XIV Jornadas de Genética e Biotecnología

IV Jornadas Ibéricas de Genética y Biotecnología

De 31/03 a 02/04

Book of Abstracts

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utad UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO DOURO
universidad de león
ABLe ASOCIACIÓN DE BIOTECNÓLOGOS DE LEÓN

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XIV Genetics and Biotechnology Conference | IV Genetics and Biotechnology

XIV Genetics and Biotechnology Conference/ IV 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 XIV Genetics and Biotechnology Conference / IV Genetics and Biotechnology Iberian Conference (XIVJGB /IV JIGB).

The main objective of the XIV JGB /IV 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 XIV JGB /IV 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.

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María Fernández-Fernández
(Outreach member)

Marta Martínez López
(Treasurer)

PROGRAM

Thursday, 31st of March

WORKSHOPS (*In person – at UTAD*)

14:30 **Prof. Ana Escudeiro and Prof. Filomena Adega**

Looking for mutations – a bioinformatic approach to solve clinical cases

15:00 **Prof. Ana Sofia Soares, Prof. Manuela Matos and Prof. Ana Cláudia Coelho**

Mycology in Microbiology: from concept to practice

15:00 **Prof. Berta Gonçalves, Sandra Pereira (Ph.D.), Ivo Oliveira (Ph.D.), Cristina Morais (Ph.D.) and Prof. Eunice Bacelar**

Abiotic stress in fruit trees and metabolic responses: a practical approach

18:00 **Prof. Raquel Chaves, Prof. Paula Martins-Lopes and Prof. Filomena Adega**

Molecular diagnosis of SARS-CoV-2

Friday, 1st of April (*morning session*)

PROGRAM

PT	ES	
09:00	10:00	**OPENING SESSION**
09:30	10:30	Plenary Conference (*online*) **Nuno Azevedo, Professor (University of Porto)** *Spatial localization and treatment of microbial cells in multispecies biofilms*
10:30	11:30	**Coffee break + Musical Moment + Posters show (digital exposition)**
		PLANT GENETICS AND BIOTECHNOLOGY SESSION
11:00	12:00	Conference (*online*) **María Ángeles Pedreño, Professor (University of Murcia)** *Deciphering the key points of the production of bioactive compounds in plant cell cultures: resveratrol case study*
11:45	12:45	Conference (*online*) **Fernando Yuste-Lisbona, Professor (University of Almería)** *Genetic regulation of meristematic function to fine-tune crop productivity*
12:30	13:30	**ORAL COMMUNICATIONS – SESSION 1 (*In person and online*)**
12:30	13:30	**Barrias S.** - *Grapevine identification in "Abandonado" vineyard using SSR-Multiplex PCR, SNP genotyping and HRM assays*
12:40	13:40	**Monteiro E.** - *Use of biostimulants as elicitors of grapevine defense against fungal diseases*
12:50	13:50	**Santos M.** - *Use of different nutrients as mitigation strategy of fruit cracking: can their application affect the gene expression in sweet cherry?*
13:00	14:00	**Correia S. -** *How cytokinin BAP affects the micropropagation of Jasione?*
13:10	14:10	**González M. -** *Production of low-gluten wheat using genetic engineering*
13:20	14:20	Discussion of all oral communications (Session 1)
13:30	14:30	Lunch

Friday, 1st of April (*afternoon session*)

PROGRAM

PT	ES	
		ANIMAL AND MICROBIAL GENETICS AND BIOTECHNOLOGY SESSION
15:00	16:00	Conference *(online)* **Ana Usié, Ph.D. (CEBAL)** *Whole genome analyses as a tool for improvement and valorisation of local livestock breeds*
15:45	16:45	Conference *(in person)* **Gabriela Almeida, Professor (Egas Moniz Institute)** *Top-down proteomics to assess microbial responses to stress conditions: aims, challenges, and outcomes*
16:30	17:30	**ORAL COMMUNICATIONS – SESSION 2 (*In person and online*)**
16:30	17:30	**Guío J.** - *Casting new light on the regulatory mechanisms of nitrogen fixation in cyanobacteria: the Ferric Uptake Regulator FurC as a new player in the regulatory network of nitrogen metabolism and heterocyst differentiation in Anabaena PCC 7120*
16:40	17:40	**Guío J.** - *Unravelling the regulatory mechanisms of transcriptional regulators in cyanobacteria: modulation of the redox states of the Ferric Uptake Regulator FurA from Anabaena sp. PCC 7120 by thioredoxin A*
16:50	17:50	**Jorge N.** - *Combination of an aerobic bioreactor with photo-Fenton process in winery wastewater treatment*
17:00	18:00	**Sousa J.** - Metschnikowia *spp.: An emergent biocontrol agent against grape fungi contaminants*
17:10	18:10	**Almeida G. and Machado E.** - *Assessment of yeast susceptibility to common fungicides used for managing powdery and downy mildew in vineyards*
17:20	18:20	Discussion of all oral communications (Session 2)
17:30	18:30	**Coffee break + Musical Moment + Posters show (digital exposition)**
18:00	19:00	**ORAL PRESENTATIONS OF SELECTED POSTERS (*In person and online*)**

Saturday, 2nd of April

PROGRAM

PT	ES	
		HUMAN GENETICS AND BIOTECHNOLOGY SESSION
09:00	10:00	Conference (*online*) **Cláudio M. Gomes, Professor (University of Lisbon)** *How does the human brain regulate protein aggregation in Alzheimer's disease?*
09:45	10:45	Conference (*online*) **Susana Ramos, Ph.D. (Gulbenkian Science Institute)** *Targeting parasite sugars in antimalarial vaccination*
10:30	11:30	**ORAL COMMUNICATIONS – SESSION 3**
10:30	11:30	**Guedes A.R.** - *Assessment of DNA damage in peripheral blood lymphocytes induced by oncologic treatments in patients with breast and colorectal cancer*
10:40	11:40	**Morais P.** - *Importance of familial studies involving balanced translocations*
10:50	11:50	**Sousa V.** - *Comet assay optimization for further application in cumulus cells*
11:00	12:00	**Gomes M.** - In vitro *assessment of the biocompatibility of clinically-available hemostatic agents*
11:10	12:10	**Lopes J.** - *A comparative study of SARS-CoV-2 molecular detection in nasopharyngeal swab* versus *saliva samples*
11:20	12:20	Discussion of all oral communications (Session 3)
11:30	12:30	**Coffee break + Posters show (digital exposition)**

Saturday, 2nd of April

PROGRAM

PT	ES	
12:00	13:00	***ROUND TABLE WITH FORMER STUDENTS (In person and online)***

Exclusive event for former and current students of the 1st, 2nd and 3rd cycles of study in the field of Genetics and Biotechnology

Alumni:

Bárbara Santos

Elisa Silva Ferrada

João Gonçalves

Juan José Vidal Nuñez

Luis Getino Alonso

Patrícia de la Madrid Salmerón

Rafaela Oliveira

Márcia Carvalho

		AWARD CEREMONY AND CLOSING SESSION (In person and online)
12:30	13:30	*Award Ceremony*
13:00	14:00	*Closing Session*

SPEAKERS

Nuno Azevedo, Professor

Nuno Filipe Azevedo graduated in Biological Engineering in 2001 at the University of Minho (UMinho), Portugal and then finished a sandwich PhD on Chemical and Microbial Technology in 2005 at the UMinho and the University of Southampton, UK (USou). After holding a Post-doctoral position at the same two institutions for 4 years, he started a Faculty Research Fellow position at the Laboratory for Process Engineering, Environment, Biotechnology and Energy (LEPABE) at the Faculty of Engineering of the University of Porto (FEUP). He is currently an Assistant Professor at FEUP. During his research career he has authored or co-authored more than 100 papers in peer-reviewed international journals (h-index 32), submitted 7 patents and co-edited two books. He has been invited to more than 20 oral presentations in national and international conferences and is regularly part of the scientific advisory committee of several international conferences. Nuno Azevedo is currently supervising or co-supervising several PhD students and postdoctoral researchers and teaching undergrad students of the Bioengineering, Chemical and Environmental Engineering Courses at FEUP.

Nuno Azevedo main research interests are to explore the potential of nucleic acid mimics for the rapid diagnosis and treatment of infectious agents, as well as multispecies biofilms. He was the leader of the EU-funded project DelNAM, a project aimed to develop a novel therapeutic approach to solve bacterial resistance to antibiotics through the delivery of antibacterial nucleic acid mimics into bacterial biofilms and cells within the human body. He was also participating as team leader in other European projects, such as “PRINT-AID-European training network for development of personalized anti-infective medical devices combining printing technologies and antimicrobial functionality” and “NanoDiaBac - Nanofluidics for ultrafast diagnosis of bacterial infections”, and is the PI or co-PI of several other national projects in these same areas. He was also a co-founder of the biotech company Biomode SA, that was granted >1.5M€ in investment funds.

María Ángeles Pedreño, Professor

Degree in Chemistry (1985) and Doctor of Sciences, Chemistry Section (1988). To complete my biotechnological training, I did a postdoctoral stay (1989-90) in the laboratory of Prof. Jean-Claude Pech, in the Plant Biotechnology Department of the Toulouse Agricultural School. In 1993, I obtained the position of Lecturer in Plant Physiology in the Department of Plant Biology at the University of Murcia and since 2006 I am Full Professor of Plant Physiology at the same University. I currently lead a research group at the University of Murcia: Biofactory Plants: Production of bioactive compounds and proteins related to pathogenesis (https://curie.um.es/curie/catalogo-ficha.du?seof_codigo=1&perf_codigo=10&cods=E005*01), focused on obtaining specialized metabolites and proteins related to plant defense. I am a numerary Academic and Treasurer of the Academy of Sciences of the Region of Murcia. I have received several awards: European Award for Young Researchers in Plant Physiology by the European Federation of Plant Biology in 1994, first Award for Applied Research in a Private Company by the European Research Centre in 2009, Award for the Lavoisier Foundation (2013, Research applied to medicine) and by the University of Murcia (2017, Award for the transfer of knowledge to private companies). I actively participate in scientific committees of the Plant Physiology Societies (http://www.sefv.es/), In Vitro Plant Tissue Culture, http://secivtv.org/) and the European Federation of Plant Biology Societies (https://www.fespb.org/), and I have organized National and International meetings related to Plant Physiology and Biotechnology. I have made more than a hundred scientific publications and led numerous national and regional projects, as well as research contracts with companies. The most current lines of research are focused on the production and characterization of bioactive compounds and proteins derived from plant cell cultures under elicitation, and on the mechanism of elicitation using genomic, transcriptomic, metabolomic and proteomic approaches. In recent years we have generated a new line of research based on the use of plant by-products, analysing compounds with bioactive potential obtained from extracts of plant residues, using different methodologies, for use in the cosmetic and food industries, as well as in the agriculture.

Fernando Yuste-Lisbona, Professor

Associate Professor of Genetics at the University of Almeria. I did my Ph.D. work on the development and mapping of PCR-based markers linked to disease-resistance genes in melon at the Plant Breeding Department of the Experimental Station "La Mayora"-CSIC, Málaga, Spain (2004-2009). During this period, in addition to pathological diagnostic and evaluation techniques, I acquired skills in genetic analysis, as well as in the development of linkage maps and quantitative trait locus (QTL) analysis. On 2010, I joined the Genetics and Physiology of Plant Development group at the University of Almeria as a PhD researcher, and I started to work in different projects aimed to get insights into the genetic and molecular mechanisms controlling reproductive development and tolerance to abiotic stress, using tomato as a model species. During my postdoctoral training, I completed stays in several research centers, among them the Max-Planck Institute for Plant Breeding in Cologne, Germany (2015-2016), where I acquired knowledge and skills in bioinformatics to carry out the analysis of multiple types of high-throughput sequencing data. Currently, I am working in two main research lines: i) the development of genomic tools in common bean, in order to identify QTLs and markers tightly linked to different agronomic traits; ii) the identification of genetic factors participating in the regulation of tomato reproductive development, as well as in the response of this crop species to different abiotic stresses, through genetic, molecular and functional approaches.

Ana Usié, Ph.D.

Ana Usié has a bachelor's degree in Technical Engineering in Computer Management (2007), an equivalent Master's degree in Senior Computer Engineering (2010) and a Master's degree in Free Software Engineering (2010), from University of Lleida (Catalonia, Spain). She then obtained her PhD degree in Bioinformatics from the Doctorate program in Molecular Health at the same university in 2014. Two month later, she moved to Portugal and continued her career at Centro de Biotecnologia Agrícola e Agro-alimentar do Alentejo (CEBAL) where she joined the Animal Genomics and Bioinformatics group. Her research work focuses on the analysis and processing of sequencing data obtained with the latest generation of NGS technologies. She has been involved in several projects working with plant and animal species, which included *de novo* assembly of genomes and transcriptomes, variants identification such as SNPs, SVs and CNVs, metagenomics and differential expression analysis, among others. During her career she had the opportunity to train and mentor new group members as well as internship students, and co-supervised bachelor' and master's theses. She is now co-supervising a PhD student. Since 2011, she has authored 19 papers in international peer review publications (7 as 1rst author), with an h-index of 7 and over 200 citations. In the last 5 years, she authored 11 papers in international peer review publications, 3 proceedings papers, 2 oral communications and 14 posters in international and national scientific meetings.

Gabriela Almeida, Professor

M. Gabriela Almeida received her undergraduate education in Chemistry at Universidade de Lisboa and a MSc. degree in Biotechnology from Universidade Técnica de Lisboa. She completed her Doctoral studies in 2003, at Universidade Nova de Lisboa.
Gabriela Almeida is Associated Professor at Instituto Universitário Egas Moniz, Researcher at CiiEM and UCIBIO where she is the head of the GB2 - Group of Biomarkers and Biosensors (http://gb29.webnode.pt/). She has 25 years of experience in higher education at several academic institutions, including Universidade do Algarve, Universidade Nova de Lisboa and Instituto Universitário Egas Moniz. From 2008 to 2012, Dr. Almeida was a Senior Research Fellow at the Chemistry Dept., Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, under the scope of Programa Ciência2007.

Her main research interests are focused on the development of point-of-use tests based on electrochemical and optical biosensors for IVD and water monitoring, and on several proteomics projects aiming at characterizing the response of microbial organisms to stress conditions. Outside the biosensor and proteomic fields, Dr. Almeida has been involved in the biochemical and structural characterization of metalloenzymes from bacterial sources. Dr. Almeida has published over 40 peer-reviewed scientific papers (h-index 22), 1 book, 3 book chapters, and 2 patents. She led 9 research projects and participated as a team member in 12 other funded projects. In addition, she has been involved in the scientific committee of Conferences, the coordination of Advanced Short Courses and Workshops, science outreach, among other activities. In recent years she has been actively engaged in technology transfer, either as a consultant, evaluator (H2020, Horizon Europe), or entrepreneur (CEO and founder of NS2: https://www.nitrogensensing.com). In 2021, she was a finalist in the Everis Awards, a program boosting innovation and entrepreneurship.

Cláudio M. Gomes, Professor

Cláudio M. Gomes, é especialista em bioquímica estrutural e tem como interesses de investigação o estudo de proteínas e dos mecanismos moleculares associados a defeitos no folding proteico e à formação de agregados, como os causadores da doença de Alzheimer. Nesta conferência vai enquadrar o estado da arte relativamente à génese de agregados tóxicos das proteínas beta-amilóide e Tau no cérebro humano no contexto da doença de Alzheimer, discutir avanços recentes do seu trabalho de investigação e desafios futuros. É autor de +125 artigos científicos com +4000 citações, tendo recebido menções honrosas nas edições 2021 e 2019 dos Prémios Universidade de Lisboa/CGD. É Professor Associado com Agregação do Departamento de Química e Bioquímica na Faculdade de Ciências da ULisboa, e coordenador do laboratório Protein Misfolding and Amyloids in Biomedicine, afiliado ao BioISI – Biosystems & Integrative Sciences Institute.

Cláudio M. Gomes, is an expert in structural biochemistry and his research is focused on the study of proteins and the molecular mechanisms associated with defects in protein folding and the formation of aggregates, such as the causes of Alzheimer's disease. In this conference, he will frame the state of the art regarding the genesis of toxic aggregates of beta-amyloid and Tau proteins in the human brain in the context of Alzheimer's disease, discuss recent advances in his research work and future challenges. He is the author of +125 scientific articles with +4000 citations, having received honorable mentions in the 2021 and 2019 editions of the Universidade de Lisboa/CGD Awards. He is Associate Professor with Aggregation at the Department of Chemistry and Biochemistry at Faculty Sciences University Lisboa, and coordinator of the Protein Misfolding and Amyloids in Biomedicine laboratory, affiliated to BioISI – Biosystems & Integrative Sciences Institute.

Susana Ramos, Ph.D.

I graduated in Microbial Biology and Genetics (Science Faculty, Lisbon University, Portugal). Since my PhD defense in Parasitology (NOVA University, Lisbon, Portugal; 2009), I accumulated 12 years of research in parasitology, in particular malaria, the disease caused by Plasmodium, anti-α-gal immunity, metabolic adaptation to infection and disease tolerance to malaria. During my PhD, at Instituto de Higiene e Medicina Tropical (IHMT, with Prof. Silveira) and the Institut de Biologie Moleculaire et Cellulaire (Strasbourg, France, with Dr. Reichaart), I focused on vector-parasite interactions, and discovered that mosquito metabolism affects *Plasmodium* sporozoite development (DOI: 10.1186/1475-2875-6-84, 10.1016/j.ibmb.2012.07.003, 10.1590/0074-0276140098). In my postdoc, since 2011, in Dr. Soares group at Instituto Gulbenkian de Ciência (IGC), I focused on host-parasite interactions. I discovered how renal heme/iron metabolism controls disease tolerance to malaria (10.1016/j.celrep.2014.05.054, 10.1073/pnas.1822024116, 10.1146/annurev-immunol-042718-041739), and malaria-associated pathogenesis, as hypoglycemia (10.1101/2021.09.08.459402) and anemia (10.1101/2022.01.12.475857). In 2017 I joined a Dr. Soares research project funded by the Bill & Melinda Gates Foundation, as the leading team member, to validate Galα1-3Galα1-4GlcNAc (α-gal) glycans as antigenic targets for the development of antimalarial vaccines, based on our previous findings that immunization against synthetic α-gal confers robust protection against *Plasmodium* infection in mice (10.1016/j.cell.2014.10.053). I have always admired how *Plasmodium* adapts to its host and vector and how the understanding of host/parasite interactions shoulders the main goal of malaria eradication. While host-parasite interactions can be exploited to prevent infection (e.g. by vaccination) and/or reduce malaria severity (e.g. by the establishment of disease tolerance), vector-parasite interactions can be targeted to block transmission (e.g. by vaccination). I believe that targeting multiple stages of *Plasmodium* development simultaneously, as likely achieved by targeting α-gal, is an innovative approach to develop antimalarial vaccines with enhanced efficacy which will provide major advances to towards the goal of malaria eradication. This is currently my main research focus, which I am excited to pursue.

CONFERENCES

Spatial localization and treatment of microbial cells in multispecies biofilms

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Keywords: fluorescence *in situ* hybridization, nucleic acid mimics, microbial detection, antimicrobial resistance, biofilms

Bacterial cells living at interfaces usually have neighbours of many different species and are able to organize themselves into complex structures named biofilms. A more effective management of these structures requires both an in-depth characterization of the spatial location of the individual cells and the use of molecules that are able to control their growth. In here, I will discuss the application of nucleic acid mimics (NAMs), also known as nucleic acid analogues, to study and manage these multispecies biofilms. More specifically, I will provide examples of works that are currently being carried out within our research team and that include: 1) coupling NAMs and fluorescence *in situ* hybridization for a more robust detection of pathogens within biofilms; 2) combination of NAMs and delivery vectors such as liposomes and cell-penetrating peptides for a more efficient treatment of individual microbial cells within biofilms.

Acknowledgments: This work was financially supported by LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC).

