in different species will be presented. Additionally, it will be introduced the graphic organization of an online atlas under preparation.

Ganoderma lucidum (Curtis) P. Karst. to fight obesity in an animal model - Preliminary results

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Keywords: Mice C57BL/6; Obesity model; *Ganoderma lucidum*; Hypercaloric diet

Obesity is a disease that has been increasing exponentially worldwide, featured by an excess of body fat added by an excessive food intake. The lingzhi mushroom *Ganoderma lucidum* (Curtis) P. Karst. has been used for an array of health benefits since it inhibits platelet aggregation, lowers blood pressure, cholesterol and blood sugar. The main objective of this work was to evaluate the anti-obesity effects of *G. lucidum* in an animal model. For this, 48 males C57BL/6 mice were acquired and divided into 5 groups: Group-1-Western Diet 0.2% Cholesterol (WD); Group-2-Western Control (WC); Group-3-WD+0.7%g/kg of *G. lucidum*; Group-4-WD+1.4%g/kg of *G. lucidum*; Group-5 WD+2.8%g/kg of *G. lucidum*. Animals were daily observed and the individual animal body weight, body temperature as well as food and water intake were recorded weekly. All ethical issues were followed (after ORBEA approval). The chemical composition of the extract was profiled by HPLC-DAD-ESI/MS. Ganoderic acid H and *p*-hydroxibenzoic acid were the main triterpenic and phenolic acids found in the extract, respectively. The animals showed no signs of disease and the body temperatures remained constant over the weeks, except for the 5th week, when they rose significantly ($p<0.05$). All groups increased mean body weight, thus Group-5 (WD+2.8%g/kg *G. lucidum*) showed the greatest weight variation, unlike Group-3, which was the group that revealed the lowest weight variation. Food and water intake were higher in animals from Group-1. Considering the preliminary results, *G. lucidum* does not seem to have a negative impact on the health status of the animals under study.

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Identification of *Salmonella spp.* and evaluation of genetic diversity with ISSRs in pork samples from slaughterhouse

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Keywords: *Salmonella, S.Typhimurium,* PCR, ISSR's, food safety.

Salmonella is a bacteria from the Enterobacteriaceae family and there are more than 2600 serotypes identified. It has become the primary cause of foodborne illness, in humans and animals, that can lead to outbreaks and even death. Therefore, the control and prevention of these pathogens are of high priority to improve the safety of the food supply. Pork meat is the main transmission vehicle of this bacteria and, commonly, the contamination develops during slaughter thus the samples of this study where collected from a slaughterhouse. Detection of *Salmonella* spp. was carried out according to ISO procedures and the cultures were stored at -20 °C in BHI and 45% (V/V) of glycerol. Thirty eight cultures were picked to BHI and incubated 24 hours at 37 °C from which we proceeded to DNA extraction. It was also used DNA from *S. typhimurium* as positive controls, the most prevalent serotype in pigs and in *Salmonella* food poisoning patients. Firstly, an identification of *Salmonella* spp. was done using specific primers followed by serotype identification using PCR that targeted specific genes of *S. typhimurium*. Afterwards, using ISSR markers, the genetic diversity of the samples was evaluated. The results confirmed *Salmonella* spp. in the thirty eight tested samples and, based on the specific gene PCR, all of them were identified as *S. typhimurium*. With the ten ISSR primers we obtained 260 markers being 245 polymorphic, resulting in a polymorphism rate of 94.23%. A dendrogram was made and revealed three different clusters. This division can be related to the sampling day at the slaughterhouse. The overall results show differences between samples that highlight the genetic variability within the same serotype.

FISH and MLPA: molecular techniques that complement Cytogenetic Diagnosis

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Keywords: Cytogenetics, Karyotyping, FISH, MLPA, Anomalies, Diagnosis.