Deciphering the key points of the production of bioactive compounds in plant cell cultures: resveratrol case study

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Keywords: Cyclodextrins, cell cultures, elicitors, magnetic nanoparticles, resveratrol

Specialized metabolites have an important role in the survival of plants, and have caught the attention of the general people, since most of them have biological activity with beneficial effects on human health, so that they are considered bioactive compounds. Plant cell cultures have been developed as a biotechnological strategy to produce bioactive compounds that are difficult to obtain or when their extraction represent a serious damage to the environment. Thus, to enhance the production of these specialized compounds highlight the use of elicitors alone or combined with magnetic nanoparticles, and plant metabolic engineering following elicitation. We will show how we have moved forward and made new discoveries based on the use of these tools and the potential that plant cells have. One of the most relevant examples is based on the use of cyclodextrins (CD), separately or in combination, with methyl jasmonate (MJ) as elicitors, which has been proven to be a very effective procedure in stimulating *trans*-resveratrol (*t*-R) production in plant cell cultures. However, the use of CD increases production costs, making its industrial exploitation economically unviable. Therefore, the development of CD recovery strategies is needed to provide a solution to their industrial use. Indeed, CD-coated magnetic nanoparticles (CDMN) together with MJ were able to increase *t*-R production in cell cultures, and CDMN were reused during three cycles of continuous elicitation, since the induction and adsorption capacity of *t*-R remained high. On the other hand, knowing the effect of the joint action between CD and MJ on inducing maximum levels of stilbene synthase (STS) expression, we designed an Agrobacterium transformation strategy by which transgenic cell lines overexpressing STS were obtained and evaluated. Some of them were able to provoke a highly enhanced production of *t*-R in the presence of CD and MJ, in comparison with the non-transgenic cell lines elicited.

Acknowledgments: *This research was part of the project PID2020-113438RB-I00 financed by the Spanish Ministry of Science and Innovation (MCIN/AEI/10.13039/501100011033 "Una manera de hacer Europa").*

Genetic regulation of meristematic function to fine-tune crop productivity

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Keywords: CRISPR/Cas, crop productivity, genome editing, meristems, quantitative trait variation, tomato

Unlike animals, where organs are formed during embryogenesis, plants develop new organs continually throughout their lifetime, an ability that relies on specialized groups of cells called meristems, whose function is to integrate a diversity of signals for coordinating stem cell self-renewal and differentiation into organ founder cells. Herein, it is shown how crop productivity can be improved and fine-tuned by exploiting combinations of selected mutations affecting meristematic function. Using tomato (*Solanum lycopersicum* L.) as a model species, it is initially described how combinations of selected mutations in multiple components of the florigen flowering pathway led to customize plant architecture and flower production. Then, it is shown the role of dosage balance among genes controlling inflorescence meristem maturation, as well as the use of genome editing technology with the aim to generate a quantitative range of inflorescence architectures thus allowing to enhance crop production. Finally, the activity of floral meristem is addressed, whose size defines the number of carpels formed in a flower, and hence affecting both fruit size and shape. Collectively, this talk seeks to address how understanding genetic networks involved in controlling meristem function will provide the best scenario for the development of knowledge-driven breeding strategies based on the use of biotechnology tools to enhance genetic variability in terms of productivity and quality related traits.

Acknowledgments: *This work was supported by the PID2019-110833RB-C31 grant from the Spanish Ministry of Science and Innovation (MICI/AEI/FEDER, UE) and the BRESOV (Breeding for Resilient, Efficient and Sustainable Organic Vegetable production) project funding by the Research and Innovation Programme of the European Union Horizon 2020 (grant agreement No. 774244).*

Whole genome analyses as a tool for improvement and valorisation of local livestock breeds

Usié A. [1,2,*], **Leão C.** [1,2,3‡], **Mendes B.** [1], **Gaspar D.** [1,4], **Hernandez F.** [5], **García-Gudiño J.** [5], **Izquierdo M.** [5], **Ramos A.M.** [1,2]

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Keywords: Whole genome sequencing, genetic variability, valorisation of local livestock breeds

Breed diversity has been shaped by a long history of domestication and selection contributing significantly to the genetic improvement of economically important traits. In the last decade, the rapid advances of high-throughput sequencing technologies have provided new and exciting opportunities to develop powerful strategies for a better understanding of the genomic basis underlying livestock phenotypical characteristics. The present availability of high-throughput sequencing together with the completion of a high number of sequenced animal genomes has enhanced the detection of genomic variants that can be used for the improvement and valorisation of the local livestock breeds genetic resources. With sequencing methods such as whole genome sequencing now is possible to target advanced research, allowing the identification of thousands single nucleotide polymorphisms (SNPs), among other genetic variants, which can be then associated with important traits. In Portugal, Alentejano is one of the most important pig breeds not only for its significant role in biodiversity and landscape conservation, but also for the socio-economic development of undeveloped and developing regions. The Alentejano pig belongs to Iberian type breeds that can be found in the “Montado/Dehesa”, a well-defined agro-sylvopastoral system habitat in the Mediterranean ecosystem. The Iberian meat and dry-cured products have a protected designation of origin (PDO) certification due to their exceptional sensorial and nutritional characteristics reaching high prices in the market. In this study, whole genome sequencing was applied to characterize the genomic architecture of several Portuguese and Spanish Iberian pig breeds in order to identify SNPs for traceability schemes and genomic population studies. Additionally, Alentejano pig individuals were analysed in order to identify SNPs associated with contrasting meat quality characteristics.

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Top-down proteomics to assess microbial responses to stress conditions: aims, challenges, and outcomes

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Keywords: Proteomics, proteoforms, microbial stress, 2D-electrophoresis, mass spectrometry

The advent of whole-genome sequencing was a breakthrough in biological sciences, giving birth to the genomic era. Afterward, the concept of characterizing a full class of biological molecules that make up a cell, tissue, or organism was rapidly extended from genes to mRNA (transcriptomics), protein (proteomics), and metabolites (metabolomics), enabling the integrative view of systems biology. Whilst genome data is static, the other "omics" approaches are dynamic, providing temporal (e.g., ms, month), spatial (e.g., cell, organelle, fluid), and developmental information. In this context, proteomics is particularly important to decipher the "whole picture" of life. By looking at the final genes products, the proteome provides information on protein modifications (proteoforms) due to alternative splicing events and/or post-translational modifications, and changes in protein abundance as a function of time, location. Therefore, there are numerous applications of proteomics to understanding cellular functions and disease-associated states at the molecular level, and how organisms respond to a given stimulus and adapt to new environmental conditions. As opposed to bottom-up proteomics techniques, top-down proteomics analyzes intact proteins without digestion, which has proved to be the foremost technology for the comprehensive analysis of proteoforms. Despite a few persisting technical challenges, the combination of 2D electrophoresis (2DE) with peptide mass finger for protein identification still offers a simple and appellative approach to discriminate complex protein mixtures. Herein, several 2DE-based proteomic studies on the molecular response of diverse microbial organisms to different condition growths, such as the adaption of *Desulfovibrio desulfuricans* to different respiratory nutrients, activation of secondary metabolism in marine actinomycetes, and secretion of antimicrobial peptides by *Saccharomyces cerevisiae* during alcoholic fermentations will be presented, thus illustrating the potential of top-down proteomics in biomarkers discovery.

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How does the human brain regulate protein aggregation in Alzheimer's disease?

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Keywords: Molecular chaperones, Amyloids, Neurodegeneration, Protein interactions, Structural Biochemistry, Biophysics, Fluorescence microscopy

Proteins are extraordinary molecules whose functions in cells are in most cases tied to a well-defined three-dimensional structure. However, despite tight biological regulation, in some conditions, proteins misfold and aggregate.
Protein aggregation into insoluble deposits is a hallmark feature of several human pathologies, including several age-related neurodegenerative dementias.
Alzheimer's Disease is characterized by the formation of insoluble deposits made by the amyloid-β and tau proteins which are deleterious for the cells in the brain causing neurodegeneration and dementia. The formation of these pathological aggregates in the brain starts many years before the first signs of dementia, steadily increasing and leading to the formation of insoluble protein deposits called plaques and neurofibrillary tangles. In this process, the early forms of aggregates called oligomers are soluble and are associated to pathological cell to cell spreading phenomena.
Understanding the basic biochemical mechanisms underlying the aggregation of these proteins and its biological regulation in the diseased brain remains largely unknown and delays the development of effective therapies. What makes a protein misfold and form insoluble toxic aggregates? Why do some proteins aggregate in specific regions of the brain and not in others? How do nervous cells regulate the formation of toxic protein aggregates?
Departing from these questions, in this presentation I will overview our recent finding on the biochemistry of amyloid-β and Tau aggregation in Alzheimer's Disease combining structural, biochemical and cellular approaches, and which uncovered brain proteins with novel chaperone functions capable to delay protein aggregation, decrease toxicity and prevent propagation of toxicity between cells (Moreira et al 2021 Nature Communications 12, 6292; Cristóvão et al 2018. Science Advances 4, eaaq1702).

Targeting parasite sugars in antimalarial vaccination

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Keywords: Malaria, Vaccine, α-gal, monoclonal antibodies, multistage targeting

Malaria, the disease caused by *Plasmodium spp.* infection, remains a leading cause of morbidity and mortality worldwide. Antimalarial vaccines have been developed to target different stages of *Plasmodium* life cycle, including pre-erythrocytic vaccines transmission blocking vaccines. However, protection afforded by available vaccines remains modest and short-lived. For example, RTS,S/AS01 blocks the initial liver stage of infection via immune-targeting of *P. falciparum* circumsporozoite protein (*Pf*CSP), with an efficacy of about 30%, far below the 80-90% estimated threshold required for malaria eradication. Vaccines targeting the *P. falciparum* transmission stages, such as the ookinete surface protein *Pf*s25, also afford incomplete and short-lived transmission-blocking activity. Alternative strategies are required to enhance the efficacy of existing vaccines or to identify antigenic targets that could induce potent antimalarial activity.

One strategy recently boosted involves the isolation of potent neutralizing monoclonal antibodies (mAb) from malaria patients, which has successfully unveiled key mechanisms underlying B cell responses in naturally acquired or vaccine-induced antimalarial immunity and led to the generation bioproducts with high prophylactic antimalarial efficacy. This is best illustrated for anti-*Pf*CSP mAb, such as the CIS43 mAb, that shows a remarkable *P. falciparum* sporozoite neutralizing activity and has recently progressed towards clinical application. We have been successful in the identification of novel antigenic targets to be incorporated in antimalarial vaccines. Our published and ongoing work suggests that targeting Galα1-3Galβ1-4GlcNAc (α-gal) glycans provides a unique strategy towards the development of a multistage vaccine to block *Plasmodium* infection and transmission, simultaneously. If successful, the combination of these strategies would significantly contribute towards the main goal of achieving sterile protection against *Plasmodium* infection and transmission, and thus, towards malaria eradication.

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ORAL COMMUNICATIONS

O1

Grapevine identification in "Abandonado" vineyard using SSR-Multiplex PCR, SNP genotyping and HRM assays

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Keywords: Grapevine identification, SSR markers, SNP genotyping, HRM

Accurate identification of older grapevine varieties can be difficult considering the existence of many synonyms, homonyms, and misidentifications. In this work, SSR-Multiplex PCR and SNP genotyping were performed in combination with HRM assays, to determine the identity of nineteen grapevines varieties present in "Abandonado", an old portuguese vineyard at Quinta da Gaivosa. The combination of the three approaches allowed the identification of eighteen out of the nineteen grapevines from the vineyard, where ten grapevines were correctly identified by ampelography and the remaining eight were misidentified. SSR and SNP data retrieved for one sample did not match any profile present in the VIVC database, but pedigree analyses suggested it may be a new variety. We obtained new information regarding three SSR *loci* for one sample identified as Cerceal Branco, that interestingly presented a red colour berry which was not expected. One other sample identified as Malvasia Preta/Preta Roxa also presented a berry colour non-coincident with its molecular identification. Both these samples might represent new somatic variants of the identified varieties by SSR/SNP profiles, but further studies are required to confirm these situations. Three HRM assays were performed to validate SSR/SNP analysis, using available reference material, representing a useful tool for varietal identification through melting curve profile comparison.

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O2

Use of biostimulants as elicitors of grapevine defense against fungal diseases

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Keywords: Biostimulants, Defense genes, Plant fungal diseases, qRT-PCR, *Vitis vinifera* L.

Climate change will impact on the severity and incidence of many fungi and fungi-like pathogens in grapevine, such as downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator*) and gray mold (*Botrytis cinerea*). These infections lead to important yield loss and tampering of wine organoleptic properties. The continuous use of synthetic chemical pesticides, can induce the development of resistance and/or as in those copper based, the accumulation of this heavy metal in vineyard topsoil. The European Commission, producers and consumers demand for more environment-friendly products. In this context, it is of utmost importance the research in innovative bio-strategies for plant protection, as the use of biostimulants elicitors of plant-defense responses.

The aim of this study was to verify the effect of plant-based biostimulants foliar applications on grapevine, by analyzing the expression of the defense genes *Gluc* and *PR17*. In a trial installed at UTAD campus, with Touriga Franca cultivar, foliar applications were made throughout 2019 and 2020 growing seasons, with nettle extract, Japanese knotweed extract, conventional fungicide, and water (control). The genes expression was evaluated in leaves sampled at veraison and maturation, in both years.

This analysis revealed that in both years, nettle and Japanese knotweed extracts elicited the upregulation of the genes *Gluc* and *PR17*, suggesting the activation of plant defense mechanisms against pathogens. These are promising results towards environment-friendly grapevine fungal diseases control.

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O3

Use of different nutrients as mitigation strategy of fruit cracking: can their application affect the gene expression in sweet cherry?

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Keywords: crop nutrition, fruit cracking, gene expression, mitigation strategies, sweet cherry

Several flesh fruits are highly affected by cracking, a severe physiological disorder that compromises their quality and causes high economic losses to the producers. Different mitigation strategies have been developed trying to reduce this disorder and also to improve fruit quality and yield of orchards. Among them, the management of crop nutrition is one of the most important. Thus, magnesium (125 g/hL and 250 g/hL), potassium (50 g/hL and 100 g/hL), calcium (150 g/hL and 300 g/hL) and seaweed, (*Ascophyllum nodosum*) based-biostimulant (75 mL/hL and 150 mL/hL) were applied at foliar level in sweet cherry trees (Cv. Burlat) located in Resende region, with the aim to increase the resistance to cherry cracking. So, to better understand how the applied compounds affect the molecular mechanisms involved in cherry cracking, the expression of some cuticle related genes was studied, namely genes related to cell wall mechanisms, such as *PaExp1*, *PaExp2*, *PaXTH*, *Paβ-Gal*, *PaEG*, *PaCYP78A9* and *PaADPG1*, and genes involved in biosynthesis and transport of cuticular waxes, such as *PaCER1*, *PaWS*, *PaKCS6*, *PaKCR1* and *PaLTPG1*. For this, total RNA was extracted from fruit exocarp and cDNA was synthetized, using fruits from all treatments at maturity stage to study the gene expression by qPCR. The results showed statistical differences among treatments for all genes. In general, higher gene expression was observed in cherries treated with calcium and seaweed based-biostimulant, suggesting that these nutrients can have an important role in preventing cracking. However, to get more accurate insights, these results should be complemented with measurements of fruits at different phenological growth stages and fruits with cracks.

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O4

How cytokinin BAP affects the micropropagation of Jasione?

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Keywords: *Jasione*, cytokinin, shoots

Jasione is a genus of short-lived, annual, biennial or perennial flowering plants native to Europe and the Middle East, belonging to the family *Campanulaceae* and order *Asterales*. *Jasione* has small blue spherical flowers gathered in the inflorescence that bloom between April and September. Cytokinin 6-benzylaminopurine (BAP) has been very effective in promoting multiplication in several species and seems to be the ideal cytokinin for multiplication of aerial parts and induction of adventitious buds. It stimulates cell division, especially in association with an auxin; in appropriate concentrations it induces the formation of adventitious shoots.

This work aimed to observe the effects of plant growth regulators, namely an auxin (NAA) and a cytokinin (BAP), on the *in vitro* growth of Jasione and its repercussions on micropropagation processes. The plant material used was already *in vitro* culture, in Murashige and Skoog (MS) medium, so disinfection was not necessary. Five different culture media were prepared: MS; MS+1 mg/L BAP; MS+2 mg/L BAP; MS+ 0,2mg/ml NAA+1 mg/L BAP; MS+ 0,2mg/ml NAA +2 mg/L BAP. The plants were kept in a photoperiod of 16 hours and a temperature of 24ºC.

Over 6 weeks, various parameters of 125 explants were evaluated, such as the number and length of shoots. The most efficient medium for shoot formation and shoot elongation was MS medium supplemented with 1mg/L of BAP and 0.2mg/L of NAA. Growth regulators have provided plant growth and development, as well as the possibility of seeking new uses or applications, and obtaining greater gains in the cultivation of different agricultural species.

O5

Production of low-gluten wheat using genetic engineering

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Keywords: gliadin, RNAi, CRISPR/Cas9, RNA silencing, celiac disease

Gluten includes a type of storage proteins located in many cereal seeds, mainly in wheat grain (*Triticum aestivum)*. In this species, gluten is composed by two kinds of proteins: gliadins and glutenins. They are responsible for some of the properties of wheat flour, including the excellent texture of its dough, which is commonly used in food industry. However, gluten causes several allergies, intestinal pathologies and autoimmune disorders, the celiac disease among them. The main treatment for patients with this latter is a gluten-free diet.

Plant Biotechnology offers an alternative solution for celiac: the development of wheat lines with low gliadin content, the most immunogenic gluten protein. The two techniques that have allowed to achieve this goal involve the silencing of gliadin genes, and they are RNA interference (RNAi) and gene editing with CRISPR/Cas9. The benefits and disadvantages of both are evaluated in this work through a bibliographical review. Transformation of wheat with RNAi caused a down-regulated expression of gliadin genes, and grains from transgenic lines contained up to 97% less gliadin than those of control lines. One of these lines, named E82, has even succeed in clinical trials with celiac disease patients, who can consume 67 g per day of bread made with flour obtained from their grains. In spite of these promising results, transgenic crops cannot be commercialized in Europe. On the other hand, CRISPR/Cas9 mutation could solve this problem since it does not introduce exogen DNA into the plant. This technique edits *T. aestivum* genome directly, preventing the expression of gliadin genes. Although gene editing has been recently developed, it has provided good results in the production of poorly immunogenic wheat. Further investigations in this area will bring this product closer to European consumers.

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O6

Casting new light on the regulatory mechanisms of nitrogen fixation in cyanobacteria: the Ferric Uptake Regulator FurC as a new player in the regulatory network of nitrogen metabolism and heterocyst differentiation in *Anabaena* PCC 7120

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Keywords: cyanobacteria, transcriptional regulation, nitrogen fixation, ferric uptake regulator

Nitrogen is an essential element for living organisms which, in spite of being the main component of the atmosphere, is limiting in ecosystems. This is because atmospheric nitrogen cannot be used directly, it must be incorporated into trophic chains through a process known as biological nitrogen fixation. This process can only be performed by a few organisms such as *Anabaena* PCC 7120, a cyanobacteria that is able to fix atmospheric nitrogen in specialized cells called heterocysts.

The identification of transcriptional regulators that control nitrogen metabolism and nitrogen fixation is of interest in order to enhance the biotechnological applications of cyanobacteria in biofertilization or biofuel production. However, the transcriptomic control of this process is rather complex and several key aspects are yet to be discovered.

FUR (Ferric uptake regulator) proteins are a family of transcriptional regulators which have traditionally been associated with the regulation of metal homeostasis. Nevertheless in cyanobacteria they have a global role, controlling various cellular processes. FurC, one of the FUR paralogs of *Anabaena* PCC 7120, seemed to be involved in nitrogen metabolism, since the global nitrogen regulator NtcA binds to its promoter region. Consequently we sought to investigate its role in the genetic control of nitrogen metabolism.

In this work, we have analysed the transcriptomic profile of a FurC overexpression strain in the absence of nitrate and we have studied its morphology and physiology under these conditions. Our results show that the overexpression of this transcriptional regulator impairs heterocyst development and reveal that FurC plays a key role on nitrogen metabolism, directly regulating the expression of important genes involved in all the steps of heterocyst formation. Taken together, these data provide a better understanding of the process of nitrogen fixation in cyanobacteria, laying the foundations for implementing and improving the use of these organisms in biotechnological applications.

O7

Unravelling the regulatory mechanisms of transcriptional regulators in cyanobacteria: modulation of the redox states of the Ferric Uptake Regulator FurA from *Anabaena* sp. PCC 7120 by thioredoxin A

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Keywords: cyanobacteria, transcriptional regulation, thioredoxin, ferric uptake regulator

The activity of transcriptional regulators is typically controlled by various mechanisms, which allow organisms to regulate gene expression in response to changing environmental or physiological conditions. The Ferric Uptake Regulator FurA from the cyanobacterium *Anabaena* sp. PCC 7120 is a global transcriptional regulator which controls many cellular processes such as iron homeostasis, photosynthesis or nitrogen metabolism, among others. Consequently, in order to be able to control such a variety of processes, its activity needs to be modulated in response to various cellular signals.

In vivo, FurA displays several redox isoforms, and the oxidation state of its five cysteines determines its activity as regulator and its ability to bind to different metabolites, such as heme or 2-oxoglutarate, which modulate its DNA binding activity. However, the precise mechanism underlying the reduction of FurA, as well as its functional electron donor, remain still unknown. As thioredoxins are essential players in thiol-based redox regulation and are involved, among many other processes, in the regulation of the activity of many redox-sensitive transcription factors, we sought to investigate the role of type-*m* thioredoxin TrxA as a potential redox partner of FurA, mediating dithiol-disulfide exchange reactions necessary to facilitate the interaction of FurA with its different ligands.

Our results demonstrate that TrxA is able to interact both *in vitro* and *in vivo* with FurA from *Anabaena* sp. PCC 7120, as it was seen in cross-linking and bacterial two-hybrid assays. Reconstitution of the electron transport chain proved that TrxA is able to reduce FurA and light to dark transitions resulted in reversible oxidation of a fraction of FurA. Furthermore, the use of site-directed mutants allowed us to propose a plausible mechanism for FurA reduction. Taken together, these results point to TrxA as one of the redox partners that modulate FurA performance.

O8

Combination of an aerobic bioreactor with photo-Fenton process in winery wastewater treatment

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Keywords: Aerobic bioreactor, long-term aerated storage, microbial growth, photo-Fenton, winery wastewater

The winery wastewater (WW) collected for this work shows high dissolved organic content (DOC = 3081 mg C/L), chemical oxygen demand (COD = 15006 mg O2/L) and total polyphenols (206.9 mg gallic acid/L). A microbial analysis of WW showed a total yeast and total bacteria concentration of $1.60x10^5$ and $1.50x10^5$ CFU/mL, respectively. To treat the WW, it was applied a combined long-term aerated storage (LTAS) with a photo-Fenton process with the aim of decrease the organic matter to legislated values by Portuguese decree-law n◦ 236/98. Three aeration periods were studied in the LTAS reactor (10, 15 and 20 h/day) under the operational conditions: air flow = 131 mg/L. min, HRT = 15 days, V = 5 L, T = 298 K, achieving a DOC removal of 60.4, 88.2 and 88.7%, respectively. With application of 20 h/day aeration time, it was observed the highest microbial growth ($1.50x10^7$ and $6.20x10^7$ CFU/mL, respectively), thus the aeration had a significant effect in microbial growth and the natural microorganisms in the WW revealed effective in DOC degradation. As a complement, photo-Fenton process was applied (pH = 3.0, $[H_2O_2]$ = 97 mM, $[Fe^{2+}]$ = 2.5 mM, V = 500 mL, agitation = 350 rpm, T = 298 K, time = 240 min) and the organic matter removal achieved the legislated values. In conclusion, the application of the combined treatment LTAS-photo-Fenton process is efficient for organic matter reduction.

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O9

Metschnikowia spp.: An emergent biocontrol agent against grape fungi contaminants

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Keywords: Non-*Saccharomyces* yeasts, *Metschnikowia* spp. diversity, antagonist activity, PCR-fingerprinting

Presently, there is an increasing demand for more sustainable and eco-friendly practices in the agricultural ecosystems. A significant amount of research has been conducted aiming the replacement, or at least the reduction, of chemical products, to mitigate the detrimental effect of spoilage/contaminants. An emerging strategy has been the use of natural products based on microorganisms, such as fungi, yeasts, or bacteria. In the winemaking industry, *Saccharomyces cerevisiae* and *Metschnikowia fructicola* are being explored as biocontrol agents against vineyard phytopathogenic fungi and grapes spoilage yeasts, respectively.
In this work, a set of 95 autochthonous *Metschnikowia* spp. strains, isolated from five different Portuguese wine regions, was studied for its potential as a biocontrol agent against four non-biotrophic contaminants fungi - *Botrytis cinerea*, *Aspergillus niger*, *Penicillium* spp., and *Mucor* spp. Genetic diversity of the yeast isolates was also evaluated through PCR-fingerprinting, using minisatellite M13 and microsatellite $(GTG)_5$, as single primers. To underlie the potential inhibition mechanism associated, two different in the *vitro* approaches were used: volatile organic compounds (VOCs) or diffusion-assay.
A large diversity of genotypic profiles was found, with no significant association with the geographical provenance of the isolates. The antagonistic behaviour detected was both yeast strain and filamentous fungi species dependent, with none of the strains being equally effective against all targets tested. Remarkably, a higher inhibitory effect was seen in the VOC assays with one of the strains being able to fully inhibit *Penicillium* spp. growth.

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O10

Assessment of yeast susceptibility to common fungicides used for managing powdery and downy mildew in vineyards

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Keywords: Fungicides, Powdery-mildew, Downy Mildew, Yeasts, Biocontrol

Powdery mildew and downy mildew are among the main fungal diseases in vineyards being responsible for significant losses in the wine industry, as a consequence of yield loss and reduced quality of infected grapes. Currently, there is an increasing trend towards the use of natural biocontrol products based on biological substances (plants or microorganisms) to reduce the traditional chemical treatments used against fungal diseases.
Recently, the efficacy of a new biocontrol product, developed within the ABCyeast project, based on a consortium of different yeast species, was evaluated in field trials in commercial vineyards in two consecutive years. The results show that the strict use of the biobaseD product was effective in controlling both fungal diseases, although its effectiveness was dependent on environmental conditions, specifically when climatic conditions were more favorable for the development of the fungal pathogens. Thus, the combination of chemical fungicides with the ABCyeast product, applied in alternation, could be an approach to overcome this limitation.
In this line, the objective of this work was to evaluate the effect of fungicides used against powdery mildew (Vivando®, Collis®, Prosper®) and Downy Mildew (Vitipec®, Cuprital®, Profiler®), on the growth and survival of the yeasts of the ABCyeast product. For this, their susceptibility to manufacturer's recommended doses of application of each fungicide, as well as a range of dilutions, was tested. All fungicides showed an inhibitory action, regardless of strain, although to different extents. The results obtained in this work are the first step in evaluating the compatibility and potential time interval between applications of the ABCyeast product and chemical fungicides, to improve disease control in a more sustainable viticulture.

Acknowledgments: This work was financially supported by ABCyeasts project no. NORTE-01-0247-FEDER-039793, co-financed by FEDER through NORTE 2020 and by Fundação para a Ciência e Tecnologia to Biosystems and Integrative Sciences Institute through contract FCT/UIDB/04046/2020.

O11

Assessment of DNA damage in peripheral blood lymphocytes induced by oncologic treatments in patients with breast and colorectal cancer

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Keywords: DNA damage, chemotherapy, radiotherapy, comet assay, breast and colorectal cancer

Genomic instability is a hallmark of carcinogenesis, which plays a key role in the conversion of a normal cell into a premalignant cell, since cells are constantly subjected to variety of agents that either directly alter the DNA sequence or cause mutations when the damage is not repaired. In this initial study, the effect of anti-neoplastic drugs and radiation used in cancer therapy of breast and colorectal cancer as agents causing DNA damage was evaluated in patients' lymphocytes. During this work, blood samples were obtained from 70 cancer patients, from Vila Real Hospital, undergoing chemotherapy and/or radiotherapy. The simple comet assay was performed to assess *in vivo* DNA damage basal levels in the peripheral blood lymphocytes of these patients, before and after a few cycles of treatment. Preliminary results show that DNA strand breaks levels of cancer patients before treatment are higher when compared to healthy individuals. After treatment administration, basal level of DNA damage was significantly increased and the number of viable peripheral blood lymphocytes, in all cancer patients, decreased as compared with their pre-treatment values. On the other hand, the variations observed in colorectal cancer biomarkers levels (Carcinoembryonic Antigen and Cancer Antigen 19.9) were not significant, and there was even a slight elevation of Cancer Antigen 15.3 levels in breast cancer patients after some cycles of treatment, suggesting that the tumour is not yet in remission. Even so, it is known that a small change in the capacity of DNA damage response may contribute to the cancer transformation. In order to clarify these findings, DNA repair capacity evaluation constitutes an important next step of this work. This information may be particularly relevant in the diagnosis and prognosis, contributing to development of new strategies to prevent and cure cancer.

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O12

Importance of familial studies involving balanced translocations

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Keywords: Cytogenetics, clinical report, translocation, diagnosis

Reciprocal translocations are one of the most frequent types of structural chromosomal abnormality occurring in 1:500 individuals in the general population. They are usually inherited, but can also occur “de novo”. Individuals carrying these translocations are phenotypically normal, but have an increased risk of infertility and phenotypic changes in their offspring, namely malformed fetuses or recurrent miscarriages, due to the production of abnormal gametes. The authors reported cytogenetic analysis of a familial history of a translocation between chromosomes 4 and 16.

A healthy couple was referred to genetic counselling because of a t(4;16)(q21.1;q22) identified in their son, after being detected during gestation in their grandson, due to advanced maternal age. Both their son and grandson are healthy, as are their other two daughters and another grandson. Family history revealed only two maternal cousins (a brother and a sister) of the woman with intellectual disability of unknown etiology. There was no family history of fetal loss or congenital anomalies.

Blood cultures were performed according to the protocols establish in the laboratory. Cytogenetic analysis followed the cytogenetic guidelines (ISCN, 2020).

Cytogenetic analysis of blood´s couple revealed the translocation t(4;16)(q21.1;q22) in the woman and a normal karyotype in the man.

To our knowledge, there is only one case with translocation t(4;16) described in a woman with small uterus and irregular menstruation, but the breaking points are different comparing to our case. Analysis of the imbalances resulting from segregation of this translocation indicated that it would be unlikely that an imbalanced embryo would be viable.

This case contributes to a better characterization of a familial translocation, since the identification of a translocation is important for the implications it has in reproduction, allowing adequate genetic counseling and prompt prenatal diagnosis.

O13

Comet assay optimization for further application in cumulus cells

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Keywords: female infertility, cumulus cells, comet assay, DNA damage

Infertility is clinically described as a reproductive system disorder characterized by the inability to achieve pregnancy after a year or more of unprotected sexual activity. Male infertility could be caused by genetic variants in the sperm genome, or by other genetic conditions, including DNA fragmentation. Recent studies have used the comet assay to assess sperm DNA damage, demonstrating that a high level of fragmentation in DNA affects reproductive ability.

Comet assay is a simple, versatile, and inexpensive method used to detect strand breaks and alkali-labile sites. Cells are lysed to form nucleoids, followed by alkaline treatment where DNA denaturation of the nucleoid occurs. In electrophoresis, the broken DNA migrates faster to the anode leading to a "comet tail" seen by fluorescence microscopy, in presence of damage. The frequency of breaks reflects the relative amount of total DNA in the tail.

These findings, in combination with recent publications, have led us to question the applicability of the comet assay in the evaluation of female infertility. Thereby the aim of this work is the optimization of the comet assay protocol in blood cells for further implementation in cumulus cells. Cumulus cells are located around the oocyte and with implications in fertilization success.

At an early stage of the work, optimization of the protocol was performed in blood samples of healthy women and all the steps of the assay were carefully tested. The results have shown that precoating, agarose concentration, type of coverslip used, pH of the lysis solution, electrophoresis time and voltage, and the use of a positive control to validate the assay are crucial. Overall, our results demonstrate that samples should be analyzed in triplicates to avoid variability of results. Following these optimization procedures, the comet assay can be implemented in cumulus cells.

O14

In vitro assessment of the biocompatibility of clinically-available hemostatic agents

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Keywords: Osteoblasts, Hemostatic agents, Cytotoxicity

Topical hemostatic sponges are an important therapeutic option to manage uncontrolled bleeding after trauma or surgical intervention. Efficacy and biosafety are the most noteworthy aspects of these agents and, given their wide availability, a systematic assessment of these parameters is of the utmost importance.
Accordingly, the aim of this work was to evaluate the *in vitro* biocompatibility of clinically available gelatin-based hemostatic agents (specifically, Hemospon®, Roeko® and Octocolagen®).
In this study, hemostatic sponges' leachables were prepared in minimum essential medium (MEM), according to the ISO EN 10993-17 standard. The human osteoblastic MG-63 cells were cultured in growth medium and then divided into the experimental groups, grown in the presence of the leachables, at concentrations of 50% and 12,5% and a control group, grown in their absence. The cell cultures were then assessed for cell viability/proliferation (metabolic activity (MTT) and DNA quantification), alkaline phosphatase (ALP) activity, mitochondrial and cellular morphology, as well as ALP histochemical staining and collagen content, at different timepoints.
Upon analysis of the results, the hemostatic agents appeared to be biocompatible and exhibited no apparent cytotoxicity, with the cells exposed to the leachables showing similar metabolic activity to that of the control, normal cellular morphology, and unimpaired collagen production. However, the Roeko® sponge showed a significantly higher ALP activity, in comparison to other hemostatic agents' leachables, demonstrating thus to have some osteogenic potential, and suggesting a prospective application in bone tissue engineering-related approaches.

O15

A comparative study of SARS-CoV-2 molecular detection in nasopharyngeal swab *versus* saliva samples

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Keywords: COVID-19 diagnostic, SARS-CoV-2, RT-PCR, nasopharyngeal swab (NPS), saliva, RNA sample stability, asymptomatic detection

COVID-19 pandemic imposed an extreme pressure on health institutions and professionals. New methods to reduce the workload of health professionals without compromising health outcomes are essential. The use of nasopharyngeal swab (NPS) sampling has some limitations, such as: the need of qualified human resources; their invasive nature and the discomfort imposed to patient; variable performance associated to the technique used; and the need of a conservation method for transport. The aim of this study was to assess the concordance in reliability, sensibility, and accuracy of SARS-CoV-2 detection, using Real time RT-qPCR, between NPS vs. passive saliva samples. A total number of 85 individuals were analysed. In general, the saliva specimens presented higher RNA concentration (average of 19.24 ng/µl and 35.94 ng/µl for NPS and saliva, respectively) and quality, but higher Ct values when compared to NPS samples (average Ct values for genes analysed: ORF1ab – 22.0 and 24.0; N- 20.0 and 24.0 and; E- 23.0 and 26.0, values for NPS followed by saliva). The diagnostic result of the presence/absence of SARS-CoV-2 presented total correspondence between the two methods, evidencing that the use of saliva samples seems as good as NPS for COVID-19 molecular diagnosis. The use of saliva samples in SARS-CoV-2 detection reduces the workload of health professionals and is less invasive for patients, thus being an excellent alternative to NPS, mainly concerning paediatric practice.

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POSTERS

P1

Behavioral and receptor alterations in a mouse model of anti-NMDAR encephalitis by active immunization

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Keywords: animal model, anti-NMDAR encephalitis, antibodies, pathogenesis, mechanism

Anti-NMDA receptor (NMDAR) encephalitis is a neurological disorder mediated by antibodies against an extracellular epitope of the GluN1 subunit of the NMDAR. Previous studies using cultured neurons and a mouse model of passive transfer of antibodies showed that the antibodies cross-link and internalize the receptors and cause behavioral alterations. Here we aim to explore the pathogenic mechanisms in a context of active immunization.

8-week-old female C57BL/6 mice were immunized with the GluN1 356-385 epitope containing peptide or saline along with AddaVax adjuvant and Bordetella *pertussis* toxin. An immunization boost was administered at day 28. Synthesis of NMDAR antibodies in serum and CSF was determined with cell-based assays, and the effects of the antibodies were assessed with confocal brain tissue immunohistochemistry and electrophysiology. Memory and behavioral alterations were assessed with a standard panel of tests: Novel Object Location (memory), Prepulse Inhibition (psychotic-like behaviour), and video-recording (stereotypies and abnormal movements).

Confocal microscopy and electrophysiological studies were performed 42 days post-immunization. Compared with control mice, those immunized with GluN1 356-385, showed increased deposits of IgG in the areas of the brain examined (cerebral cortex, cerebellum and hippocampus). These findings were associated with a significant decrease of total cell-surface surface and synaptic NMDAR clusters, and impairment of hippocampal long-term potentiation (LTP). Accompanying symptoms included memory deficit, psychotic-like behavior, and stereotypies characterized by repetitive episodes of walking backwards and compulsive grooming.

This model of active immunization causes alterations similar to those observed with antibodies from patients with anti-NMDAR encephalitis and expands the model of passive transfer with the development of aberrant motor behaviors. The model will help to determine the immunobiology of the disease and how pharmacological interventions (e.g., allosteric modulation of NMDAR) can be used as an adjuvant to immunotherapy.

P2

Detection of cell cycle and chromosomal anomalies on bread wheat seeds upon exposure to a 2,4-D-based herbicide

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Keywords: *Triticum aestivum* L., mitotic cycle, cytotoxicity, chromosomal anomalies

Some of the herbicides widely used in bread wheat (*Triticum aestivum* L.) cultivation to control broadleaf weeds are mainly constituted by 2,4-Dichlorophenoxyacetic acid (2,4-D). Beyond the weeds, the herbicides can induce toxicity in non-target species and the crop.
This work aimed to evaluate the cytotoxicity induced by a commercial herbicide based on 2,4-D in bread wheat's root mitotic cell cycle. The seeds were imbibed in distilled water (control, 0 ppm) and aqueous solutions of herbicide whose dosages were determined based on the 2.4-D concentration: 250, 500, 750, and 1000 ppm, for 3h, 6h, and 24h.
The germination rate decreased with the augment of 2.4-D dosage and exposure time. No seeds germinated on the 24h x 1000ppm treatment. We observed irregularities at interphase mainly affecting the nucleoli and irregular mitotic cells in all phases during this work. The Mitotic Index (MI) and the Percentage of Dividing Cells with Anomalies (%DCA) differed significantly ($p<0.05$) among treatments. The lowest average MI was found after 24h of exposure and the highest %DCA at 750 ppm. Most of the mitotic cells were in prophase and metaphase, suggesting cell cycle arresting in these phases in response to cytotoxicity. Except for telophase, per mitotic phase, irregular cells with different anomalies were observed. Stickiness was one of the most common anomalies detected in all mitotic phases. C-mitosis was also recurrent, indicating hinder of spindle formation. Our data showed that the 2.4-D concentration was the most statistically significant factor ($p<0.001$) inducing cytotoxicity. The appearance of nucleolar and chromosomal abnormalities in response to herbicide exposure is indicative that it may inhibit rDNA transcription, protein synthesis, root and plant growth in wheat, affecting its development, yield, and quality.

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P3

Influence of Bov-B LINE retrotransposon on the evolution of Iberian Peninsula snake karyotypes

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Keywords: Transposable elements, Serpentes, Bov-B LINE, *in silico* mapping, DNA-FISH, phylogenetic analysis, RT-qPCR

Transposable elements (TEs) are segments of repetitive DNA with the ability to move within a genome. Retrotransposons belong to the family of TEs and have the capability to be transferred within the same species or even to different species, which may lead to an increased size of the genomes in a short period of time. There are three major reptile groups: Crocodilia, Testudines and Lepidosauria, in which Serpentes (snakes) belong to Lepidosauria group. The repetitive DNA content varies significantly between Serpentes species, being retrotransposons the largest family of repetitive sequences that contribute to genome size and activity. The Bov-B LINE retrotransposon belongs to one of the most abundant retrotransposon families in vertebrates that demonstrate evidence of horizontal transfer. Discovery in *Bos taurus* (BTA) and thought to be Ruminantia specific it was later found to be highly conserved in Serpentes. The Bov-B LINE retrotransposon was analysed in Squamata genomes in order to better understand it's involvement with the regulation and evolution of Serpentes. An *in silico* mapping and phylogenetic analysis of these retrotransposons was carried out in squamate reptile genomes. The DNA-FISH of Bov-B LINE reinforced the *in silico* results by demonstrating that this retrotransposon was dispersed throughout all chromosomes of BTA. Finally, the quantification of the number of copies of this retrotransposon, through RT-qPCR, and the analysis of variants using HRM led to important preliminary results, like for example the discovery of seven different variants of Bov-B LINE in the species analysed. With the integration of all obtained results, it is possible to highlight the importance that retrotransposons may have in the evolution and regulation of Serpentes.

P4

Antibiotic Resistance: Genomics for a global crisis

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Keywords: Antibiotic resistance, genomics, resistant strains, *Streptococcus pneumoniae*

Antibiotic resistance could be defined as the capacity that certain pathogenic bacteria strains acquire, which enables them to survive exposure to antibiotics.
This is not a new phenomenon. On the contrary, in the last few decades, it has been observed a significant increment in antibiotic-resistant strains, which through accumulated mutations have adapted to the continuous exposure to antibiotics.
The phenomenon can be explained by the bacteria's adaptation to the surrounding environment. This process can happen as a consequence of horizontal gene transference (HGT) or through mutations, being the later the only source of new variability.
The World Health Organization has stated that the emergence and dissemination of antibiotic-resistant bacteria can have unimaginable consequences.
Resistance to β-lactamic antibiotics is especially worrying because many of the pathogens which were treated with them, such as *Streptococcus pneumoniae, have* already evolved into multidrug-resistant (MDR) strains.
Thus, detection of potential sources of mutations is necessary to stop or decline this process. Molecular techniques such as cloning, PCR, genome, and transcriptome analysis, have been used to determine what genes from random bacterial DNA libraries contain resistance-associated mutations. The problem is that using the current technology, detection of every mutation in every single bacterial strain is not feasible.