Cytogenetics aim is to establish individual´s karyotype in order to diagnose any chromosomal aberrations. However, when the anomalies are smaller than 5-10Mb, they are not detectable by conventional karyotyping and it is necessary to use molecular techniques, such as Fluorescent *in situ* Hybridization (FISH) and/or Multiplex Ligation-Dependent Probe Amplification (MLPA). FISH technique is based on the hybridization of complementary, single-stranded DNA on fixed metaphase chromosomes or interphase nuclei. It localizes chromosome rearrangements in specific cells or tissue types and can provide results even when tissue is insufficient or unsatisfactory for karyotyping. A labeled probe and the target DNA are denatured and the complementary sequences are subsequently hybridized. Fluorescent signals of the specific probes are visible by the use of a fluorescent microscope. MLPA is a semi-quantitative technique that compares samples of patients with controls in order to detect genomic gains and losses. This technology is extremely versatile in its applications, flexible in its target loci, suitable for high throughput testing, efficient, and cost-effective. The target sequences are amplified by a PCR reaction and then the fragments are separated by capillary electrophoresis. The peak areas are quantified and compared with control samples, reflecting the relative copy number of the corresponding target sequence in that sample. The technique used in each situation depends on specific needs. FISH consists in a more targeted approach, since it requires a previous knowledge about the chromosome aberration being analyzed and, therefore, is not a screening tool. On the other hand, MLPA, as semi-quantitative method, is not able to detect balanced alterations, since it can only detect changes in probes copy number. Despite their utility and efficiency these techniques do not replace conventional karyotyping, they complement it, in order to provide a more accurate diagnosis.

ABX464 influence in HIV RNA: a different perspective

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Keywords: Retrovirus, Anti-inflammatory, CBC, CBP80, Argonaute2, miR-124, RISC

HIV-1 is the retrovirus responsible for acquired immunodeficiency disease syndrome (AIDS). ABX464 (Abivax464) is a small size molecule orally administrated that has shown strong anti-inflammatory effects in the DSS-model for inflammatory bowel disease, by preventing the replication of HIV virus. This molecule stimulates splicing of the virus by binding to the CBC (cap binding complex), which will promote the recruitment of CBP80. ABX464 has a specific dual ability to generate anti-inflammatory miR-124 and spliced viral RNA may be applicable in the treatment of both inflammatory diseases and HIV infection. The objective of this work was to discuss and show that ABX464 not only has this effect and this way of action but can also bind to other molecules. We could conclude that, doing comparative analysis of different articles, and reading various information about the topic and analysing all the information we collected. So, we conclude that in the presence of this molecule there will be a more efficient production of RISC, and the production of this complex is important for miRNA incorporation so that the mRNA could bind to it, and that way the translation will not happen.

Do tumor-associated macrophages promote feline injection site sarcomas progression?

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Keywords: Tumor-associated macrophages, feline, feline injection site sarcomas

Tumor-associated macrophages (TAMs) are key components of the tumor microenvironment that have been associated with proliferation and progression in several tumor types. Feline injection site sarcomas (FISS) are peculiar malignant tumors of mesenchymal origin which arise in sites of injections or implantation of medical devices. Despite the many studies, the role of macrophages has not yet been studied on these tumors. To elucidate the biological significance of TAMs in feline injection site sarcomas, a comprehensive assessment of the tissue distribution of TAMs was performed. TAMS were retrospectively analysed using MAC387 immunohistochemistry (Clone MCA874G, AbDSerotec, Kidlington, UK; 1:100 dilution) in 58 FISS. Tumors were grouped according to their differentiation, the presence of necrosis and mitotic count. Other histopathological features such as the presence of giant cells and lymphocytic inflammatory infiltration were also evaluated. A variable number of macrophages were observed diffusely between neoplastic cells. In general, macrophage infiltration was scarce in 29 tumors; moderate in 18 tumors and in 11 cases it was considered high. TAMs density was significantly associated with tumor differentiation ($p<0.001$), mitotic count ($p=0,003$) and necrosis ($p=0,03$). By contrast, no association was observed between TAM density and the presence of giant cells and lymphocytic inflammatory infiltration. Thus, the best-differentiated tumors, with a low mitotic count and absence of necrosis have a low MAT, while the most undifferentiated tumors, more proliferative and with necrosis have, in general, a greater number of MATs. Our results suggest that, during FISS progression, TAMs may induce tumor cell aggressiveness and proliferation.