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P5

Exo-lip: A novel therapeutic approach for Alzheimer's disease treatment

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Keywords: exosome-like liposomes; neurodegenerative diseases; blood brain barrier; gene delivery; tau protein; acetylcholinesterase

Alzheimer's disease (AD) afflicts 50 million people worldwide. Moreover, this number is dwarven by the projected 152 million people with age above 65 years old that are expected to suffer from this disease in 2050.
It is difficult to point out the initial potentiator for the development of this disease. However, it is widely accepted that the accumulation of hyperphosphorylated Tau protein into neurofibrillary tangles is responsible for a great part of the toxicity and damage done to neuronal cells.
One promising therapy consists in the use of siRNAs to decrease the production of this protein in pathological conditions. However, one of the setbacks of this approach is the difficulty of an effective and safe delivery of these molecules in the brain, due to the presence of physiological barriers such as the blood-brain barrier (BBB) and short circulation lifetime. Liposomes have been used to achieve efficient delivery of molecules, including nucleic acids, into the brain. We developed a novel exosome-like liposomal vector (exo-lip), that conjugates the ease of production and the high encapsulation efficiency of siRNA that most cationic liposomes present, with the high biocompatibility that naturally-occurring exosomes confer.
Exo-lip systems have a small size of 150.5 ± 2.5 nm and a polydispersity below 0.2. They present a high storage life, only destabilizing after 3 months at 4^{o} C and have high encapsulation efficiency (above 95%). These systems show little to no cytotoxicity in L929 and SH-SY5Y cell lines. The silencing of tau gene expression by appropriate siRNAs will potentiate even further the therapeutic interest of this novel type of nanovectors.

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P6

Cytotoxicity effects of glyphosate in the root mitotic cell cycle of bread wheat 'Nogal'

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Keywords: Bread wheat, cell cycle, cytotoxicity, glyphosate, mitotic index

Bread wheat is one of the most cultivated cereals around the World. The intensive use of herbicides in wheat cultivation to control weeds may also induce phyto- and cytotoxicity in the crop. Glyphosate is one of the main compounds present in herbicides formulations, and its toxicity has been debated for the past few years. Contaminating soils with glyphosate may impair germination or root growth due to cytotoxicity. Therefore, it is essential to evaluate the effects of glyphosate-based herbicides in wheat´s root mitotic cell cycle. Chromosomes are very responsive to external stimuli and abiotic stresses such as exposure to glyphosate, leading to anomalies and decreased cell division rate. The mitotic index (MI) and the percentage of dividing cells with anomalies (%DCA) are reliable indicators to assess cytotoxicity induced by toxic agents or other abiotic stresses. In this work, we imbibed seeds in different concentrations of a commercial glyphosate-based herbicide (corresponding to 250, 500, 750 and 1000 ppm of glyphosate) for a short period to evaluate its effects in the root mitotic cell cycle of the bread wheat variety 'Nogal,' by comparison with control (non-treated seeds).
The increase in glyphosate concentration led to a significant decrease in MI. Most of the mitotic cells were in prophase, indicating arresting of the cell cycle. Contrarily to what is expected, higher average values of %DCA were found in the lowest concentrations of glyphosate solutions (250 ppm and 500 ppm), which decreased in the higher concentrations (750 ppm and 1000 ppm), suggesting a saturation point to the plant followed by a putative recover or activation of an adaptation mechanism. The mitotic cells showed various types of mitotic spindle and chromosomal anomalies previously detected in other plant species exposed to toxic agents, highlighting the harmful effect of glyphosate.

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P7

Serum Biochemistry reference intervals for European hedgehog (*Erinaceus europaeus*) in Northern Portugal – a preliminary study

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Keywords: European Hedgehog, Serum Biochemistry, Reference intervals

The European Hedgehog (*Erinaceus europaeus*) is an insectivorous mammal with a wide geographic distribution that has been suffering a reduction in population density due to several anthropogenic factors. Clinical biochemistry is mainly used to monitor disease development and reaction to treatment. Biochemical reference intervals are used to help identifying anomalies. Its determination is of particular importance, since without knowing what is normal, it is difficult to determine the disease state. This preliminary study aims to determine serum biochemistry reference intervals in the species *Erinaceus europaeus*. For this data the registry of the Clinical Pathology Laboratory (LPC) of the Veterinary Hospital of the University of Trás-os-Montes and Alto Douro (HVUTAD) between January 2017 and December 2021 was analyzed. In the study were included 22 healthy animals (10 males and 12 females); 11 were adults and 11 were young. The parameters Glucose (mg/dL), Total Proteins (g/dL), Albumin (g/dL), ALT (U/L), Alkaline Phosphatase (U/L), Creatinine (mg/dL), Urea (mg/dL), Phosphorus (mg/dL), Total Calcium (mg/dL) and Gamma-GT (U/L) were analyzed. Considering the age factor, statistical significant differences were observed for ALT between males and females (p=0,028, higher in males). Considering the gender factor, statistical significant differences were observed for Total Proteins (p=0.023, higher in adults) and for Gamma-GT (p=0.019, higher in young animals). No other statistical significant differences were observed for the remaining parameters. Our study allows us to conclude that for the species *Erinaceus europaeus* it seems to be appropriated to further analyze the effect of gender and age in normal biochemical parameters, as for some of them statistical significant differences can be observed.

P8

Hematological reference intervals for European hedgehog (*Erinaceus europaeus*) in Northern Portugal – a preliminary study

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Keywords: European Hedgehog, Hematology, Reference intervals

The European Hedgehog (*Erinaceus europaeus*) is an insectivorous mammal with a wide geographic distribution. Due to climatic changes and other factors such as the elimination of rural areas, a considerable number of animals of this species live now in urban areas close to humans where they are exposed to several anomalies that may result in hematological and biochemical changes. So, this species has been suffering a reduction in population density due to several anthropogenic factors. For this reason, it is important to deepen the knowledge of this species, especially at the laboratory and clinical level. Hematological reference intervals are used to help identify anomalies. The calculation of reference intervals for a given species is of particular importance, since without knowing what is normal, it is difficult to determine the disease state. The main objective of this work is to carry out a study of hematology in the species *Erinaceus europaeus* in order to calculate hematological reference intervals and further investigate the effect of age and sex on these values. For this retrospective study, data from the registry of the Clinical Pathology Laboratory (LPC) of the Veterinary Hospital of the University of Trás-os-Montes and Alto Douro (HVUTAD) between January 2017 and December 2021 were analyzed. For this study 34 healthy animals were included (21 males and 13 females). Considering the age, 13 were adults and 21 were young. All the parameters included in the hemogram were analyzed for all the animals. No statistical significant differences were obtained between adults and young animals, nor between males versus females ($p>0,05$). Our study allows us to conclude that for the species *Erinaceus europaeus* it seems to be sufficient to determine hematological values in general, without the need for specific values regarding sex or age factors.

P9

Cytogenetic analysis of products of conception in CHTMAD genetics laboratory

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Keywords: Cytogenetics, miscarriage, diagnosis

Of all recognized pregnancies, about 10-15% end in clinical miscarriage, mostly toward the end of the first trimester. There are different risk factors for the occurrence of spontaneous abortions, such as maternal age, presence of gynecological pathology and chromosomal abnormalities, are responsible for 50% to 70% of miscarriages and among these, aneuploidies, like trisomy 16, monosomy X and polyploidy, are the most common.
The aim of this study was to analyze the incidence of chromosomal abnormalities in products of conception in the Centro Hospitalar Trás-os-Montes e Alto Douro (CHTMAD).
Between January 2003 and December 2021, a total of 1027 products of conception samples were received at the Genetics Laboratory. Cytogenetic analysis was performed according to standard techniques and followed the cytogenetic guidelines (ISCN, 2020).
The study included 1027 samples. Cell growth was obtain in 84% of the samples. Cytogenetic analysis detected chromosomal abnormalities in 287 samples (34%), being the numerical anomalies the most frequent (273 cases). Among aneuploidies, monosomy X (47 cases) and trisomy 16 (35 cases) were the most common. There were 14 cases with structural anomalies, of which eight have un- or balanced translocation and of these two were inherit (in an unbalanced form).
The rate of chromosomal abnormalities detected was slightly lower than that described in the literature. However, monosomy X and trisomy 16 have an incidence similar as described by other authors.
Cytogenetic analysis of products of conception is important for an attempt to discover the cause, becoming crucial for a better couple genetic counseling.

P10

Immunohistochemical expression of tensin-4/CTEN in squamous cell carcinoma in dogs

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Keywords: CTEN, immunohistochemistry, canine SCC, tumour

C-terminal tensin like (tensin-4/CTEN) is a distant member of the tensin family. It is described as an oncogene in many cancers, however, its role on squamous cell carcinoma (SCC) is poorly understood. In order to report, for the first time, the clinical value of CTEN in canine SCC, we investigated by using an immunohistochemistry protocol, 45 SCC sections, from dogs with different breeds, genders and ages (8.9 ± 3.6 years).
CTEN expression was observed highly strong in the cytoplasm and in the basal layer of the non-tumoral epidermis. Immunopositivity revealed that CTEN expression is higher in grade III tumours (100% immunopositivity) ($p<0.0001$), evidencing a strong correlation between CTEN expression and the histological grading of the tumour, and a possible involvement in tumour progression. Based on our results, CTEN is proposed as an oncogene in canine SCC tumours and a promising biomarker and a therapeutic target in SCC.

P11

The Breaking Point: Impact of Reciprocal Translocations on Male Infertility

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Keywords: Andrology, Male infertility; Cytogenetics; Reciprocal Translocations

Chromosomal abnormalities are an important etiologic factor regarding male infertility. The frequency of numerical or structural defects in infertile males varies from 2,2 to 15,2%, in which sex chromosome abnormalities and Robertsonian translocations are the most common. Reciprocal translocations have a much lower incidence and are associated with low sperm count (oligospermia) or even absence of sperm (azoospermia). Azoospermia is to be expected if sex chromosomes are involved, while oligospermia is more frequent in translocations involving only autosomes. Spermatogenesis in these cases is believed to be impaired by two main processes: damage of critical genes to spermatogenesis in the breakage and recombination of chromosome translocation, and through spermatogenetic arrest. However, normal semen parameters can still be found in such carriers. Chromosomal breakpoints seem to dictate whether the male infertility is pregestational (failure to produce a fertilized ovum), gestational (embryo loss after fertilization) or both.

Here we report four different cases of male infertility involving autosome-only reciprocal translocations with different semen profiles. Three male patients with a t(4,22)(p16.1;q11), t(4,10)(q31.3;p15) and a t(11,22)(q14.2;q13.1) with altered semen parameters and a t(6,8)(p23;q21.3) with normal semen profile.

All the above four translocations have been previously associated with male infertility. And, although with different breakpoints in play, t(4,22) and t(6,8) have been described in patients with normal semen profile but with gestational infertility; t(11,22) has been reported, so far, only in oligozoospermic males and t(4,10) exhibits both forms of infertility. Regarding the discovered breakpoints, we found three associated with infertility: 4p16.1 with pregestational infertility, 4q31.3 with gestational infertility, and 10p15 with both. Uncovering these delicate associations between translocations breakpoints and male infertility can provide invaluable insights into the process of spermatogenesis.

P12

Defining new tools for detecting mutations in the EGFR gene in liquid biopsies from patients with Non-Small Lung Cancer

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Keywords: NSCLC (Non-Small Cell Lung Cancer), TKIs, cfDNA, EGFR, HRM, NGS

In non-small cell lung cancer (NSCLC, most common type of lung cancer) EGFR stands out as the gene most frequently mutated, mainly in Tyrosine Kinase domain's region. For that reason, EGFR gene is a therapeutic target to the EGFR-TKIs (EGFR-Tyrosine Kinase Inhibitors) and its mutations can correlate with sensitivity or resistance to EGFR-TKIs, thus functioning as predictive biomarker of drug response. Frequently, it can occur the gain of resistance-related mutations (p.T790M) in EGFR gene during the treatment with EGFR-TKIs.
Currently, the diagnosis of NSCLC patients is performed by analyzing the molecular profile (using PCR and NGS techniques) of solid tumor biopsies, which is an invasive and painful process and sometimes impossible to perform, due to the location of the tumor.
This work aims to develop a simple method for detecting mutations associated with different types of therapeutic responses in liquid biopsies of NSCLC patients, which is less invasive and more sensitive than the techniques now used. For this, the main mutations of the EGFR exons were mapped and primers were designed to amplify these regions. Extraction of cfDNA from liquid biopsies from different healthy donors were proceeded. Furthermore, PC9 NSCLC cell line that have the EGFR p.E746_A750delELREA mutation was used as a control and to test the sensitivity of the different assays. The cfDNA samples were amplified by Multiplex PCR and the presence of the p.E746_A750delELREA was verified by NGS in all the samples. This data was confirmed by Sanger Sequencing. Also, HRM (High Resolution Melting Temperature) assays were performed as a faster and more sensitive detection alternative. We achieve a sensitivity of 0,05% (fraction of mutant allele) to detect changes in exon 19 and it was also possible to identify the mutation previously analyzed by NGS (Exon19, p.E746_A750delELREA).
This test will help the clinician to choose the most appropriate therapy, both at the beginning and during the follow-up of the case since other mutations can be acquired during treatment.

P13

Fatal Familial Insomnia- more than people who stop sleeping

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Keywords: FFI, PRNP, mutation, prion

Fatal familial insomnia (FFI) is a rare genetic degenerative brain disorder that affects the thalamus, the part of the brain that controls the sleep-wake cycle. Typically, the symptoms begin between the ages of 40-60 years and, other than sleep disturbance some of those symptoms are weight loss, balance problems, hallucinations, delirium, and dysautonomia preceding motor and cognitive deterioration. These symptoms tend to get worse over time. Because this disease is so rare, there is little information about its risk factors.

FFI is a prion disorder showing autosomal dominant inheritance and is specifically associated with asp178-to-asn mutation of the PRNP gene. This mutation is caused by a SNP, which is the alteration of a single nucleotide, in this case a guanine is replaced by an adenine in the PRNP gene that is located in the short arm of chromosome 20.

The average age at onset is 40 years and their life expectancy is 7 to 73 months after the symptoms become apparent.

There is no cure for this disease and its treatment is based on its symptoms and only serves to alleviate them. However, researchers are working hard toward effective treatments and preventive measures.

P14

Municipal wastewater treatment by ultrasound-Fenton processes for organic matter and biomass reduction

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Keywords: Biomass reduction, chemical oxygen demand, municipal wastewater, ultrasound-Fenton.

The release of poorly treated (or untreated) municipal wastewater (MW) into receiving rivers and streams puts the resource-constrained communities at risk of waterborne diseases, such as amoebic dysentery, cholera, typhoid fever, hepatitis A and poliomyelitis. Therefore, in this work, a municipal wastewater was collected, and advanced oxidation processes (AOPs) were applied with the aim of (1) compare the efficiency of Fenton, ultrasound (US) and ultrasound-Fenton (US-Fenton) processes, (2) optimize US-Fenton process operational conditions and (3) evaluate the organic matter and biomass reduction. The US-Fenton process was optimized with variation of pH, H_2O_2 and Fe^{2+} concentration and cavitation time. Under the best operational conditions (COD = 8512 mg O_2/L, pH = 4.0, [H_2O_2] = 30 mM, [Fe^{2+}] = 2.0 mM, total solids (TS) = 3.25 g/L, volatile solids (VS) = 1.92 g/L, cavitation time of 60 min (3 s ON/ 5 s OFF), amplitude (A) = 40%, temperature = 298 K), it was achieved a COD removal of 94.8 and a VS/TS of 0.13.

In comparison, the application of Fenton and US revealed for MW treatment, a COD removal of 25.9 and 17.3%, respectively and a VS/TS of 0.14 and 0.24, respectively. The COD removal results were fitted with a pseudo-first order kinetic rate and the synergy of the US-Fenton process achieved 83.3%, therefore, the combination of ultrasound with Fenton process has synergetic effect. In conclusion, the US-Fenton process is an efficient process for MW treatment, with high organic matter and biomass reduction.

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P15

Bacillus thuringiensis: a short review about its insecticidal properties

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Keywords: *Bacillus thuringiensis*, *cry* gene, Cry proteins, insecticide, Bt crops

Bacillus thuringiensis (Bt) is a gram positive microorganism, that resides in a broad range of environments; it can be pathogenic for some invertebrates (mainly insects of the order Lepidoptera, Diptera and Coleoptera) due to the presence in the genome of this bacterium of the *cry* gene that codifies for a crystal-like protein called Cry (or Bt), which is an δ-endotoxin produced during its sporulation cycle. This protein is very toxic when it gets in contact with the gastrointestinal (GI) tract of these invertebrates, due to the formation of pores in the cell membranes that lead to cell lysis and the death of the insect. This is the reason why historically, Bt has been used as an insecticide, and the reason why nowadays the *cry* genes are being used to create plague resistant GMOs, specially Bt maize, which expresses the *cry1Ab* gene.

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P16

The Role of Biosensors in Pulmonary Cancer Diagnosis

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Keywords: Non small lung cancer cells, biomarkers, diagnosis, biosensor

Lung cancer is amongst the most common cause of cancer deaths worldwide, mainly because of its asymptomatic status in early stages, thus usually it is only detected at an advanced stage of the diseases. NSCLC (Non-small cells lung cancer) represents about 85% of all lung cancers.
The identification and characterization of molecular changes involved in cell transformation from normal to malignant is of critical importance, and can help to improve prevention measures, early detection, and even monitor the efficiency of treatments. Knowledge of the patient's tumour characteristics and genetics will greatly enhance personalized prognosis and selection of the ideal treatment for each individual patient. Early diagnosis can be achieved using human fluids that can be easily assessed (e.g. blood, urine, saliva) for routine screening for the presence of biomarkers linked to NSCLC. The list of biomarkers is continuously being updated and can be used in different technological platforms which allow their detection. The use of biosensors, which are bioanalytical devices that can provide specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) can be a way to achieve a quick, cheap, and reliable result. A comparison amongst different technological tools presently used in NSCLC diagnosis will be shown, alongside with new technological solutions: Biosensors, that can help overcome some of the limitations found in the previous.

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P17

History of the evolution of the human genome

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Keywords: Human genome, Evolution, Speciation, Human evolution, Future evolution, Human history, Genetic variability

Evolution is the constant adaptation of species to the current environmental conditions. These adaptations are only possible due to the genetic variability within species, which is created by the occurrence of random mutations in the individuals' genomes. These mutations go from single nucleotide polymorphisms (SNPs) to insertions or deletions of short sequences (INDELs) to larger mutations such as chromosome alterations (mostly segmental duplications, deletions, inversions, and translocations). Besides mutations, repetitive sequences, such as satellite DNA and transposable elements, are also a great cause of genetic variability due to their ability to mobilize within the genome, restructuring the genome, creating new genes or altering gene regulation.

The evolution of the human species, and its speciation, were conducted by the occurrence of these mutations and their selection by adaptative pressures that were fixated or deleted, as they provided an advantage or disadvantaged to survival and reproduction. Human specific characteristics such as bipedalism and the language development are examples of characteristics that remained by positive selection.

The genetic pool of the human species has been enlarged not only by random mutations but also by the interbreeding between different hominid species that lived in the same time window. This was due to hominid populations' migrations and occurred mainly between *Homo sapiens*, *Homo neanderthalensis* and, to a lesser extent, *Homo denisova*.

Studying the evolution of our genome is very important not only to know from where we came, but also to try to predict where we are going, the path that led to what we are and the next steps on our evolutionary direction, keeping in mind the possible future environmental conditions, as well as the growing lack of selective pressure due to technological progress.

P18

The two sides of the coin: *Salmonella enterica* serotype *Typhimurium*

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Keywords: *Salmonella enterica* serotype Typhimurium, Cancer, Ames Test, Salmonellosis.

Salmonella enterica serotype Typhimurium (*S.* Typhimurium) is a Gram-negative bacterium belonging to the *Enterobacteriaceae* family, within the *Gammaproteobacteria* class. It is a unicellular, flagellated, facultative anaerobic organism and is shaped like short thick bacillus.
This bacteria is known for being the main cause of food poisoning in humans that can be transmitted by zoonosis. The features that made this kind of *Salmonella* so pathogenic are the ones related with its physiology: through the flagella they approach to the host cell; then they form biofilms to survive in adverse conditions and finally with their secretion system they encroach the host.
Despite the fact that it can cause salmonellosis, this serotype is also used as a tool to valuate if a substance is mutagenic based on the Ames test and as a possible new therapie to fight against cancer. This fact was confirmed in mice with a tumour delayed metastasis, where increased life expectancy in its attenuated form was observed Its application in humans is still being investigated. Nevertheless, the possibility of using this bacteria in cancer treatment has gained importance in recent years.