The CAM assay as a model to study the angiogenic potential of biomaterials

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Keywords: Meniscus; Intervertebral disc; Vascularization; Angiogenesis; CAM assay; Silk; Methacrylated Gellan-Gum; Tissue engineering

Meniscal lesions and intervertebral disc degeneration (IVD) are two of the most frequent orthopedic lesions with a deleterious effect on patient's life quality. There are clinical solutions for these pathologies, but they are ineffective in the long term and in restoring the biofunctionality of the native tissues. Therefore, the development of novel Tissue Engineering (TE) approaches that address the regeneration of these cartilaginous tissues is in great need. Vascularization is a critical factor in the regeneration of vascularized tissues ensuring the viability and function of tissue substitutes. The objective of this work was to study the angiogenic response of three-dimensional silk and silk/elastin scaffolds and disc-shaped methacrylated gellan-gum hydrogels for TE applications by using the chicken embryo chorioallantoic membrane (CAM) assay. The CAM assay is a recurrent assay to study angiogenesis, being used in TE as an intermediated step between *in vitro* and *in vivo* models, which consists on the implantation of biomaterials on the CAM of developing chicken embryos. The angiogenic response is further evaluated through quantification of blood vessels converging toward the implanted biomaterials and by histological characterization through hematoxylin & eosin staining and immunohistochemical detection of lectin. The angiogenic potential of silk and silk/elastin scaffolds and the non-angiogenic properties of methacrylated gellan-gum hydrogels are evidenced in this work. The different angiogenic abilities exhibited by the tested materials allows to equate their combination in the same three-dimensional structure, in order to mimic the different vascular and avascular regions present in complex tissues such as meniscus and IVD.

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Immunoexpression of MMP-9 in canine malignant tumours

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Keywords: MMP-9, canine, tumor, mammary

Text Female dogs develop mammary tumours with a biologic and histopathologic behaviour similar to Human Breast Cancer (HBC), that's why it is a suitable model in study of these neoplasias. Metalloproteinase (MMP) are a family of endopeptidases zinc dependent with over 20 different types. MMP-9, in particular, plays a vital role in tumour cells invasion and metastases. The subexpression of MMP-9 has been observed in HBC but scarce documented in canine mammary tumours (CMT). Our objectives are to evaluate MMP-9 immunoexpression in benign and malign CMT and to stablish its relationship with tumor clinicopathological characteristics and survival of the animals.
In this study, MMP-9 expression was evaluated in 30 canine malignant mammary tumors, using the immunohistochemistry technique. The immunoexpression of MMP-9 was evaluated according to its presence in the stroma and the intensity and extent of labelling in tumor cells. A statistically significant relationship was observed between their presence in the stroma and the mitotic index (IM) ($p = 0.021$), the Histological Degree of Malignity (GHM) ($p = 0.008$) and metastasis in the lymph nodes ($p = 0.004$). There was also a statistically significant relationship between the intensity of staining in neoplastic cells with the GHM ($p = 0.024$) and the presence of metastases in the lymph nodes ($p = 0.025$). Regarding the extension, no statistically significant relationships were observed. It can also be concluded that the high intensity of MMP-9 expression in neoplastic cells, as well as diffuse positivity in stromal cells, affect the survival of animals, being associated with a worse prognosis ($p = 0.012$ and $p = 0.042$, respectively). Our results are in line with some studies that were carried out in human medicine and veterinary medicine, suggesting an association between overexpression of MMP-9 and the acquisition of characteristics of greater biological aggressiveness by the tumor.

Preliminary study on oxidative stress genes in zebrafish liver exposed to simvastatin

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Keywords: simvastatin, zebrafish, oxidative stress, catalase, superoxide dismutase, semi-quantitative PCR

In the last years there has been an increase number of pharmaceuticals compounds detected in aquatic environments. One of these compounds is simvastatin, which is a drug used for the treatment of hypercholesterolemia, by decreasing the synthesis of cholesterol. Previous studies have shown that when Simvastatin gets into aquatic environments, even at low concentrations, it has a high bioaccumulation potential in living organisms, affecting for example embryo development, cells membrane stability and metabolic activity, and also inducing mortality. For the simvastatin ecotoxicological effects testing, the recommended and widely used zebrafish (*Danio rerio*) model was chosen. One important aspect that makes zebrafish a good model is that this species has a close phylogeny to mammals regarding simvastatin target pathways. The liver, as one of major organs involved in metabolism and detoxification processes and therefore sensitive to oxidative stress, was selected to evaluate the gene expression of catalase and superoxide dismutase. Catalase (*Cat*) and superoxide dismutase (*Sod*) can be used as oxidative stress biomarkers due to their rapid response to chemical contamination and environmental stresses. The aim of this preliminary study was to identify alterations on the *Cat* and *Sod* gene expression in response to different concentrations of simvastatin. For this purpose, fish were exposed during 90 days to simvastatin concentrations ranging from 8 to 1000 ng/L and the genes expression profiles were evaluated by semi-quantitative PCR.

Detection and evaluation of virulence factors of *Staphylococcus aureus* in a slaughterhouse

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Keywords: *Staphylococcus aureus*, meat, slaughterhouse

Staphylococcus aureus is one of the main pathogens found in meat for consumption and can cause severe illness in humans and animals. The virulence of *S. aureus* depends on the presence of certain genes like *nuc*, genes encoding enterotoxins, *qacA*, *coa*, *tsst*, etc. These bacteria cause mainly gastroenteritis but can cause other serious problems owing to its resistance to antimicrobials and limited treatments available. The aim of this study is to detect and to confirm, the presence of *Staphylococcus aureus* in samples from a slaughterhouse to determine where the contamination occurs and if there are parts that are more prone to this contamination. Posteriorly, the identification of the virulence genes present in the samples will be done. The samples were obtained from a slaughterhouse and collected from different parts of the pig carcass. The bacteria isolation, according to ISO procedures, started with BHI medium, after that we put them in petri dishes to evaluate the purity and typical aspect. A pure and typical colony was chosen, transferred to BHI and incubated 24 hours at 37 ºC, followed by DNA extraction and a Polymerase Chain Reaction, with specific primers, for identification of the specie. The presence of *Staphylococcus aureus* was confirmed in all samples tested and the study will be followed by the analysis of virulence genes.

Effect of green-synthesized magnesium oxide@hydroxide (MgO@MgOH) nanoparticles in the osteoclastic differentiation of macrophages

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Keywords: Green synthesis, MgO@MgOH nanoparticles, osteoclast differentiation

Plant based green synthesis approaches are gaining importance due to their simplicity, cost-effectiveness and ecological compatibility. Plant extract based synthesis of metal oxide nanoparticles (NPs) facilitates the production of non-toxic NPs due to the presence of various phytochemicals and biochemical compounds that are beneficial for biomedical and pharmaceutical applications. Due to the relevance of magnesium (Mg) in bone metabolism and remodelling, this work describes the effect of green-synthesized magnesium oxide@hydroxide (MgO@MgOH) nanoparticles in the osteoclastic differentiation of THP-1 differentiated macrophages. Rose Hip extracts were used for the synthesis of MgO@MgOH nanoparticles from two magnesium precursors (magnesium nitrate Mg $(NO_3)_2$ -Mg-NO and magnesium chloride $MgCl_2$-Mg-Cl). Suspended THP-1 cells were differentiated into adherent THP-1-derived macrophages through induction with phorbol 12-myristate 13-acetate (PMA) for 48 hours. At this stage, MgO@MgOH NPs were added (1, 10 and 100 µg/mL) to the adherent macrophages and remained in the culture for a further 6 days. Adherent macrophages induced into osteoclasts with M-CSF and RANKL (two osteoclastogenic factors) were used as control. NPs-treated and control cultures were characterized at 24 hours and 6 days for osteoclastic markers (tartrate-resistant acid phosphatase (TRAP) activity and staining, presence of multinucleated cells and formation of actin rings). Results showed that, overall, the green synthesis approach demonstrated potential for developing highly stable MgO@MgOH nanoparticles. Additionally, these NPs, synthesized from the two magnesium precursors, either in the absence or in the presence of Rose Hip extracts, increased the tested osteoclastic parameters after 6 days of exposure, compared to that observed in the M-CSF+RANKL-induced osteoclasts. As bone remodelling comprises the concerted action of the osteoclasts and the osteoblasts, experiments performed in a more representative model, i.e, co-cultured osteoblasts and osteoclasts, are being performed to analyse the integrated effect of MgO@MgOH NPs in bone cells.

From bench to bioterium and back again: Development of a U1 snRNA-based therapy for Mucopolysaccharidosis IIIC

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Keywords: Mucopolysaccharidosis IIIC, *HGSNAT,* U1 snRNA, Mice