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P19

Mucor, *Aspergillus* and *Penicillium* isolated From Invertebrates in Garden Soil - A Public Health Perspective

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Keywords: Soil, Earthworm, Fungi, Health

The invertebrate Earthworm *Lumbricus terrestris* is native to Western Europe. It is considered invasive as it is widespread globally, tolerant of a variety of transport and climate conditions. A study was carried out on 10 samples of Earthworm (*Lumbricus terrestris*) collected in gardens in order to study the fungal microbiota. Samples were analysed using routine mycological methods. Samples were inoculated by apposition of the animal's body on Potato Dextrose Agar and Sabouraud Dextrose agar and incubated at 25°C and 37°C for 3 to 7 days. The fungi present in the different samples were *Mucor* spp. (100%), *Mucor circinelloides* (1/10), *Aspergillus* (9/10), *Aspergillus niger* (8/10), *Aspergillus ochraceus* (1/10), *Penicillium* spp. (1/10). Since theses isolates have potential of infection in humans and animals, more studies need to be performed to understand the importance of these invertebrates to human in a One Health Perspective.

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P20

Isolation of Dermatophytes in Small Ruminants from the Northeast of Portugal

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Keywords: Sheep, Goats, Portugal, Fungi, Health, Herds

The aim of this study was to carry out a screening of dermatophytes in small ruminants from herds in the Northeast of Portugal. Fur and scale samples were collected using the Mackenzie technique and sent to the Medical Microbiology Laboratory. The samples were inoculated in Dermathophyte Test Medium® and microscopic identification was performed using dichotomous keys. In this study, 47 hair samples from 42 (89.4%) sheep and 5 (10.6%) goats were studied. Most of the animals in the sample were 3 years old (n=15; 31.9%) and 4 years old (n=12; 25.5%). In terms of breed, the sheep were all Churra breeds, and goats were Serrana breeds. All small ruminants had lesions. Dermatophytes of the genus *Microsporum* were isolated in 2 samples from 2 Churra breed animals aged 4 years. Dermatophytes were isolated from samples from lesions on the snout and back. The occurrence of dermatophytosis in this study was 4.3%. This study allowed to increase the knowledge about dermatophytosis in small ruminants from the Northeast of Portugal.

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P21 Dermatophyte Surveillance in a Cat Shelter from the North of Portugal

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Keywords: Cat, Dermatophytosis, *Microsporum canis,* Shelter

Dermatophytosis (ringworm) is a common and highly transmissible fungal skin infection of cats. Dermatophyte carriage data are crucial for assessing epidemiology and designing potential control strategies in shelters.
An epidemiological dermatophytosis survey was carried out in cats without clinical signs at one shelter in Northern of Portugal, between October and December 2021. Fur samples (n = 34) were collected from 34 shelter cats using the toothbrush technique. Dermatophyte culture was performed using Dermatophyte test medium®. The Petri dishes were handled under sterile conditions and incubated at 28°C for up to 21 days. One dermatophyte from the species *Microsporum canis* was identified in one female cat out of the 34 cats (2.9%; 95% CI: 1.2 - 4.6%).
These results imply that dermatophyte shedding is rare in cats admitted to the studied shelter. Considering the scarcity of epidemiological reports in Portuguese shelters, these results could be a useful contribution towards the diagnosis and prevention in shelters.

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P22

Is dermathophytosis a disease of concern in shelter dogs? Preliminary studies

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Keywords: Dermatophytosis, Dog, *Microsporum canis,* Shelter

Dermatophytosis is one of the most important zoonotic skin diseases. Dermatophytes have the ability to invade keratinized tissue to produce superficial mycoses in both humans and animals. Three ecological groups of dermatophyte species are recognized namely anthropophilic, zoophilic and geophilic. According to the new classification dermatophyte species have been grouped into seven genera: *Trichophyton, Epidermophyton*, *Arthroderma*, *Nannizzia*, *Microsporum*, *Paraphyton* and *Lophophyton*. The infections caused by dermatophytes are among the most frequent human and veterinary dermatologic infections. Fur samples were obtained from 122 from dogs of a shelter; 58 (47.5%) of them were males and 64 (52.5%) were females. Dermatophyte culture was performed using Dermatophyte test medium® in Petri dishes. The Petri dishes were handled under sterile conditions and incubated at 28°C for up to 21 days. The only dermatophyte isolated was *Microsporum canis* (0.81%; 95% CI 95%: 0.0 - 1.69%). The occurrence of dermatophytosis in this study was low. This study allowed increasing the knowledge about dermatophytosis in shelter dogs of the Northeast region of Portugal.

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P23

Fabry's disease: not a 7-headed beast

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Keywords: Rare genetic disease, alpha-gal enzyme, heredity, symptoms, diagnosis

Fabry disease is a rare genetic disorder associated with a problem in the production of the enzyme alpha-galactosidase A, which leads to an accumulation of GL-3 in lysosomes, which in turns into damages cells gradually, causing health problems in different organs. It is currently known that the GLA gene, responsible for the production of the alpha-galactosidase A enzyme is found on the human X chromosome, thus causing a differential distribution of this disease between men and women, which can be transmitted from parents to children.

It is associated with a variety of symptoms from gastrointestinal problems such as diarrhea or vomiting, heart or kidney problems to psychological problems such as depression, thus making it difficult to identify the disease. The accumulation of glycolipids also causes the formation of non-cancerous (benign) skin lesions (angiokeratomas).

Due to the fact that it is a progressive disease with no cure, which can start in childhood or develop into adulthood, diagnosis as early as possible is crucial, so that its progression can be monitored and symptoms controlled.

This work aims to inform more about this disease, it lists various symptoms, diagnosis options and treatments in order to minimize it's progression and improve the quality of life of patients, thus demystifying this "7-headed *beast*".

P24

Bacteriophages: How to tackle the antibiotic crisis

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Keywords: phage therapy, bacteriophages, antibiotics, MDRO

For a time, the appearance of microbial resistance against certain antibiotics did not represent a significant problem since a wide variety of antibiotics were still available. However, its overuse and globalization have resulted in the emergence of multidrug-resistant organisms (MDRO). Infections caused by these organisms are expected to provoke up to 10 million direct deaths per year by 2050, stating the necessity of novel approaches to counter this problem.

Phage therapy was first suggested by D'Herelle more than 100 years ago as the use of bacteriophages or their proteins to clear bacterial infections. To this effect, phage selection remains essential and relies on aspects like viral lifecycle and bacterial range specificity.

Bacteriophages can be selected after consecutive adaptation cycles against a specific pathogen (personalized phage therapies). Despite its good results, this process is time and resource-consuming, and therapies like phage cocktails are also considered. These consist of bacteriophages targeting different receptors on the bacterial surface, and, in addition to being easier to develop, also exert less selective pressure.

Bacterial hosts can still acquire phage resistances, however, these are far less frequent than resistance for antibiotics. Furthermore, phage resistances generally involve fitness costs that range from higher sensitivity against immune serum to the restoration of antibiotic susceptibility, which points to combined therapies as the most successful approach.

Although there are no extensive trials regarding safety concerns, clinical results have shown infection clearing before with no adverse effects. However, the possibility of bacteriophages disrupting the host's microbiota needs to be considered since this situation has been reported previously.

The growing number of MDRO infections evidences the need for antibiotic-free treatments. In particular, phage therapy should continue being researched due to its high pathogen specificity and promising clinical results regarding infection clearing.

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P25

Assessment of acorn infusion in a canine mammary cancer cell line by alkaline comet assay

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Keywords: Canine mammary cancer, acorn infusion, DNA damage

Canine mammary carcinomas (CMCs) are frequently associated with aggressive biological behaviour; surgical treatment remains the main therapeutic approach and advances in novel medical treatment should be developed. Acorn from *Quercus suber* has been used in folk medicine, given its potential pharmacologic effects. The aim of this study was to evaluate the effect of acorn infusion (AI) in a primary canine mammary cancer cell line.

A seven-year-old intact female dog was presented with a palpable 2 cm tumour at the right inguinal mammary gland, which was histologically classified as an invasive carcinoma-and-malignant myoepithelioma, histological grade II. Cells were isolated from the fresh tumour tissue and a primary cell culture was established. The AI was prepared with addition of 80 mg of powder in 10 mL of boiled water and left to stand at room temperature for 10 min, and then filtered. Cells were exposed to several AI concentrations (200, 400 and 800 µg/mL) for 48h and 72h. The alkaline comet assay was performed and visual scoring was the method used to evaluate DNA damage.

An increase in DNA repair was observed between 48h and 72h at 200 µg/mL [74.00±11.90 vs 46.50±12.30 arbitrary units (AU)] and 400 µg/mL (69.25±14.30 vs 55.33±3.30 AU). In contrast, a decreased DNA repair was observed at 800 µg/mL (43.00±9.68 vs 51.50±6.22 AU). These data showed that AI presented anti-genotoxicity activity since it contributed to the reduced DNA damage in a dose-dependent way. These findings point to potential novel treatment approaches for CMCs, based on the use of natural compounds. Further investigations should be performed to investigate the molecular mechanisms associated with acorn efficacy differences.

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P26

A Bugs Life with Fungi – Biodiversity Study

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Keywords: Survey, Bugs, Fungi, Health

Insects are responsible for diseases in humans, animals and plants and it can result in major epidemics. Insects can be vectors of diseases caused by viruses, bacteria, protozoa and even fungi. A preliminary study was carried out with the aim of survey which medically important fungi can be isolated from insects. Five insects were studied, European earwig (*Forficula auricularia*), Ant (Family *Formicidae*), Fly (*Musca domestica*), Mosquito (Family *Culicidae*), and Blowfly (Family *Calliphoridae*) in order to study the fungal biodiversity in these animals. Samples were sent to the Medical Microbiology Laboratory of the University of Trás-os-Montes and Alto Douro, where cultures were performed in on Potato Dextrose Agar and Sabouraud Dextrose agar and incubated at 25 ºC and 37 ºC for 3 to 7 days. The terrestrial insects, European earwig and Ant presented *Mucor* spp. and *Aspergillus* spp. respectively. Regarding Blowfly, both *Mucor* spp. and *Aspergillus* spp. where isolated. In relative to the Fly it was isolated *Mucor* spp., *Aspergillus* spp. and *Penincillium* spp. The Mosquito was the insect that presented the greatest variety of isolated fungi (*Mucor* spp., *Aspergillus* spp., *Cladosporium* spp., *Alternaria* spp.). This study allowed increasing the knowledge about fungal biodiversity in insects and their potential as fungal vectors.

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P27

Talaromyces marneffei in animals of a Portuguese shelter. One Health implications

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Keywords: *Talaromyces marneffei;* One Health; Shelter

Talaromyces marneffei is a thermally dimorphic fungus that is endemic in some parts of Asia. The clinical significance of *T. marneffei* is associated with fatal systemic mycosis in immunocompromised patients, as those infected with human immunodeficiency virus (HIV). The aim of this study was to evaluate the occurrence of *T. marneffei* in the fur of shelter animals in Northeast Portugal.

A convenience sample of fur of 156 animals belonging to a shelter was examined. The species were cats (21.8%) and dogs (78.2%). The samples were inoculated in Potato Dextrose Agar medium and Sabouraud Dextrose Agar medium and incubated at 25ºC and 37ºC for 3-7 days. *Talaromyces marneffei* were identified in culture from 2 (1.28%) female stray dogs. The occurrence in dogs was 1.6% (CI 95%: 0.71 – 2.49%).

These animals never travelled to Asia. This finding proves the presence of the pathogen in dogs in Portugal. Further research is required to better understanding the relevance of dogs and their significance for public health as for immunocompromised patients, in a One Health approach.

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P28

Molecular and cytogenetic analysis of satellite DNA 1 in different human cell lines

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Keywords: Satellite DNA; HSAT1; copy number, FISH, chromosome rearrangements

Satellite DNA (satDNA) sequences are tandemly repeated sequences located mainly in the centromeric and pericentromeric regions of the chromosomes, being the main constituent of constitutive heterochromatin. These sequences have been the subject of growing interest by the scientific community since they represent an important organizational component of the human genome, and have been correlated with the occurrence of frequent chromosomal rearrangements like Robertsonian translocations. However, a large part of these repetitive sequences still remains under characterized, namely the satellite DNA 1 (HSAT1), that is distinguished by being the most abundant AT-rich fraction in the human genome. Since the initial studies on this satDNA, that describe its monomer sequence and map HSAT1 on the short arm of the acrocentric human chromosomes, not many information has been gathered. Moreover, HSAT1 has been underrepresented in the reference genome as it was shown recently.

Here, we present a molecular and cytogenetic characterization of HSAT1 in terms of its abundance and physical location in different normal and cancer cell lines. This was achieved by performing qPCR to determine the absolute copy number of this satellite in different cell lines and by using fluorescence *in situ* hybridisation (FISH) to map HSAT1 in the chromosomes of the cell lines analysed. Our results show HSAT1 representativity in the genomes is variable and that this satellite its colocalized with rearranged chromosomes breakpoint regions, indicating that these sequences are potential keyplayers in chromosomal instability.

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P29

ROS generation in plant-pathogen interactions

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Keywords: ROS, H_2O_2, plant pathogen, innate immunity, PTI, RBOHs, apoplast

In plant-pathogen interactions, the goal of the pathogen is to colonize its ecological niche, generally, a host tissue where it can proliferate and cause physiological deregulation and pathological processes. Microorganisms can either penetrate through the plant surface or take advantage of physical injuries or natural openings such as stomata to reach the plant's interior. Once inside, microorganisms can move through the host tissue and spread to other plant parts.

However, plants have developed some defence mechanisms to counteract the pathogens. There are natural preformed barriers such as cell walls, cuticular lipids, or secondary metabolites as a first defence layer. In addition, plants can detect pathogens through pattern recognition receptors (PRR) by recognizing microbe-associated molecular patterns (MAMPs), which are structures related to microorganisms' survival that are widely conserved. In addition, plants can detect damage-associated molecular patterns (DAMPs), molecules derived from the pathogen lytic activity in plant cells.

This pathogen recognition will induce a plant response, denominated pattern-triggered immunity (PTI). This plant response is mediated by the alkalinization of the surrounding tissue to the infection point, the activation of ion fluxes across cell membranes, a *de novo* synthesis of antimicrobial phytoalexins, and the generation of reactive oxygen and nitrogen species (RONS).

Interestingly, the plant ROS producing enzymes include plant NADPH oxidases (NOXs), which are ROS generation respiratory burst oxidase homologs (RBOHs) located in the plasma membrane. Other ROS producing enzymes include cell wall ionic or covalently bound peroxidases and apoplast polyamine oxidases. As a result of their enzymatic activity, these ROS-producing enzymes generate the superoxide anion radical (0_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$). When combined, these reactive oxygen species will overcome the redox homeostasis mechanisms activated in the microorganisms, causing eventually bacterial death.

Here, the ROS producing mechanism used by plants facing pathogens is explained in detail, highlighting the most important ROS producing enzymes and their mechanism of action.

P30

Genomic Template Stability assessed in almond trees of cv. 'Vairo' treated with biostimulants and boron-based fertilizers

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Keywords: Almond, plant biostimulants, ISSR, iPBS, IRAP, RAPD, REMAP

Almond is highly produced in the NE of Portugal, where late frosts during flowering, low precipitation, and high temperature in summer affect productivity and quality. In addition to late-flowering cultivars, plant biostimulants (PBs) and boron-based fertilizers have been used to improve cell division, vegetative growth, photoassimilates rate, and nutritional status of almond trees. Despite the wide use of PBs in multiple food crops, the scientific evaluation of their effects is scarce. Our team previously analyzed the effects of PBs and boron-based fertilizers in the mitotic cell cycle of almonds and verified the intensification of cell division without significant anomalies. This work focused on the molecular characterization of three-years-old almond trees of cv. 'Vairo' was treated with two PBs (based on seaweed extract and free amino acids) and two boron-based fertilizers (applied on soil and leaves) in a rainfed orchard (NE Portugal) using leaf samples collected through the summer of 2019 in treated trees. Three monthly applications of individual PBs based on seaweed extract (AN), amino acids (AA), and boron ethanolamine (BE) and a unique application of boron on the soil (BS) were made. The molecular stability was assayed by comparing with untreated trees using ISSR, RAPD, IRAP, REMAP, and iPBS markers. The molecular data achieved in 'Vairo,' under the edaphoclimatic conditions where it was studied, revealed that AA, BE, or BS treatments induced higher molecular stability, corroborating our previous cytogenetic results.

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P31

Genetic Variability of Dermatophytes Isolated in Wildlife

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Keywords: Dermatophytes, ISSR-PCR, Polymorphism, Wild Animals

Dermatophytes are aerobic fungi that infect keratinized areas, being responsible for dermatophytosis, the most common fungal infection. Since these fungi' morphological and cultural characteristics are insufficient to distinguish them, as they are very close morphologically and physiologically, the microbiological analysis was complemented with a molecular approach. For the lab diagnosis, the samples were isolated from different sources (different wild animals), which were previously grown in DTM medium and then were subcultured to PDA medium. After their growth, the colonies were subjected to staining by lactophenol with cotton blue for microscopic identification. Due to time constraints, only four cultures of *Nannizzia nana* isolated in foxes were analyzed, one of them presented a different color than usual. Total DNA was extracted from the four isolated cultures to perform the molecular analysis, followed by ISSR-PCR. The analysis of the selected ISSR primers allowed to calculate the polymorphism rate and perform a dendrogram using the SM coefficient and the UPGMA method, showing the phylogenetic relationship of the samples under study. The four samples presented about 43% of polymorphism rate among them, and in the dendrogram, two clusters were formed. One cluster had only one sample, being the most phylogenetically distant, while the other cluster had two groups, one with two samples and the other with only one sample. Contrary to what was expected, the most phenotypically different sample is not the most phylogenetically distant.

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Geographic origin and botanical sources: Influence in phytochemical composition and biological activity of honeys

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Keywords: Honey, Geographical origin, Phytochemical composition, Antioxidant capacity

Honey is an important carbohydrate food source as the only available natural sweetener, being used and recognized as a product with nutraceutical qualities and high and diverse medical potential, including antioxidant capacity. In this food matrix, this property is generally associated with its content in phenolic compounds. The geographical origins and botanical sources influence the content in bioactive compounds, being the phenolic profile different if the honey is from different geographical and botanical sources. Therefore, the aims of this study are to identify and quantify the content in total phenols, *ortho*-diphenols, and flavonoids, as well as antioxidant capacity in honey originated from different floral sources of different regions of Portugal and find a correlation.

To evaluate the content in phenolic compounds several assays were employed, as well as antioxidant property including radical scavenging ability (ABTS, DPPH) and ferric reducing power (FRAP) in several honey samples. The content in phenolic compounds in this study yields extremely favourable findings, ranging from 0.154 to 1.212 mg GA g^{-1} for the total phenol content, from 0.049 to 1.033 mg GA g^{-1} for *ortho*-diphenols, and from 0.014 to 0.410 mg CAT g^{-1} for flavonoids content, also verifying antioxidant properties (0.0004-0.0227 mmol Trolox g^{-1}, 0.0002-0.0047 mmol Trolox g^{-1}, and 0.0005-0.0111 mmol Trolox g^{-1}, for ABTS, DPPH, and FRAP, respectively). This fact contributes to the increased economic and/or industrial interests on account of the applications of these components in the food, cosmetics, and pharmaceutical industries, being this natural sweetener a product with several salient therapeutic properties.

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P33

Combination of cytogenetics, genomics and bioinformatics methodologies for the detection and study of complex chromosome alterations

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Keywords: Cytogenetics, Bioinformatics, Chromosomal abnormalities, Chromosomics, Breast cancer

In the past few years, cytogenetics has been remarkably evolving through the development of numerous multidisciplinary techniques. Such progress allowed not only the improvement of the resolution obtained by classical methods, but also the increase of knowledge about genomes. Therefore, the integration of strategies based on PCR (Polymerase Chain Reaction), FISH (Fluorescence *in situ* Hybridization) and CGH (Comparative Genomic Hybridization) was crucial to increase the resolution in the identification of complex chromosomal abnormalities. Nonetheless, the association between cytogenetics and other areas such as bioinformatics and genomics proves to be essential to the intensive study and comprehension of genomes. As a result, the term “chromosomics” was proposed, being an important perspective to be considered in chromosome and genomic studies. Hence, the aim of our work consists in the analysis of a breast cancer case, thus highlighting the need of using bioinformatics and cytogenetic tools in the identification and study of chromosomal abnormalities.