Genetic therapy directed towards the correction of RNA missplicing is being investigated at basic research and in late-stage clinical trials. Many mutations that change the normal splicing pattern and lead to aberrant mRNA production have been identified in Lysosomal Storage Disorders (LSDs). Mucopolysaccharidosis IIIC (MPS IIIC) is one of those LSDs caused by mutations in the *HGSNAT* gene that encodes an enzyme involved in heparan sulphate degradation. Approximately 55% correspond to 5' splice-site (ss) mutations thus constituting a good target for mutation-specific therapeutic approaches. Recently, we have demonstrated in fibroblast cells that a modified U1 snRNA vector designed to improve the definition of exon 2 5'ss of the *HGSNAT* can restore splicing impaired by the mutation c.234+1G>A. Presently, our goal is to evaluate the therapeutic potential of that modified U1 snRNA *in vivo* by testing it in mice expressing the human splicing defect. For this purpose, full-length constructs were generated by cloning the wild-type (wt) or the mutated *HGSNAT* splicing-competent cassettes into the pcDNA 3.1 backbone. The subsequent transfection of wt and c.234+1G>A *HGSNAT* total cDNA constructs in COS-7 cells allowed us to confirm that they reproduce the splicing process observed in healthy control and patient cDNA's. Therefore, both plasmids are ready to be expressed in mice to test the therapeutic effect of the modified U1 snRNA. According to the successful protocol reported by other researchers, we will use those constructs to generate mice expressing the human c.234+1G>A mutation in the liver and test its modified U1-mediated rescue *in vivo* by analysing the presence of the normal transcripts/proteins after 48h. Currently, we have already started these experiments and we expect to succeed in the *in vivo* correction of the MPS IIIC c.234+1G>A splicing defect, proving the feasibility of this U1 snRNA-mediated strategy for the treatment of MPS IIIC patients.

Hydrodynamic injection in C57Bl/6J mice: lateral effects

P47

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Keywords: Mice C57Bl/6J, hydrodynamic injection, intravenous administration

The hydrodynamic injection is often used to achieve the rapid distribution of substances in the body. This technique has the advantage that the administered dose reaches a nearby systemic circulation quickly. This procedure is performed in laboratory animals in the lateral tail veins, and consists on the administration of a volume of 7 to 10% of the animals' body mass. The administration should be done in a maximum of seven seconds. The objective of this work was to analyze the adverse effects of the hydrodynamic model in C57Bl/6J male mice (authorized by ORBEA). For this, in an initial phase, we used 10 animals, with an average body mass of 25 g, to which we administered 2.5 ml of saline solution. Then, we performed the same procedure on 15 animals, with an average body mass of 25 g, to which we administered 1.7 ml. All injections were made by the same person and at the same period of the day. All 15 mice injected with 1.7 ml of saline survived and were sacrificed after 48 hours. Regarding the group administered with 2.5 ml of saline solution, 70% of the animals died. Before dying, the animals showed changes in breathing (dyspnea), in the color of the mucous and in the aspect of the eyes. During the necropsy, it was identified splenic (33.3%) and liver spots in 27% of the animals, and 7% of the mice presented splenic and hepatic spots simultaneously. Based on these results, we can conclude that the hydrodynamic injection technique gives origin to a high mortality rate when performed with the volume of 10% of the body mass, besides, it causes macroscopic changes in the organs. In the future, we intend to perform this technique in anesthetized animals.

Toxicological effects of pulegone and eugenol in laboratory animals: a pilot study

P48

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Keywords: essential oil; mice; toxicology

Pulegone is found in essential oils and is used to flavour food, beverages and dental products. Eugenol is found in plant extracts and is used in dentistry with analgesic and antiseptic properties. This work aims to evaluate the effect of these compounds in the variation of the body mass, food and water consumption in FVB/n mice. For that, thirty 15-week-old female mice were used, divided into six experimental groups: group 1 (control group); group 2 (599 mg/kg of pulegone+509 mg/kg of eugenol); group 3 (1197 mg/kg of pulegone+1017 mg/kg of eugenol); group 4 (1796 mg/kg of pulegone + 1526 mg/kg of eugenol); group 5 (1526 mg/kg of eugenol) and group 6 (1796 mg/kg of pulegone). The values of these compounds were calculated based on the intake of an adult man. After one week of acclimatization, the animals received the respective concentrations of the compounds incorporated in their diet, for two weeks. Body weight, average water and food consumption per animal were recorded weekly. An increase in body weight was observed in all groups over the weeks except for group 4, which showed an accentuated decline (24.08g±2.35 in the last week). There was a greater increase in mean body mass in the group 3 (28.93g±1.68). The average food consumption was lower in group 4 (2.75g) and higher in group 2 (6.88g) when compared to the control group (4.79g). Concerning the mean water consumption, group 4 had a higher consumption (8.92g) when compared to the control group (5.12g). Eugenol and pulegone seems to affect the animals' body weight as well as the food and water consumption, in the highest concentration.