P34

Isolation of *Aspergillus felis* in a Dog From a Shelter - A Emerging Agent of Invasive Aspergillosis in Humans, Cats, and Dogs

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Keywords: *Aspergillus felis;* Dog; One Health; Shelter

Aspergillosis, a mycosis caused by infection with fungi belonging to the genus *Aspergillus*, occurs in a diverse range of human and animal hosts. In humans, aspergillosis is increasingly diagnosed due to the introduction of novel immunosuppressive regimens among patients. *Aspergillus felis* is a fungus that has been reported in chronic invasive pulmonary aspergillosis in humans, invasive fungal rhinosinusitis in cats and disseminated invasive aspergillosis in dogs. The disease in all host species was often refractory to aggressive antifungal therapeutic regimens.

A convenience sample of 156 animals belonging to a shelter was examined for the presence of fungal mycobiota in the fur. The species were cats (21.8%) and dogs (78.2%). The samples were inoculated in Potato Dextrose Agar medium and Sabouraud Dextrose Agar medium and incubated at 25ºC and 37ºC for 3-7 days. *Aspergillus felis* were identified in culture from 1 animal (0.64%), a male dog. The occurrence in dogs was 0.81% (CI 95%: 0.0 – 1.69%).

Since, *A. felis* is an emerging agent of invasive aspergillosis in cats, dogs and humans is required to better understanding the relevance of the isolation of the fungus in the fur and their significance for public health in a One Health approach.

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P35

Aspergillus isolated from the skin of a Mara (*Dolichotis patagonum*)

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Keywords: Rodents, Argentina, Skin, Fungi

Mara (*Dolichotis patagonum*) or Patagonian Hare are rodents that inhabit arid areas of grass and low shrubs. It is an endemic species of Argentina. They are animals that resemble a cross between a rabbit and a small deer, with short greyish brown fur and a white ventral area, long and slightly pointed ears.
In order to monitor the distribution of medical importance fungi in animals, samples were collected from the fur of a Mara from a Portuguese zoo.
The Mackenzie technique was used to collect the samples. Samples were sent to the Medical Microbiology Laboratory of the University of Trás-os-Montes and Alto Douro, where cultures were performed in Dermatophyte Test Medium® (DTM®) and Potato Dextrose Agar (PDA). After the fungus was isolated, it was inoculated in Brain Heart Infusion Blood Agar, for the search for hemolysis.
Dermatophytes were not isolated. The only medically important fungus isolated was the *Aspergillus* genus. Virulence factors such as hemolysins were not found. Knowing the *Aspergillus* genus and its distribution and host variety is extremely important. *Aspergillus* spp. is an opportunistic saprophyte widely distributed in nature. It can cause a wide range of illnesses ranging from allergies to invasive infections in animals and humans. Invasive Aspergillosis is the most serious infection caused by *Aspergillus* spp. It mainly affects the lungs of immunosuppressed patients and is able to migrate to deep organs.

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P36

Isolation of the Fungi with Medical Importance *Acremonium* and *Aspergillus* in the fur of a Panthera (*Panthera pardus*) - A One Health Approach

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Keywords: Wild Cats, Zoo, *Aspergillus*

In order to monitor the distribution of medical importance fungi in animals, samples were collected from the fur of a Panthera (*Panthera pardus*) from a Portuguese zoo.
The Mackenzie technique was used to collect the samples. Samples were sent to the Medical Microbiology Laboratory of the University of Trás-os-Montes and Alto Douro, where cultures were performed in Dermatophyte Test Medium® (DTM®) and Potato Dextrose Agar (PDA). To detect virulence factor in *Aspergillus* genus, the fungus was inoculated in Brain Heart Infusion Blood Agar, for the search for hemolysis.
Dermatophytes were not isolated. The only medically important fungus isolated was the *Acremonium* genus and *Aspergillus* genus. Virulence factors such as hemolysins were not found in the isolate of *Aspergillus*. Species of *Acremonium* are commonly found in soil, decaying vegetation. Infections in humans typically develop following traumatic inoculation and it has a significant role as a cause of onychomycosis. Also, have been reported invasive infections such as osteomyelitis, sinusitis, arthritis, peritonitis, and less frequently central nervous system infections.
Aspergillus spp. is a fungus widely distributed in nature. This genus as medical importance because causes allergies and invasive infections in animals and humans. This study contributes to the knowledge of mycobiota in the skin of wild cats.

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P37

CDKN1C gene in Beckwith-Wiedemann Syndrome

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Keywords: Beckwith-Wiedemann Syndrome, Imprinting, *CDKN1C* gene

The chromosomal region 11p15 encodes several growth-promoting and growth-inhibiting factors, as well as imprinted genes that regulate fetal and postnatal growth, and thereby playing an important role in human growth and development. Deregulation of imprinted genes in the 11p15 region could cause Beckwith-Wiedemann Syndrome (BWS; OMIM #130650) through a few different mechanisms leading to either primary epigenetic alterations or genetic alterations that modify the relative contribution of parental alleles. Imprinting abnormalities on the 11p15 region are the most common cause of BWS, and the presence of pathogenic variants in the cyclin dependent kinase inhibitor 1C (*CDKN1C*) gene accounts for 5-10 % of BWS cases.

CDKN1C gene is a negative regulator of cell proliferation that spans 1943 bp (NM 000076.2) and comprises three exons, two of which are protein coding (protein $p57^{KIP2}$). It inhibits cyclin-dependent kinase complexes (CDK) during the G1 phase of the cell cycle. *CDKN1C* is an imprinted gene localized in the short arm of chromosome 11 (11p15) and is mono-allelically expressed by the maternal allele in the majority of organs. The $p57^{kip2}$ protein is expressed in several tissues including female tissues like placenta and has therefore been implicated in embryonic development.

Some authors defend possible correlations between the molecular etiology and the phenotypic traits, such as the presence of pathogenic variants in the *CDKN1C* gene related with an increased risk of childhood cancer and specific phenotypic traits including cleft palate, umbilical hernia and brain abnormalities.

The pathogenic variants in the *CDKN1C* gene related with BWS diagnosis, the variable allelic expression and associations with certain symptomatic traits make *CDKN1C* an important gene to study within the scope of this syndrome. The purpose of this project is, therefore, to implement the research of *CDKN1C* gene within the context of BWS.

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In vivo biological evaluation of a collagen membrane in a diabetic rat model

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Keywords: Diabetes, Biocompatibility, Biomaterial, Inflammatory Response

Diabetes is a highly prevalent metabolic disease, characterized by increased blood glucose levels, due to a deficiency in the insulin-secreting function of the pancreas and/or a deficient action of insulin on target tissues, which results in complications that impact most body systems. Chronic hyperglycaemia negatively affects bone regeneration and remodelling and increases the susceptibility to several diseases such as periodontal disease and osteoporosis. As such, this study aims to assess the influence of diabetes in the biological response to a subcutaneously implanted collagen membrane focusing on the characterization of the local inflammatory response. In this study, 15 male Wistar rats were used. The diabetic condition was induced in 9 animals via intraperitoneal administration of 55 mg/kg of streptozotocin (STZ), and 15 days later, collagen membranes (Evolution Std, OsteoBiol®, Tecnoss, Italy) were subcutaneously implanted in all animals. The animals were then divided into four groups: Control (Ctr48h; n=3), Diabetes (Ctr48h; n=3), Control (Ctr3w; n=3) and Diabetes (Ctr3w; n=6). Following the implantation (48 hours and 3 weeks), blood samples were collected and analyzed for serum markers, and the skin samples with implanted collagen membranes were processed for histopathological analysis. Diabetic animals showed higher serum values of urea, creatinine, alkaline phosphatase (AP) and alanine aminotransferase (ALT) when compared to the respective control group. In the histopathological analysis, the implanted collagen membranes showed a higher degree of degradation in the diabetic animals than in the control, especially at 3 weeks. We also observed a higher presence of inflammatory infiltrate in the control groups in comparison to diabetic groups, demonstrating the occurrence of a moderate inflammatory reaction in the control animals and a scarce one in the diabetic animals.
The features observed in our work present relevant clinical implications. Therefore, further studies should be carried out in order to elucidate the mechanisms involved in the increased degradation rate of collagen-based biomaterials in diabetic conditions, and its association with the modulation of inflammatory response.

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Analysis of enterotoxin genes in pig samples from a slaughterhouse

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Keywords: *Staphylococcus aureus*, enterotoxins, food safety, pork meat

Staphylococcus aureus is a commensal bacterium that colonizes the skin and mucosal membranes, thought it can also act as an opportunistic pathogen being known to be one of the main causes of nosocomial and community-acquired infections. Besides the ability to cause severe infections and diseases, *S. aureus* also presents the potential to develop antimicrobial resistance, thus increasing the risk to human health. The expression of virulence factors, such as enterotoxins, defines the pathogenicity of *S. aureus* and the degree of infection. Hence, the aim of this study was to identify the specie *Staphylococcus aureus* from 16 samples of pigs surfaces collected at a slaughterhouse along with the study of enterotoxin genes and their frequency. The application of PCR technique with specific primers, and subsequent agarose gel electrophoresis, allowed the recognition of *S. aureus* in collected samples with the detection of *nuc* gene. Multiplex PCR allowed the identification of genes encoding enterotoxins – *sed*, *seg*, *sei*. According to our results, *sed* gene wasn't found in any samples, *seg* gene was presented in 62.5% of samples (10/16) and *sei* gene had a prevalence of 50% (8/16). With this study it was possible to recognize the high prevalence of virulence genes in *S. aureus* from pork meat, as well as confirm that the majority of samples are enterotoxigenic.

P40

A Smiling Future: Angelman Syndrome

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Keywords: Gene dysfunction, neurodevelopmental disorder, rare diseases, nervous system, healthcare, attenuation treatments

Angelman syndrome is a complex genetic and rare disorder that affects 1 in 12000 to 20000 individuals and, in spite of allowing a normal life expectancy, this disease disturbs mainly the nervous system and it is characterized by a delayed development, intellectual disabilities, severe speech impairment and mobility, becoming noticeable in early childhood and increasing its severity over the years. Also, it can cause epilepsy and features like small head size.

The loss of function of UBE3A gene, located in chromosome 15 is the cause of Angelman Syndrome. Usually, people inherited one copy of the gene from each parent, that is activated in most of body tissues, except some regions of the brain. In these areas, only the maternal copy is activated, thus events of chromosome changes or mutations constitute an issue.

Despite all the symptoms and features, children with this syndrome often have a happy and excitable personality, with frequent smiles and laughter.

Reports from parents, when their child is diagnosed with this rare disease, state feelings of hopelessness and devastation. However, from that moment, these children, their families and caregivers are not alone anymore, receiving a better and specialized follow-up, to help increase their capacities and autonomy, not just from clinical support, but also from associations that assist in their daily challenges.

The diagnosis is often challenging, consisting of clinical and laboratory findings, followed by molecular genetic testing.

There is yet no cure for the disease and management is mainly symptomatic, such as anti-seizure medication, various therapies (physical, occupational, communicational, behavioural) and dietary changes. Nowadays, there are a number of associations that fund new researches focused in gene replacement therapy, paternal gene activation and downstream or symptomatic therapy.

P41

Staphylococcus aureus virulence genes: detection and identification in samples from food establishments

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Keywords: *S. aureus*, food safety, foodborne illness, enterotoxin genes, hemolysin alpha gene, toxic shock syndrome toxin 1 gene

Staphylococcus aureus is a commensal bacterium, with up to 30% of humans presumed to be persistent carriers. The bacterium can cause invasive, life-threatening infections, which means it is of utmost importance for food handlers to take all necessary precautions and avoid cross contamination of the food[1]. Infections with methicillin-resistant *S. aureus* strains have become more common, especially in nosocomial settings. The potential damage that this bacterium can cause depends on the virulence genes it possesses. The aim of this study was to identify and confirm the presence of *S. aureus* strains and determine which virulence genes were present in 13 samples obtained from food handlers' establishments. After performing swabs in the hands of food handlers, 71 *Staphylococcus aureus* isolates were obtained and were stored at -20ºC, after their cultivation in Brain-Heart Infusion (BHI) broth for 20-24 hours and addition of 45% glycerol. Using PCR, followed by agarose gel electrophoresis, the presence of the bacterium was confirmed in all the selected samples. Three enterotoxin genes - *sed*, *sei* and *seg* – were tested in Multiplex PCR. Separate PCR reactions were used to test for the hemolysin alpha gene (*hla*) and for the toxic shock syndrome toxin 1 gene (*tsst-1*). The 13 isolates contained virulence genes *seg, sei, hla*, and 12 (92%) contained the *tsst-1* gene. None contained the *sed* gene. Enterotoxins typically cause vomiting and diarrhoea, as they interfere with bowel function. The presence of these genes is worrying, as these microorganisms were collected from the hands of food handlers and cross-contamination of foods is highly likely if hands' hygiene was not properly performed. It can lead to outbreaks of Staphylococcal foodborne poisoning.

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Conventional cytogenetics and FISH analysis of 10 samples with a suspected diagnosis of Multiple Myeloma in CHTMAD

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Keywords: Multiple Myeloma, Conventional Cytogenetics, FISH

Multiple myeloma (MM) is a heterogeneous clonal malignancy of plasma cells accounting for 300 to 500 new cases per year, 15% of hematologic malignancies, and 1% of all diagnosed cancer cases in Portugal. MM is characterized by cytogenetic and molecular abnormalities with significant impacts on prognosis. Chromosomal abnormalities are present at diagnosis and can evolve during the progression of MM. Metaphase karyotyping and fluorescence in situ hybridization (FISH) are considered the standard diagnostic procedures performed in clinical practice. When conjugated, the detection rate of chromosomal abnormalities is around 90%. The most frequent cytogenetic alterations are aneuploidies, reciprocal translocations involving the immunoglobulin IgH gene such as t(4;14) and t(11;14), monosomy or deletions in the long arm of chromosome (#) 13, loss of the short arm of #17 and gains in the long arm of #1. The authors present conventional cytogenetics (CC) and FISH results of 10 samples referred to Cytogenetic Laboratory of CHTMAD, with a suspected diagnosis of MM. CC and FISH were performed according to standard techniques. FISH probes included del(13)(q14), del(17)(p13), t(4;14) and t(11;14). Cytogenetics analysis followed the standard guidelines (ISCN 2020). In the 10 analysed samples, 5 presented chromosomal alterations associated with MM [aneuploidies, del (13q), del (17p)]. The FISH technique not only confirmed the results obtained by conventional cytogenetics but also detected alterations not founded by CC, namely t(4;14). However, CC allowed the identification of other abnormalities, such as a rearrangement in #11 and a case with a complex karyotype that has prognostic value (associated to poor prognosis and disease evolution). The results obtained are consistent with those described in the literature and revealed the importance of CC analysis together with FISH in the genetic study to predict treatment responses and prognosis in patients with a suspected diagnosis of MM.

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Genetic characterization of *Quercus rubra* and *Quercus petraea* provenances

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Keywords: *Quercus rubra*, *Quercus petraea*, Genetic analysis, ISSR

The species *Quercus rubra* and *Quercus petraea* belong Fagaceae, are widely distributed throughout North America and Europe, respectively. *Quercus rubra* was introduced around Europe through animal dispersal, especially in mountainous areas, while *Quercus petraea* is more common in hillside areas.
The present study was developed in the Vegetal Citogenomic Laboratory of the University of Trás-os-Montes e Alto Douro (UTAD) in the framework of the *REINFFORCE* Project, (*Resource infrastructures for monitoring, adapting and protecting european atlantic forests under changing climate*). The main objective of this was to analyze the diversity and genetic relationships of 28 individuals from different origins of *Quercus rubra* and *Quercus petraea*.
In this study, 8 ISSR *primers* from the *University of British Columbia* (UBC) were used for the amplification of 11 samples of *Q. rubra* and 3 ISSR *primers* for the amplification of 17 samples of *Q. petraea*
Molecular data obtained with the ISSR marker were used in the construction of UPGMA method of genetic similarity, which confirmed the efficacy of *primers* used to discriminate individuals by provenance for *Q. rubra*, but for *Q. petraea* the *primers* used were ineffective in discriminating individuals by provenance.
In conclusion, this research enabled the selection of *primers* for the amplification of ISSRs in *Q. rubra* and *Q. petraea*. The selected *primers* demonstrated the potential for polymorphism detection among the 28 individuals and species and provenance discrimination.

P44

Molecular epidemiology and phylogenetic studies of dermatophytes of animal origin

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Keywords: Dermatophytes; Epidemiology; Phylogeny: Polymorphism, ISSR

Dermatophytes are a group of filamentous pathogenic fungi that invade and infect keratinized tissues. The main objective of this study was the detection, identification, and characterization of dermatophytes samples at the microbiological, phylogenetic, and molecular levels.
The samples were collected using the Mackenzie technique in the Laboratory of Medical Microbiology at UTAD, were inoculated into Dermatophyte Test Medium (DTM) and Potato Dextrose Agar (PDA). In this study, 15 samples of animal origin were analyzed. Through the application of the Lactophenol with Cotton Blue technique, it was possible to identify 4 of these samples, from wildlife origin, as dermatophytes, more specifically *Nannizzia nana*, a zoophilic and geophilic dermatophyte fungus that affects animals, mainly pigs. These 4 samples were then transferred to sterile distilled water for later molecular analysis in the UTAD Applied Molecular Genetics laboratory. For this, DNA extraction was performed using the Plant/Fungi DNA Isolation Kit, and then, PCR reactions were performed using 14 ISSRs primers. The occurrence of dermatophytes in this study was 26.6%. At molecular level, it was possible to observe around 58% of polymorphism rate. The analysis of the dendrogram showed that despite all the samples being of the same species, there was an evident separation of the 4 samples into two main groups with a correlation coefficient about 0.62. This study allowed to increase the knowledge about the phylogenetic and epidemiological level of this species in animal samples.

P45

Effect of growth regulators on mint micropropagation

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Keywords: Mint, Growth regulators, Micropropagation, BAP, NAA, MS medium

The Green Mint, or common mint, scientific name *Mentha spicata,* is a perennial herbaceous plant of the family *Lamiaceae*. The fact that the essential oils present mainly in leaves, can be used for medicinal and culinary purposes, makes it one of the most grown plants all over the world.
Micropropagation, one in vitro culture technique for plant multiplication, allows to obtain from "mother plants", many genetically equal plants free of contamination, in a short time. This can be important if we want to have plant available throughout the year
Under this work, five parameters were evaluated over a period of six weeks: number of nodes, number and size of shoots and roots. To evaluate the effect of growth regulators on the micropropagation of mint, specifically of cytokinin BAP (6-Benzylaminopurine) and auxin NAA (α-naphthaleneacetic acid), explants were subjected to five different culture media: MS medium (Murashige & Skoog); MS + 1 (mg/L) BAP; MS + 2 BAP; MS + 1 BAP + 0.2 NAA; MS + 2 BAP + 0.2 NAA. Finally, they were placed in walk-in culture chambers at 24ºC. Regarding the number of shoots in the different media, the ones with the highest number of shoots were the media MS + 1BAP and MS + 2BAP + NAA. Contrary to expectations, regarding the size of the shoots, number of nodes and number and size of roots, MS medium presented the plants with the best characteristics. Over these six weeks, all parameters evaluated increased gradually.

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Biochemical and genetic evaluation of *Castanea sativa* Mill. provenances

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Keywords: *Castanea sativa*, ISSR, biochemical parameters, climate change

The evaluation of the global warming effects on forest growth requires prior and improved knowledge on the specific responses of each species to different climate changes that may arise. Thus, the identification of the changes that occur during the interaction organism–environment (biochemical, physiological, or morphological responses) allows us to characterize and understand how a certain species can survive in unfavourable environmental conditions.

The chestnut is a monoecious plant of great size and longevity, integrated into the deciduous oak, which grows well in temperate climates, tolerating severe winter colds but very sensitive to late frosts. *Castanea sativa* Mill. is predominant in Portugal, where it has a relevant place at the socioeconomic level with interesting characteristics not only from a nutritional point of view but also related to the beneficial health effects associated with its consumption.

The provenances studied in this work are inserted in the Arboretum of the University of Trás-os-Montes and Alto Douro (UTAD), which in combination with the REINFFORCE project, provided an area for cultivation within a framework of analysis and study of all discriminatory features. The aim of this work was to evaluate the intraspecific genetic and biochemical diversity of *Castanea sativa* Mill. individuals, from two populations belonging to different European regions, using molecular ISSR markers as well as the quantification of the content of photosynthetic pigments, phenolic compounds, soluble sugars, and starch. The study of the biochemical parameters revealed no statistically significant differences between sources and the obtained concentration for each parameter was concomitant with well-adapted organisms. On the other hand, the genetic assessment revealed the existence of genetic variability between the two populations under study, which supports that they belong to different locations.

P47

A study of the *TP53* gene in renal carcinomas of roe deer

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Keywords: Roe deer, *TP53, Capreolus capreolus*, p53 protein, PCR, immunohistochemistry, renal carcinoma

Roe deer is one of the most abundant ungulates and a major game species in Europe. However, detailed reports on its pathological conditions are infrequent. In the present work we describe two renal carcinomas from roe deer using immunohistochemical and molecular methodologies. Mutations occurring in the *TP53* tumour suppressor gene have been described as one of the most important prognostic factors of this type of cancer. Two renal carcinomas of roe deer from the archive of the Histology and Pathological Anatomy Laboratory were analysed by immunohistochemical and molecular evaluation. Immunohistochemical stains for broad-spectrum cytokeratin (clone AE1/AE/3, Dako), vimentin (clone NCL-L-VIM-V9, Novocastra) and p53 (clone DO-7, Thermo-Scientific) were performed. For molecular evaluation, DNA extraction was performed from paraffin-embedded tumour samples. PCRs were performed by amplifying a small fragment of exon 4 of *TP53* gene.

In terms of molecular characterisation, we were successful in obtaining a 265 bp amplicon corresponding to exon 4 of the *TP53* gene. As for the immunohistochemical characterisation, we detected a p53 protein staining, higher in the 2003 case (9.5%) compared to the 2019 case (3.2%). We also obtained vimentins and cytokeratins staining.

The clarification of cancer's molecular pathways through the characterisation of spontaneous tumour models in different species is crucial, promoting an increase in knowledge about the changes that cause the onset of renal carcinoma.

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P48

Molecular Identification of *Dictyocaulus capreolus* in roe deer

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Keywords: *Dictyocaulus*, parasites, ITS2, *Capreolus capreolus*, roe deer, multiplex PCR

Dictyocaulus spp. are important parasites of the respiratory tract of domestic and wild ruminants. The characterization of the parasite's host ranges, geographic distribution and pathological findings are of extreme importance. The genus *Dictyocaulus* is represented by several species, including *D. capreolus*.

Thirty four roe deer were necropsied at UTAD between 2017 and 2021. Adult lungworms were collected and the morphological and molecular characterization were performed. DNA extraction was done from intact specimens followed by amplification of a region of ITS2 that can distinguish *Dictyocaulus* species. Sequencing of the amplicons of two specimens was performed.

The morphological analysis showed nodular lesions and/or areas of lung consolidation in nine animals (26.5%), with one animal presenting *Dictyocaulus* sp. in the bronchi. A parasitic pneumonia was confirmed histologically in eight animals (23.5%), characterized by the presence of nematode larvae and eggs, concomitant with inflammation and smooth muscle hypertrophy. The molecular method based on multiplex PCR revealed a single amplicon with a length compatible with *D. capreolus*. The purification and sequencing of this product allowed us to analyse a sequence with 388 bp. The BLAST analysis confirmed that this sequence belongs to *D. capreolus* with the accession number MZ746962.1. A polymorphic site (C/T) was highlighted in position 101, confirming that the two individuals under analysis were heterozygotes.

It is important to validate molecular markers with potential application in systematic, population genetic or molecular epidemiological studies. The correct diagnosis of lungworm species and a deep knowledge of host ranges and transmission patterns of *Dictyocaulus* spp. are crucial for reducing the risk of cross-transmission between wildlife and livestock species.

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P49

Generation of Porcine Reproductive and Respiratory Syndrome (PRRS) resistant pigs

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Keywords: PRRS, CD163, SRCR5, PAM, PMM, PMBC

Porcine Reproductive and Respiratory Syndrome (PRRS) is an infectious disease that causes major economic losses worldwide. It is caused by the PRRS RNA virus (PRRSV), which infects porcine cells of the monocyte-macrophage lineage. Several factors involved in the entry of the virus into the cell have been identified, including the scavenger receptor CD163 or haptoglobin scavenger receptor, which is expressed in specific subtypes of macrophages, such as those of the respiratory system. Its extracellular portion has 9 cysteine-rich domains (scavenger receptor cysteine-rich, SRCR), of which SRCR5 appears to be involved in the infectious process.

The CRISPR/Cas9 system was used to delete exon 7, which codes for the SRCR5 domain. Microinjected zygotes were transferred into the oviduct of recipient females and 32 piglets were born. Four (12.5%) were obtained with the expected deletion, of which three were mosaic and only one, pig 345, showed the precise exon deletion. Pigs 310 and 345 were crossed to obtain an F1 consisting of 6 heterozygous, 4 wild-type, 1 homozygous (630) and 1 biallelic (629) pigs.

Peripheral blood monocytes (PBMC) from ΔSRCR5 animals showed full differentiation potential towards macrophages (PBMC-derived macrophage, PMM). No significant difference in CD163 expression was observed between wild-type and modified animals, while CD163 retained its biological function of haemoglobin-haptoglobin uptake.

Subsequently, pulmonary alveolar macrophages (PAMs) and PMMs were inoculated with the 3 subtypes of PRRSV-1 and 2 strains of PRRSV-2. These tests demonstrated that macrophages from ΔSRCR5 pigs are resistant to the virus.

The potential generation of ΔSRCR5 pigs could lead to an increase in global livestock production and an increase in animal welfare.

P50

Study of genotoxicity mechanisms arising from subacute exposure tramadol

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Keywords: *In vivo* essays, Tramadol, Genotoxicity, Hepatotoxicity, Neurotoxicity

Tramadol use is associated with adverse reactions and multiorgan toxicity. The 8-hydroxiguanosin (8-OHdG) is a biomarker of oxidative stress damage at the DNA level, so its quantification allows estimating the damage in this nucleic acid. In turn, the *apurinico/apyrimidinic endonuclease* 1 (Apex1) gene for a DNA-reparation enzyme, thar is produced when there's oxidative damage.
This research study aimed to investigate whether exposure to tramadol, at its maximum recommended daily dose, during the period of 14 consecutive days, causes damage on genetic material in liver cells and the cerebral cortex of Wistars rats.
Samples of liver tissue and cerebral cortex of Wistar rats (*n* = 6) subacute exposed to tramadol 50 mg/kg and from a control group (*n* = 6) administered analogously with saline solution. Oxidative stress at DNA level was evaluated by quantifying 8-OHdG, using the Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation) (Cell BioLabs). Apex1 gene expression was analyzed in cells of the cerebral cortex by real-time quantitative PCR using NZSpeedyTM Green Supermix (NZYTech). The 18S ribosomal RNA (18S rRNA) coding gene was used as a housekeeping gene for loading control purposes.
It was found that the liver cells and cerebral cortex of the group of animals exposed to tramadol had, respectively, a concentration of 8-OHdG 2.0 and 1.8 times higher than those of the control group. It was found that the Apex1 gene was 1.8 times more expressed in the cells of the cerebral cortex of the tramadol animals compared to the control animals.
The results suggest that the damage caused to the DNA of liver cells and the cerebral cortex, by repeated exposure to a clinically relevant dose of tramadol, manifests itself predominantly at the chemical/oxidative level. In this sense, it reinforces the need for a careful prescription of opioids.

P51

Analysis of variability and genetic structure of a roe deer population using mtDNA: preliminary results

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Keywords: Roe deer, populations, genetic structure, mitochondrial DNA

Capreolus capreolus, commonly known as roe deer, is a native mammal from Europe, that belongs to *Cervidae* family. This herbivore has a widespread geographical distribution which extends from the Iberian Peninsula to the north of Scandinavia, being found as well in Turkey, Israel, and Jordan.

In the latest four centuries there have been a significant number of fluctuations in this deer population, especially due to anthropogenic activities, like hunting, which led to a decrease in size and distribution of roe deer. These alterations combined with translocations of animals of the same species, can drive to meaningful consequences on the genetic structure, diversity, and fitness of populations. Therefore, the study of the diversity and genetic structure of roe deer populations is of great importance.

Several studies have been carried out, using mitochondrial DNA (mtDNA) as a molecular marker, since its ease to amplify, due to its number of copies in a cell, is cheap to work and have lower recombination.

The aim of this work is to estimate the genetic diversity and differentiation of roe deer individuals using mtDNA. In this poster we present some of the preliminary results using 30 samples from different geographical areas of roe deer genomic DNA.

P52

Genetic characterization of new hybrid chestnut rootstocks applying ISSRs and iPBs markers

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Keywords: *Castanea sativa;* ColUTAD, ISSR, iPBS, genetic characterization, *Phytophthora cinnamomi*

Chestnut crop (*Castanea sativa* Mill.) is currently considered a strategic activity in the Portuguese economy, especially in the mountainous lands of the interior North side of Portugal where crop alternatives with economic sustainability are scarce.
Due to Anthropogenic Climate Changes (APC), new abiotic and biotic challenges have appeared, which have caused the decline of chestnut orchards. Thus, the spread of the diseases of this species was accentuated, highlighting ink disease, caused by the soil oomycete *Phytophthora cinnamomi* Rands. Finding and producing chestnut trees resistant to these diseases resulting from breeding programs must be a priority.
The aim of this study, developed at the University of Trás-os-Montes and Alto Douro, is to characterize genetically the chestnut hybrid generation (F2 generation) with resistance against ink disease to be in candidates to new rootstocks. In order to carry out this work, fifteen samples were collected from plants resulting from controlled pollinations between the hybrid ColUTAD and *C. sativa* (F2 generation) and one sample of the ColUTAD clone (from F1 generation). After DNA extraction from leaves and amplification of molecular markers, “Inter-Simple Sequence Repeat” (ISSR) and “inter-primer binding site” (iPBS), a total percentage of polymorphism of 92% and 98% were detected, with each marker respectively. Through the analysis of the results of the dendrogram based on the combination of the totality of the data of the ISSR and iPBS markers, a level of similarity between the 16 individuals of 38% was observed, with jaccard coefficient values varying from 0.38 to 0.75.
This work allowed to have a preliminary genetic characterization of this material that resulted from the introgression of *C. sativa* genes into the ColUTAD hybrid rootstock, genotypes that are closer to the national varieties without losing resistance to the ink disease.

P53

Searching for a new purpose of old drugs in COVID-19 fight: targeting the S protein by Molecular Docking

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Keywords: COVID-19, pandemic, spike protein, drugs, molecular docking, bioinformatics

In 2019, the world was struck by the first pandemic of the 21st century caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). COVID-19 belongs to the *Coronaviridae* family and is known for its high spread capability. This RNA virus uses its spike protein to enter the cell by binding to the angiotensin-converting enzyme 2 (ACE2), at this membrane protein receptor binding domain (RBD) of the host cell, thus causing infection.

The severity of the pandemic along with the initial lack and afterward incomplete effectiveness of vaccines led to an increased need for therapies. To fasten this process, molecular docking is being used for the repurposing of existing drugs due to the great advantages of this computational tool. This methodology allows to in silico identify the drugs with the highest potential to either block the spike protein of the virus, or the ACE2 of the host cell.

In this work we present a brief review on the recent findings of some virtual screening studies focused on the candidate compounds that present the capacity to inhibit the spike protein (RBD, thus preventing infection). According to these studies, 12 compounds were selected, being their effectiveness tested by molecular dynamics simulation. The final steps will be to validate these results in animal experiments and clinical trials, but this powerful bioinformatics' strategy greatly decreases the response time and costs of a therapy so urgently needed.

P54

Pre-harvest application of calcium and *Ascophyllum nodosum* on sweet cherry: effect on cuticle related genes in fruits with and without cracks

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Keywords: Calcium, cherry cracking, gene expression, seaweed, sweet cherry

Sweet cherry tree is one of the most important crop worldwide, producing fruits with high economic importance and also one of the most affected by cracking, a severe physiological disorder that can occur during fruit development and ripening, affecting its commercial value. Thus, sweet cherry trees from cultivar Burlat installed in an orchard located in Resende region were selected to study this disorder. The effect of pre-harvest application of calcium (150 g/hL and 300 g/hL), seaweed (*Ascophyllum nodosum*) based-biostimulant (75 mL/hL and 150 mL/hL) and a combination of both nutrients (300 g/hL of calcium and 150 mL/hL of seaweed) on gene expression was studied. Once the cracking can occur due to genetic factors, fruits with and without cracks were collected at maturity stage, total RNA was extracted from fruit exocarp and then was reverse transcribed to cDNA, in order to study the expression of some cuticle related genes, such as *PaExp1*, *PaExp2*, *Paβ-Gal*, *PaEG* and *PaPIP1;4*, by qPCR. The studied genes presented statistical differences among different treatments and also among fruits with and without cracks. In general, higher gene expression was observed in fruits treated with the low dose of calcium (in fruits with and without cracks) and also in cherries without cracks treated with the high dose of seaweed, suggesting that these nutrients have considerable effect in molecular mechanisms involved in cherry cracking. However, these results need to be complemented with more phenological growth stages of fruits and other cracking related genes.

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P55

Safety of an elderberry (*Sambucus nigra* L.) extract: an oxidative DNA damage study

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Keywords: Elderberry, genotoxicity, comet assay, oxidative damage, natural colorants

Elderberry (*Sambucus nigra* L.) is a plant that has long been used in folk medicine. Its berries are highly concentrated in flavonoids, particularly anthocyanins, well-known for their colorant and antioxidant properties. Given that oxidative DNA damage increases the likelihood of modifying genomic function, the elderberry's antioxidant properties are particularly interesting. The aim of this study was to evaluate the degree of DNA damage (and oxidative DNA damage) in mice given an elderberry extract (EE) to assess its potential as a natural colorant.

The anthocyanin profile was determined using HPLC-DAD-ESI/MS. The study was approved by the Portuguese Veterinary Authorities. Twenty-four eight-week-old female FVB/n mice were divided into four experimental groups (n=6/group) and given three different EE concentrations dissolved in drinking water for four weeks: Group(G-)II (12mg/mL), G-III (24mg/mL), and G-IV (48mg/mL), while G-I (control) received only tap water. The alkaline (pH>13) comet assay was carried out in mononuclear blood cells, with and without formamidopyrimidine DNA glycosylase (FPG) treatment. This enzyme converts oxidized purines into DNA single-strand breaks, allowing the detection of oxidative DNA damage. The comets were classified by visual scoring.

The predominant anthocyanins detected were cyanidin-3-*O*-sambubioside-5-*O*-glucoside and cyanidin-3-*O*-sambubioside. The genetic damage index was similar in all experimental groups (p>0.05), but, with the addition of Fpg (GDI_{Fpg}), G-III had significantly lower values (p=0.04) than the control group.

We conclude that the extract was not genotoxic and, as expected, appeared to protect from oxidative DNA damage, with a favourable effect on GDI_{Fpg}. These results suggest that this extract might be used as a natural colorant.

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P56

FA-SAT molecular and cellular characterization in human cancer cells

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Keywords: FA-SAT DNA, FA-SAT RNA, human cancer cells, molecular characterization, cellular characterization

Satellite DNA sequences (satDNA) were considered as junk DNA for many years. However, recent studies have proved that satDNA sequences are transcribed yielding satellite non-coding RNAs (satncRNAs), being involved in several cellular pathways and in diseases, such as cancer.
FA-SAT is the major satDNA sequence of the domestic cat. Recently, it was proved that FA-SAT is conserved and transcribed in several Bilateria species, including human. The FA-SAT DNA and RNA profiling in cat mammary tumors revealed interesting insights about its regulation by epigenetic mechanisms. In which concerns to its cellular function, FA-SAT ncRNA interacts with PKM2 protein in the cell nucleus, making the switch between proliferation and apoptosis both, in cat and human cells. However, in human cancer cells, the molecular and cellular profiling of FA-SAT needs to be further studied.
In this sense, we designed a comprehensive approach, using 3 human non-tumor cell lines and 12 human cancer cell lines from different type of tumors to trace the FA-SAT DNA and RNA profile. Regarding the FA-SAT DNA levels (analyzed by real time qPCR) in human cell lines, no biological significant changes were observed. However, the FA-SAT RNA amount (quantified by real time RT-qPCR) revealed to be decreased in two tumor cell lines HeLa, H1975 and a non-tumor cell line GM12878. Furthermore, no correlation between the FA-SAT DNA and RNA levels was found, suggesting its regulation by epigenetic mechanisms. Also, we analyzed several cell lines by RNA-FISH, and it was possible to verify that, despite its different FA-SAT transcription levels, its nuclear location is maintained in human cell lines, similarly to the reported for cat cell lines.
Here, we present the FA-SAT DNA and RNA profiling in human cancer cells. This is the first step for understand the role of this essential key player in cellular pathways and in the oncogenic process.

P57

Semiquantitative analysis of genes *PaEXP1* and *PaADPG1* under foliar application of potassium and magnesium in sweet cherry

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Keywords: Cracking, Cv. Burlat, gene expression, magnesium, potassium, sweet cherry

Due to its chromatic, aromatic and nutritional attributes, and its effects in human health, the fruits of *Prunus avium* L., known as sweet cherry, have a particular interest in different parts of the world. Cracking is considered the major problem in cherry production since it affects many cherry cultivars with a high incidence. It's known that the expression of genes involved in cell wall modifications may be associated to cracking in sweet cherry, such as *PaEXP1* and *PaADPG1* genes. *PaEXP1* encodes an expansin, a non-enzymatic protein whose their increased expression can leads to an increase in fruit size before the ripening phase, while *PaADPG1* encodes a polygalacturonase, a pectin degrading enzyme required for fruit abscission.

The goal of this work was to better understand how the application of different concentrations of potassium (50 g/hL and 100 g/hL) and magnesium (125 g/hL and 250 g/hL) affects the expression of these two genes, through a semiquantitative analysis, in *Prunus avium* L. fruits collected at the green/red and red maturation phases. For this, total RNA was extracted from fruit exocarp and then cDNA was synthetized. To validate the results, a housekeeping gene was used as control, which maintained their expression to all treatments and maturation stages. The results showed differences among treatments and maturation stages for *PaEXP1*, presenting, in general, higher expression in red stage. For *PaADG1*, the results were similar in all treatments and maturation stages, however, treatments with potassium appears to lead to a higher expression.

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P58

Evaluation of the presence of *tsst* and *hla* virulence genes in samples from a slaughterhouse

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Keywords: *Staphylococcus aureus*, virulence genes, food safety, meat

Staphylococcus aureus is one of the main pathogens found in meat for human consumption, being the main cause of several nosocomial infections and diseases. From its antibiotic resistance, comes an increased risk to human health.
The expression of several genes, such as the *nuc* gene, the *hla* gene and the *tsst* gene, defines the degree of pathogenicity of this bacterium. The aim of this study was the identification of *Staphylococcus aureus* from 16 samples of pigs surfaces at slaughterhouse, as well as characterizing the genes mentioned above and their frequencies.
To detect the presence of *S. aureus* in the samples collected, i.e., identification of the *nuc* gene, as well as to identify the *hla* and *tsst* genes, the PCR technique with specific primers and subsequent electrophoresis in agarose gel, were used.
According to the obtained results, the *hla* gene was predominant, with a prevalence of 75%. The *tsst* gene was present in 56.3% of the samples.
With this work, it was possible to conclude that *Staphylococcus aureus* was present in several samples, highlighting the elevated prevalence of virulence genes, which demonstrates the risk of contaminated pork coming to consumers.

P59

Identification and evaluation of virulence genes in samples of the *Salmonella* spp. genus

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Keywords: *S. Typhimurium*, *S. Enteritidis,* virulence, *sopB*, *stn*, infection

Salmonella is a genus of Gram-negative bacteria which is responsible for a high number of foodborne bacterial infections. This microorganism is the most common infection agent in animals raised for commercial purposes, which causes great economic losses. The infection caused by *Salmonella* represents a major public health problem worldwide due to hygienic and sanitary conditions, availability of water and food treatment, being the main transmission route through the contaminated food of animal origin. This genus of bacteria has a very complex virulence system, since there are several genes involved. These virulence genes can be classified into two categories: genes located on the chromosomes and genes located on the plasmid that contribute to virulence. In this study, 11 samples of the *Salmonella* genus obtained from swine carcasses were analysed. All samples were tested using the PCR method for six genes associated with virulence: *stn, spvB, sopB, orgA, spaN* and *sipB*. The last three genes were analysed simultaneously in a Multiplex PCR reaction. All samples had a positive result for the *sopB* gene and a negative result for the *spvB* gene. The *stn* gene was detected in 10 of the 11 analysed samples. Additionally, in the Multiplex reaction, only one of the samples showed a negative result for the three studied genes. The results of the study on virulence genes showed the pathogenicity of *Salmonella* serovars and the challenges it presents in the swine production chain. *S. Typhimurium* and *S. Enteritidis* represent the serovars that are most often isolated in pork, therefore, those that are most associated with the disease in swine and humans.