Molecular docking: applications in drug discovery

P49

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Keywords: Bioinformatics, Molecular docking, Drug discovery, Pose; Score

Molecular ligand-protein docking is a computational method used to predict the interaction between a ligand and a protein surface, usually the active center. This prediction is calculated by obtaining the best binding conformation, usually called pose, between the ligand and the protein, and then scoring the binding conformation by calculating a predicted binding energy value, usually called score. Because the interaction mechanism between ligands and proteins is a dynamic system there is wide variety of structural parameters that influence the score result. There are many algorithms, each one with its own characteristics, advantages and disadvantages. The score values are estimates and are based on the degree of the protein-ligand interaction affinity, applying various assumptions and simplifications. Furthermore, to obtain score values there are multiple formulas that evaluate all the chemical interactions existing between the two molecules and determinate energy value corresponding to all interactions as a whole. Molecular docking is an established *in silico* structure-based method widely used in the drug discovery process. In this presentation we will discuss the usefulness of docking in the identification of novel compounds of therapeutic interest and in predicting ligand-protein interactions, at a molecular level, between potential drugs and the protein targets of interest.

Protrombine Time and Activated Partial Thromboplastin Time Reference Intervals in dogs

P50

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Keywords: PT, aPTT, Reference Intervals, Dog

Reference intervals (RIs) are basic tools used in medicine and laboratories to interpret patient laboratory test results, allowing healthy and unhealthy individuals differentiation. Once Ris can be influenced by internal factors like age, sex or breed as well as factors such as environment, lifestyle and time of the year or laboratory methods. Ideally, a laboratory should establish RIs specific for its method and local population. For this purpose, at Laboratório de Patologia Clínica do Hospital Veterinário da UTAD we perform our own RI study to establish RIs specific to protrombine time (PT) and activated partial thromboplastin time (aPTT) in our dog's population, respectively 6,7-10,8´´ and 9,0-14,8´´. Thus, in order to determine whether our results differ from those of the literature: PT (6,4 a 7,4´´) and aPTT (9 a 11´´), we compared both. According to the American Society for Veterinary Clinical Pathology reference interval guidelines, the acceptance criterion was as follows: after the elimination of outliers, less than 10% of the results fell outside the literature PT and aPTT established RIs. The results showed that for PT 79.3% and for aPTT 77.1% of our results are outside of those mentioned in the literature. Considering the criterion mentioned, literature intervals shouldn´t be accepted, demonstrating the importance of creating internal RIs. The new established RIs are now in use at Hospital Veterinário da UTAD.

Impact of ncRNA on human diseases related with autophagy dysregulation

P51

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Keywords: Non-coding RNAs (ncRNA), long non-coding RNA (lncRNA), autophagy, diseases, bioinformatics.

Non-coding RNAs (ncRNA) are RNA molecules that are not translated into proteins. Although some of these RNAs are well known for a long time, as ribosomal RNA (rRNA) or transfer RNA (tRNA), in the last years, mainly due to the ENCODE project, a huge number of ncRNA have been identified in the human transcriptome and in many other eukaryotic species. These elements have been shown perform extremely important functions in terms of structure and genome regulation. Long non-coding RNA (lncRNA) are a class of ncRNA relatively abundant in all cells and involved in numerous indispensable cellular mechanisms, such as autophagy regulation. Autophagy is an essential catabolic process that helps to keep cells in homeostasis through the breakdown of damaged or unwanted proteins and dysfunctional cytoplasmic organelles. This process either display a protective or harmful role in the cell, depending on their activation state and other cellular conditions. The relationship between lncRNAs and autophagy has been shown to be involved in the progression and possibly the prevention of many neurological, cardiac or liver diseases. In this presentation we intend to explore the ncRNA impact in some diseases related with autophagy dysregulation, knowing that the functionality of these ncRNA depends largely on the secondary and tertiary structures that these molecules adopt in a healthy and in a disease cellular context and how bioinformatics tools to predict these structures can be placed at the service of knowledge and health.