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Phylogenetics relationships within the genus *Vigna* inferred by SSR markers

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Keywords: Legumes, germplasm diversity, phylogenetic relationships, microsatellites

Climate changes cause several losses in crops' production and promote the occurrence of new pests and diseases. These negative impacts can be mitigated by the development of new varieties, with greater tolerance to high temperatures, droughts, low soil fertility and resistance to pests and diseases. The genetic diversity evaluation of underused cultivated germplasm and wild relative crops will be crucial to obtain new varieties. Microsatellites (SSRs), are the molecular marker per excellence for these studies.

The genus *Vigna* belongs to the family Leguminosae and includes more than 150 cultivated and wild species distributed throughout the world. This genus has great variability and contains some of the most important cultivated legume species worldwide, as cowpea (*Vigna unguiculata* L. Walp.) and mung bean (*Vigna radiata* L. Walp.), which are of great social and economic importance across Africa and Asia.

To assess the genetic diversity and phylogenetic relationships in the genus *Vigna*, a set of three SSR loci was analysed in 33 accessions, including four different *Vigna* species and six subspecies of *Vigna unguiculata*. A total of 12 alleles and 16 genotypes were detected, of which eight were considered unique. The three loci amplified proved to be polymorphic and the total number of alleles ranged from two to five alleles per locus. The dendrogram obtained revealed a coefficient of similarity varying from 45 to 100%. This study allowed to assess the diversity within the genus *Vigna* and *Vigna unguiculata* subspecies and to analyse the phylogenetic relationships between them. The study will be complemented in the future with the analysis of a greater number of SSR loci.

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COVIFENZ®, the first plant-based vaccine against Covid-19

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Keywords: Agroinfiltration, Medicago, molecular pharming, *Nicotiana benthamiana*, virus-like particle

On the 11th march 2020, the World Health Organisation declared Covid-19 as a pandemic that spread across 114 countries. Since then, more than 450 million people have been infected and more than 6 million people from all around the world have died. At that moment Medicago, a Canadian pharmaceutical company, claimed that they would be able to produce a new vaccine just 3 weeks after the antigenic sequence of the virus was known. Two years later, on 24th February 2022, Health Canada announced the approval of COVIFENZ®. Actually, the objective of this bibliographic work is to explain how this vaccine is obtained and its results.

COVIFENZ® vaccine contains recombinant virus-like particles (VLPs) synthesised in plants. VLPs mimic SARS-Cov 2 viruses because their coats (capsids) are very similar and have the Spike glycoprotein. Nevertheless, they have no genetic material inside. So, VLPs are not infective although keep intact the ability to promote a good immunogenic response.

Medicago produced the VLPs in *Nicotiana benthamiana* plants because this species offers many advantages as bioreactors in molecular pharming. Plants are infiltrated with *Agrobacterium tumefaciens* strains genetically modified in such a way that the transient and heterologous expression of the vector in plants give rise to the efficient synthesis of VLPs.

Dosage contains apart from VLPs (3.75 µg), AS03 adjuvant (0.25 mL) and excipients, held under cold chambers between 2-8 ºC. 0.5 mL are administrated via intramuscular and its efficacy was determined about 71% after Phase 3. In addition, there is zero risk because plant pathogens don't affect humans and vice-versa.

The antigen is then uptaken by Antigen-presenting Cells (APCs), so response is granted to be optimum as it induces both T cells and antibodies production by B cells, it is also secure for all ages due to plant origin.

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Probiotics as a solution in the research of new anti-infectives

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Keywords: Probiotics, resistance, bacteria, *Bacillus* spp, microbiome

It is widely known that antibiotics resistance has increased significantly in recent times. Consequently, treating infectious diseases will suppose a real challenge in the following years, predicting a dire future for global health. As a result, the need for new alternatives to treat diseases generated by antimicrobial resistance strains is now urgent. New alternative strategies have arisen to fight multidrug resistant bacterial infections, including probiotics.

Probiotics are non-pathogenic microorganisms that significant benefits for the host, and they are widely used as a food supplement.

Probiotics can modify the human microbiome preventing infections caused by multidrug-resistant pathogens. These microorganisms may attach to the intestine´s surface and compete for resources other bacteria need to survive.

We aim to prevent the multidrug-resistance problem by using probiotics. Our final purpose is to find a bacterial strain that has an inhibitory effect on the growth of pathogenic bacteria and has a broad range of action. The *Bacillus* genus is a sporulating group of bacteria with great potential. It can proliferate at 37C, and its spores can survive low pH and physiological concentrations of bile salts to prevail in the digestive tract.

Here, we selected 20 different *Bacillus* spp. strains and tested their antimicrobial effects in different bacteria strains of *Escherichia coli, Pseudomonas fluorescens, Xanthomonas or Enterobacter cloacae.* Moreover, we identified the maximum concentration of bile salts in which the vegetative *Bacillus* spp. cells could survive and the minimal pH in which they could maintain their viability.

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Evaluation of fungal biodiversity in objects belonging to cats- Analysis by the Mackenzie technique

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Keywords: Fungi, cats, Mackenzie technique

In this study, 19 samples from 18 objects belonging to 19 cats and one sample from a cat hair were analysed.
For the collection of samples was used the Mackenzie technique or toothbrush technique. After the collection, all the samples were sent to the Laboratory of Medical Microbiology of the University of Trás-os-Montes and Alto Douro, where cultures were performed in Dermatophyte Test Medium® (DTM®) and Potato Dextrose Agar medium.
Concerning about the animals to whom the objects belonged, 11 (57.9%) were females and 8 (42.1%) were males, aged between 1 and 15 years. As for breed, 3 (15.8%) were European Shorthair, 15 (79.0%) were undetermined and 1 (5.3%) was Siamese. As for the lesions, only one animal had lesions. These animals belonged to the municipalities of Alfândega da Fé (n=8; 42.1%), Macedo de Cavaleiros (n=3; 15.8%), Vila Flor (n=7; 36.8%), and Vimioso (n=1; 5.3%).
The objects where the samples were collected were bedding (n=17; 89.5%) and the collars (n=1; 5.3%), and 1 sample of hair from the cervical area (n=1; 5.3 %).
Fungal growth was observed in 73.7% (n=14) corresponding to 13 objects and the animal hair sample. In this study, 4 genera of filamentous fungi were isolated. No Dermatophytes were isolated. In 3 (15.8%) samples it was not possible to identify the isolated fungi and in 5 (26.3%) no isolation was obtained.
In these samples, the fungal genera isolated were Aspergillus spp. (n=5; 26.3%) and Penicillium spp. (26.3%), Alternaria (15.8%), Scopulariopsis (10.5%).
The isolated fungal genera were opportunistic, however, they all have infective potential in human and veterinary medicine. This study contributes to the knowledge of fungal biodiversity in cats and the environment that surrounds them.

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Effect of calcium application on sweet cherry quality and expression of genes involved in cracking

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Keywords: Fruit cracking; calcium; *Prunus avium* L.; sweet cherry; gene expression.

The sweet cherry is a fruit of high economic interest due to its health-giving qualities and its appealing appearance and flavor. However, due to climate change, such as periods of heavy rainfall and elevated temperature during the growing season, it has led to an increase in fruit cracking which leads to serious losses for producers. Cherry cracking is currently one of the biggest concerns for producers; this is not only associated with environmental factors but is also conditioned by genetic and biochemical factors. To prevent cracking several approaches have been developed, including the application of calcium by foliar spraying. The aim of this study was to study the effect of calcium application on cherry quality of the Burlat cultivar and on the expression of genes involved in cracking. Calcium was applied at pre-harvest at two different concentrations (150g/hL and 300g/hL). Subsequently, several physical and chemical parameters (weight, dimensions, total soluble solids content, pH, titratable acidity and maturity index), texture parameters (epidermal rupture strength and firmness), cracking index, histological parameters (cuticle thickness, epidermal cell thickness, epidermal cell wall thickness, hypodermal cell thickness and hypodermal cell wall thickness) were determined and the expression analysis of PaExp1 and PaExp2 genes was also performed. The results of this work indicated that the lowest calcium concentration (150g/hL) proved to be more advantageous, as it led to a lower fruit cracking index, as it increased the expression of *PaExp1* and *PaExp2* genes and increased the thickness of epidermal cell, hypodermal cell and hypodermal cell wall.

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Clinical cytogenetics of dog: Protocol optimization for chromosome analysis

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Keywords: Cytogenetics, Dog, Metaphase, Chromosomes, Optimization

Dog cytogenetic analysis is used to diagnose sexual development disorders and cancers. The canine karyotype is very complicated to analyse, since it has a high number of chromosomes (2n=78) as well as a similarity of size and bands, especially in the smaller autosomes. The methodologies to obtain metaphases with high-resolution are poorly described in this specie. The protocol involves several steps, from seeding (with the addition of mitogens that stimulate lymphocytes), between 48 to 72h, addition of colcemid (to stop cell division), hypotonic shock, to fixative (for better individualization of chromosomes) and scattering. It is also possible to add reagents that intercalate in the chromosomes to make them more distended [such as ethidium bromide (EB)]. The goal of this study was to find the best conditions to acquire high-resolution chromosomes in canine blood samples.
The exposition time (48 and 72h) and the type of mitogen used [(phytohemagglutinin (PHA) and pokeweed (PO)] were investigated. Furthermore, two different quantities of colcemid (200 or 150 µL) and EB (5.7 or 3.5 µL) for 1 and 2h, were used.
In this study no metaphases were obtained with PO, regardless the time of exposure. In contrast, PHA showed more quantity of metaphases especially with 72h of exposure. Better chromosome spreading was observed with 150 µL of colcemid for 1h instead of 2h and when compared 200 µL in both times. Regarding the use of EB, with the highest concentration few metaphases were observed, although with more distended chromosomes.
The results obtained showed that well-spread chromosome could be obtained using 150 µL of PHA (for 72h), 150 µL of colcemid and 3.5 µL of EB, both added for 1h.
This study serves as the foundation for a standard technique that will be effective for future canine cytogenetic studies.

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Effect of calcium and seaweed extracts in the expression of fruit cracking related genes in *Prunus avium* L.

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Keywords: bioestimulants, calcium, cherry, cracking, gene expression

Cracking in sweet cherry (*Prunus avium* L.) is a physiological disorder that occurs near the harvest due to rain events, which causes large financial losses to the producers. Several orchard management practices have been applied to reduce the severity of this disorder, such as the foliar application of calcium and seaweed extracts. In this study, a lower and a higher dose of foliar spraying of calcium (150 g/hL and 300 g/hL) and a seaweed extract of *Ascophyllum nodosum* (75 mL/hL and 150g/hL) were applied in sweet cherry trees, cultivar Sweetheart. Moreover, the expression of fruit cracking related genes, were analysed in fruits without cracks from all treatments. Total RNA was extracted from fruit exocarp and then cDNA synthesis was performed. A semiquantitative analysis of the *PaKCS6*, *PaKCR1*, *PaLAS2*, *PaATT1*, *PaWS*, *PaLTPG1* (involved in cuticular wax biosynthesis) and *PaPIP1; 4* (aquaporin) genes, was performed. The treatments performed in this study had an impact on the expression of genes related to waxes and water transport. The results showed differences in the gene expression for some treatments. Cherries treated with seaweed extract showed higher expression than cherries treated with calcium for the genes *PaKCR1*, *PaATT1*, *PaWS*, *PaLTPG1* and *PaPIP1;4*. Lower doses of both products increased the expression of *PaWS*, *PaLTPG1* and *PaPIP1;4.* No differences between the lower and the higher doses were found for the gene *PaKCR1*. The genes *PaKCS6* and *PaLACS2* showed low and similar expression in all the treatments. These results revealed that the application of both compounds positively affected the gene expression and could be a tool to mitigate cherry cracking.

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Analysis of genetic diversity and similarities between *Mucor circinelloides* and *Talaromyces marneffei* based on ISSRs

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Keywords: ISSR, Molecular markers, Variability, Fungi

Mucor circinelloides is one of the most common species of *Mucorales* that can cause fatal infection in immunosuppressed individuals. *Talaromyces marneffei* is an emerging opportunistic pathogen that also has the potential to infect immunosuppressed individuals. The aim of this work is to study the genetic variability of these fungi.
Two samples of *M. circinelloides*, one from a vertebrate and another from an invertebrate, and a sample of *T. marneffei*, from a wild mammal, were analyzed. Genomic DNA was extracted from the fungal samples and PCR, using six ISSR primers, was performed to analyse the genetic diversity. A binary matrix was constructed based on the presence/absence of bands on the agarose gel. A dendrogram using the UPGMA method and SM coefficient was done and allowed to infer about the genetic variability and similarity of the samples. The primers with the highest number of bands were the UBC880 and the UBC888 and the lowest number was obtained with the UBC881 primer. The primer with the highest number of unique bands was the UBC835 and the one with the lowest number was the UBC880 primer. In general, a high percentage of polymorphism was obtained with all the primers, being 100% the maximum of polymorphism, observed with the UBC881 primer. It was found that the samples of *M. circinelloides* showed a high intraspecific variability, despite being grouped in the same cluster, different from the cluster of *T. marneffei*.

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Fungal Biodiversity and Wildlife in The One Health Approach

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Keywords: Wildlife, One Health, Variability, Fungi

The identification of fungi in wild animals is important, especially in game animals that can be in contact with humans, since these can be carriers of fungi that cause infectious diseases.
Twenty-nine wild mammals belonging to 10 species were studied: deer (n=5; 17.2%), rabbit (n=7; 24.1%), weasel (n =1; 3.4%), genet (n=2; 6.9%), wild boar (n=2; 6.9%), otter (n=1; 3.4%), fox (n=2 ; 6.9%), mouse (n=5; 17.2%), mole (n=3; 10.3%), deer (n=1; 3.4%).
The Mackenzie technique was used to collect the samples. Samples were sent to the Medical Microbiology Laboratory of UTAD, where cultures were performed in Dermatophyte Test Medium® (DTM®) and Potato Dextrose Agar (PDA).
Isolation of filamentous fungi was obtained in 86.2% of the samples. Dermatophyte fungi were not isolated. In total, 8 genera of filamentous fungi were isolated, in ascending order: *Aspergillus* spp. (n=10; 34.5%), *Cladosporium* spp. (n=8; 27.6%), *Mucor* spp. (n=7; 24.1%), *Alternaria* spp. (n=4; 13.8%), *Fusarium* (n=3; 10.3%), *Penicillium* spp. (n=3; 10.3%), *Talaromyces* (n=1, 3.4%), *Curvularia* (n=1, 3.4%). In 24.1% (n=7) of the samples there were fungi whose genus could not be identified, and in 17.2% (n=5) unidentified yeasts were isolated. The yeast *Rhodotorula mucilaginosa* was isolated from a rabbit, a deer and a mouse. It was possible to identify 3 species of filamentous fungi: *Aspergillus niger* was isolated in 17.2% (n=5) of the studied samples, *Mucor circinelloides* in 6.9% (n=2), and *Talaromyces marneffei* in 3.4% (n=1) of the samples.
This study allowed increasing knowledge about fungal diversity in wild mammals and contributes to increasing knowledge from a One Health perspective.

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WORKSHOPS

Looking for mutations – a bioinformatic approach to solve clinical cases

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Keywords: Bioinformatics, Variant analysis, Diagnosis, Prognosis, Case studies

In recent years, scientific research has revealed an increasing number of variants associated to different diseases. Particularly, cancer mutations' identification is a very active field and new discoveries are made every day.

Sequence technologies as Sanger and Next-generation sequencing are widely used for mutation detection in clinical settings. These mutations have been used for disease diagnosis, prognosis, therapeutic decision, follow up of patients and risk population assessment in hereditary syndromes.

Bioinformatics analysis of the data generated from sequencing technologies is a critical process involving base calling, read alignment, variant identification, and annotation. During this process, the sequence information is compared to a reference sequence to identify whether there are any variants in the targeted sequences. The annotation and interpretation processes are set to identify each variant and their possible clinical significance.

In the present workshop, the participants will be challenged to solve clinical cases, identifying the presence/absence of mutations through the analysis of sequence data using bioinformatics' software and web-based tools. The search in databases and genome browsers will allow the interpretation of the detected mutations and the discussion of disease diagnosis, prognosis, and therapeutics.

Mycology in Microbiology: from concept to practice

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Keywords: Fungi, Diagnosis, One Health

Fungi are eukaryotic organisms that are the main responsible for the decomposition of organic matter, however, some of them have pathogenic properties. Filamentous fungi are ubiquitous and can be a risk to public health, as they are opportunistic fungi with pathogenic potential, especially for immunosuppressed individuals.

This workshop aims to provide tools to understand the importance of fungi, their presence in our environment and their potential as infectious agents for human and animal health. The basis of the identification and characterization of fungi will be presented and explored in practical laboratory experiments, namely routine mycological identification and the use of molecular biology as complementary diagnostic techniques.

Abiotic stress in fruit trees and metabolic responses: a practical approach

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Keywords: Abiotic stressors, Biostimulants, Climate change, Fruit trees, Mitigation strategies

The most recent climate projections point to a decrease in water availability, an increase in air temperature, and the occurrence of extreme phenomena, such as excessive rainfall near the harvest periods. Consequently, significant economic losses occur due to a strong reduction of the commercial value of the fruits. Abiotic stresses such as extreme temperatures, drought, salinity, and UV-B radiation are the foremost limiting factors for crop productivity. Under the current climate changing scenario and also due to the increase of global trade in fruit to meet consumer demand for regular supply of high-quality fruit, it is important to understand the relationship between preharvest treatments with biostimulants and the physiological behaviour of fruit trees. Although no consistent literature is available about the effect of those substances, such as glycine betaine (GB) and *Ascophyllum nodosum* (AN), on the physiological performance of fruit trees, these compounds might be a new and innovative solution to increase the crop ability to tolerate stressful environments. The accumulation of osmolytes such as GB (quaternary ammonium compound) in cells can stabilize the structures by maintaining the integrity of membranes against the damaging effects of abiotic stresses via osmoregulation or osmoprotection. Seaweed based biostimulants, like AN, are composed of several components, such as plant hormones, proteins, sugars, vitamins, humic substances, and phenolic compounds. Several published reports suggest that biostimulants improve plant productivity by increasing the minerals assimilation and the photosynthetic activity, reducing the transpiration rate and the fruit-cracking incidence. This workshop will provide an update on recent studies focusing on the physiological responses to changing environmental conditions at different fruit tree levels. Specifically, we will address how we can determine plant stress responses taking advantage of new technologies to link physiology and omics approaches.

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Molecular diagnosis of SARS-CoV-2

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Keywords: RT-PCR, SARS-CoV-2

Since the first outbreak of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a devastating pandemic has been affecting countries all over the world. RT-qPCR is a diagnostic method that allows the qualitative detection of the viral RNA. This method is used to identify the presence of the virus by detecting parts of its genome, after steps of specimen collection and RNA isolation. RT-qPCR is considered to be the most sensitive and specific test to diagnose SARS-CoV-2 infection and is characterized for its early viral infection detection competence, as this technique is efficient and capable of detecting a small number of copies of SARS-CoV-2.
In this practical workshop we intend to show and approach the following issues:
- Experimental procedures for the molecular diagnosis of SARS-CoV-2 - RNA extraction and RT-qPCR;
- Results analysis following the OMS and DGS guidelines;
- Data Report - SINAVE Lab;
- General discussion.

Participation number: 18 (3 groups of 6 persons)
Formation: 2 Hours
Starting: 18h. End: 20h.

ACKNOWLEDGMENTS

The Organizing Committee thanks all those who contributed for the accomplishment and success of the XIV Jornadas de Genética e Biotecnologia/ IV Jornadas Ibéricas de Genética y Biotecnología (XIV JGB/ IV JIGB), namely:

The University of Trás-os-Montes and Alto Douro that annually supports the realization of this event.

The University of León, Spain, that also supports the realization of this event and betted with UTAD in the accomplishment of this event.

The Honour Committee and the Scientific Committee for their active participation and presence in the event.

The speakers for their willingness to accept our invitation, to enrich this event with their conferences, and for the transmission of scientific knowledge.

The Professors and researchers who presented Workshops at this event.

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All colleagues and students from other universities for participating in this event making it an event open to all the international and national scientific communities.

All the participants for demonstrating interest in joining this event and for some of them presenting their scientific work enriching greatly these days. Without you, this event would be meaningless.

The students of the Secondary Schools who participated in this event presented their Junior Posters.

The sponsors that support partially this event.

Overall, to all who have contributed to the success of the XIV JGB/ IV JIGB.

Thank you very much.

The Organizing Committee of the XIV JGB/ IV JIGB

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