New potential regulators during xylem differentiation: Phosphatase and Tensin Homolog and Annexins

P52

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Keywords: *Arabidopsis thaliana,* phosphoinositides, vascular development, abiotic stress resistance, programmed cell death, PTEN, ANN

In higher plants such as *Arabidopsis thaliana*, xylem and phloem are functionally specialized conducting tissues which form the vascular system and allow the exchange among distantly-separated organs. In order to acquire their conductive function, xylem cells undergo a highly regulated differentiation program. This process, which results in the degradation of xylem protoplasts, includes secondary cell wall (SCW) deposition and programmed cell death (PCD). Previous work from the group led to the identification of a phosphatase family, the so-called PHOSPHATASE AND TENSIN HOMOLOG (PTEN), as key regulators of xylem formation. In the current project we focused on characterizing the role of PTEN2 in xylem differentiation. Firstly, transgenic plants overexpressing *PTEN2* were generated. We found that this overexpression alters SCW deposition and PCD, impairing xylem differentiation. Protein interaction analysis performed in the lab identified some members of the ANNEXIN (ANN) family as potential PTEN2 interactors. In particular, we assess the biological relevance of ANN1/2/3/4 and PTEN interaction in xylem differentiation. Our results suggest a possible interaction between ANN2 and PTEN3. Furthermore, confocal microscopy analysis of *ANN*2 expression patterns shows that this gene is expressed in the vascular cylinder, indicating a potential functional role in the vascular tissues. Since ANNs are known to play a role during abiotic stress resistance, drought stress resistance of *pten2pten3* and *ann2* knockout mutants has been examined. Interestingly, we found that both *pten2pten3* and *ann2* are more sensitive to drought stress, suggesting a possible role of these genes in abiotic stress responses. Overall, our data show that the overexpression of *PTEN*2 impairs xylem differentiation. Moreover, it may interact with ANN2 and thereby be necessary for the regulation of xylem development during abiotic stress.

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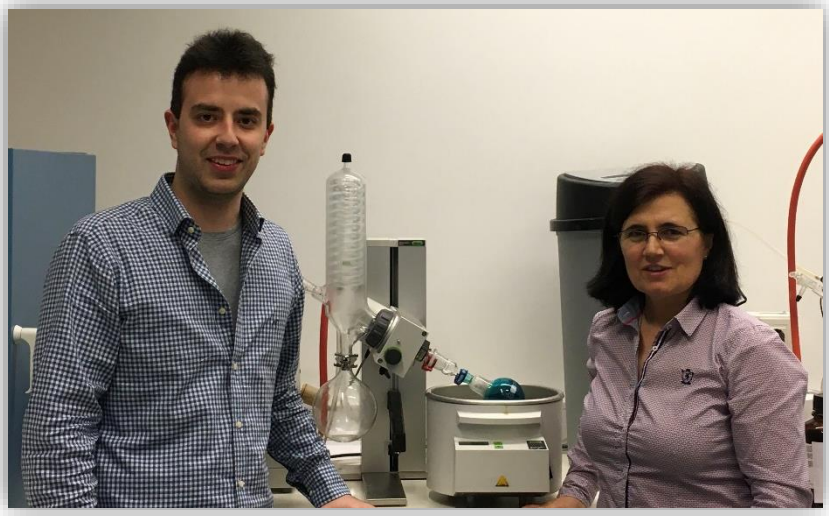
Workshops

How to extract, isolate and identify plant-based secondary metabolites with biotechnological and pharmacological relevance?

Eurico Lima, Lucinda Vaz Reis

Secondary metabolites are organic substances produced by organisms through the secondary metabolism pathways that have been widely studied concerning their pharmaceutical and biotechnological application. A practical example of these applications is the combination of paracetamol with caffeine, which resulted in a pharmaceutical formulation that, in the migraine treatment, results significantly better than the formulation without the plant metabolite. Besides, metabolites have been broadly studied in terms of hemisynthesis, since studies show that small structural changes in these natural origin compounds can significantly increase their potency and effectiveness in biological systems. Thus, the impact of natural compounds has gradually expanded in the various subjects of current science, in order to find ways to improve the well-being and health of society worldwide, as well as to solve economic and environmental problems. This workshop aims to make future geneticists and biotechnologists understand the importance of these metabolites, as well as establish the first contact with recurrent methodologies in the extraction of these organic compounds. Natural resources such as cinnamon, commonly used in all branches of cooking, black tea, typically used in the preparation of infusions, black pepper, a spice used as a condiment, and citrus fruits, will be the targets of study in this workshop. To this end, methods such as steam distillation, extraction using Soxhlet, and sublimation, reflux and crystallization techniques will be put to the test by participants in an attempt to extract metabolites with high relevance in biological systems such as caffeine, piperine, limonene and cinnamaldehyde. Finally, in a more theoretical approach, several techniques for the isolation (various types of chromatography) and characterization (e.g., mass spectrometry and nuclear magnetic resonance) of these metabolites will be presented.



Molecular Genetics – Genotype vs Phenotype

Manuela Matos, Marlene Santo, Ana Sofia Soares, Ana Cláudia Coelho

Recent advances in molecular biology allow the development and application of different tools and concepts of molecular genetics to diverse areas of knowledge. This workshop intends to provide an overview of molecular genetics applications in a wide variety of areas and with different objectives. A short presentation of the applications and several examples will be presented and used as basis for discussion and analysis in a phenotype *vs* genotype perspective. The participants will have the opportunity to observe and characterize, in the lab, different phenotypes of plants and fungi and discuss the importance of the molecular genetics and the genotypes analysis.

Introduction to Phyton and R programming for applications in Bioinformatics

Irene Oliveira, Eduardo Pires

Nowadays, a considerable amount of biological data is being generated in all sectors of society. As a result of this, there is a high demand for professionals with multidisciplinary knowledge and skills in the areas of biological data analysis as well as a high level of computer program expertise. Among the numerous existing informatics applications and software for analysing and interpreting data and Phyton stand out as the most cited in the literature and are the most suitable for those who want to create their own computer tools and data analysis apps for easy and quick integration into research. is a free software program in continuous evolution that allows performing advanced statistical procedures, data manipulation, and alternative graphics to those commonly obtained with other software, mainly with the use of *Bioconductor* (*Open Source Software for Bioinformatics- https://www.bioconductor.org/*) that currently manages 1823 libraries in the area of Bioinformatics. Phyton proved to be a fundamental tool for the analysis of this type of data, progressing at the same time as the analytical techniques, standing out in the last decade as the preferred language in the area. It is intended to present the potential of both software briefly, illustrate their use in some analyses of genetic and biological data, basic commands and packages. Participants must bring computer with an Internet connection and, if possible, R software (https://www.r-project.org/), RStudio (https://rstudio.com/) and Phyton (Anaconda: https : / / www.anaconda.com/) already installed. Some biological problems in R and Phyton and resolution, based on predefined scripts, will be presented to support the execution of programming procedures.

The history of silkworm production and new technologies

Jorge Azevedo

The sericulture was originated in the province of Shanxi - China, in the Neolithic period, as demonstrated by the discovery of silkworm cocoons dating from 2600-2300 BC. The Chinese domesticated silkworms, *Bombyx mor*i, L. - today considered as the animals most dependent on the care of humans, without which they will not survive. The history of sericulture in the Iberian Peninsula began with the Arab expansion of the 8th century, with a lack of authentic documents regarding the introduction of silkworm and the manufacture of silk fabrics in Portugal; and it practically ended in 1884/5, with the worldwide expansion of artificial fibres. The traditional techniques of silkworm breeding in Portugal include the definition of the places and equipment used in the insect facilities, as well as the care to be taken with the mulberry leaves used exclusively in the feeding of these insects. The new technologies include the rearing of breeds of silk production of better quality, as well as the production of mulberries more adapted to food, agriculture, energy production and medicine. The multiple uses of silkworms are briefly: (1) the larvae, given the high content of protein, amino acids, fatty acids and vitamins, are being used for food and pharmacology; (2) pupae are used in cooking and as food for birds and fish, and the oil residue that is extracted from it is used for cosmetics and the feeding of birds, pigs and fish; (3) butterflies, rich in bioactive ingredients, are used in the textile and pharmaceutical industries; (4) eggs are used in the pharmaceutical industry; (5) excreta are used, together with mulberry leaves, for animal feed; (6) silk, made up of two proteins - fibroin and sericin, is extracted from the cocoons and used in the textile industry, medicine, cosmetics and in the production of biomaterials - tissue engineering and regenerative medicine.

Clinical genomics of the periodontal disease

Carlos Viegas, João Requicha

In this workshop, starting from a brief theoretical introduction, we will go down to the operating room where we will accompany a patient's surgery with manifestations of the disease. We will take advantage of the surgical time to collect biological samples to be treated later in the laboratory. At the end, a round table will be held to discuss the role of clinical genomics in improving health service provision, namely in the definition of individualized prophylactic and therapeutic strategies.

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Thank you very much,

The Organizing Committee of the XII JGB / II JIGB

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