II Symposium of Biotechnology

15th and 16th December 2025

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Book of Abstracts

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Dear participants,

It is our great pleasure to welcome you to the **II Symposium of Biotechnology**, held at the University of Évora. This event brings together researchers, professionals, and students for a dynamic scientific program designed to promote knowledge exchange and active discussion over the course of the meeting.

Following the success of the first edition, this Symposium is dedicated to all those who work in or have an interest in the diverse fields of Biotechnology. The scientific program reflects the multidisciplinary nature of this area, covering topics related to molecular and cellular biotechnology, plant, animal and microbial biotechnology, biomedical applications, and biotechnological innovations with industrial and environmental relevance. Renowned invited speakers will contribute with their expertise, fostering discussion on recent advances and emerging challenges in Biotechnology.

The II Symposium of Biotechnology takes place at the University of Évora, providing an inspiring academic setting that encourages scientific interaction and collaboration. The Organizing Committee, on behalf of the University of Évora, warmly welcomes all participants and hopes that this meeting will offer an excellent opportunity to share research results, establish new collaborations, and strengthen existing scientific networks.

We would like to express our sincere gratitude to the members of the Scientific Committee for their essential contribution to the organization and quality of this Symposium. We also thank all participants for their involvement and enthusiasm.

We hope that you enjoy the scientific program of the II Symposium of Biotechnology and take the opportunity to appreciate the historic city of Évora, a UNESCO World Heritage site. This book of abstracts contains detailed information about the Symposium, including the scientific program and the list of participants.

Welcome to Évora!

The Organizing Committee,

Hélia Cardoso
Lénia Rodrigues
Catarina Campos
Ana Catarina Sousa
Mónica Marques
Catarina Estêvão
Rita Pires

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Scientific Programme

Day 1 – 15th of December

08h30 | **Participant registration**

09h00 | **Opening session**
- Prof. Ana Paula Canavarro, Vice-Rector for Education and Pedagogical Innovation
- Prof. Fernando Carapau, Director of the School of Science and Technology
- Prof. Diogo Figueiredo, Director of the Biology Department
- Prof. António Manuel Pereira, Director of the Course Committee of the Bachelor's Degree in Biotechnology
- Prof. Hélia Cardoso, Responsible for the Curricular Units of Biotechnology and Genetic Engineering and Biotechnology

09h30 | ***Keynote lecture*: Plant biotechnology: reality and beyond**
- Prof. Jorge Canhoto, CFE, University of Coimbra

10h30 | **Regulatory mechanisms of somatic embryogenesis mediated by secreted molecules: the olive tree as a case study**
- Rita Pires, MED, University of Évora

11h00 | **Genome editing as a tool to protect plants from a changing environment: tackling stress responses in rice**
- Tiago Lourenço, ITQB NOVA, NOVA University of Lisboa

11h30 | **Coffee break and poster session**

Lunch Break

14h30 | **Biopolymers and bioactives: a natural partnership**
- Hugo Duarte, CERES, University of Coimbra

15h00 | **Plant biotechnology and nutrigenomics: activation of defence mechanisms through induced resistance**
- Raquel Navarro Sempere, Ideagro (Alltech)

15h30 | **Microalgae as sources of bioactive compounds: applications in skeletal biology**
- Paulo Gavaia, CCMAR, University of Algarve

16h00 | **Coffee break and poster session**

18h30 | **Closing of the 1st day**

Day 2 – 16th of December

08h30 | **Participant registration**

09h00 | **Are fermented foods a contemporary healthy and sustainable panacea?**
- Marta Laranjo, MED, University of Évora

09h30 | **Algae production in Portugal: general remarks**
- Joana Silva, Arborea Biofoods

10h00 | **Innovative strategies to optimize biopharmaceutical processing and formulation**
- Ana Paula Tavares, CICECO, University of Aveiro

10h30 | **Biotechnology, therapeutic translation and entrepreneurship: an integrated perspective on neuroscience research**
- Mariana Fiadeiro, RISE-Health, University of Beira Interior

11h00 | **Coffee break and poster session**

12h30 | **Closing session and best poster awards**

13h00 | **Closing of the II Symposium of Biotechnology**

Plenary Section

Plant Biotechnology: Reality and Beyond

Jorge Canhoto

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Plant biotechnology encompasses a diverse set of methodologies that enable the conservation, modification and breeding of plants, thereby contributing to a more productive, cost-effective and environmentally sustainable agriculture. These technologies facilitate the development of plants with enhanced tolerance to both biotic and abiotic stresses, thus addressing the challenges posed by climate change. In parallel, their potential extends to several industrial sectors, including the pharmaceutical, food, cosmetics and fragrance industries, through the production of high-value bioactive compounds.

Many biotechnological approaches rely on the ability to culture and multiply plants *in vitro*, as these systems are essential for generating genetically transformed or genome-edited plants, for producing double haploids used in breeding programmes, for cloning genotypes of interest and for synthesising economically valuable secondary metabolites. In addition to their direct impact on agricultural productivity and innovation, plant biotechnology plays an important role in biodiversity conservation and in mitigating the environmental impacts of agriculture, particularly through the propagation and reintroduction of threatened species and the reduction in the use of plant protection products.

Despite this potential, and its direct contribution to meeting the United Nations Sustainable Development Goals and the objectives of the European Union, such as the European Green Deal and the Farm to Fork Strategy, the application of biotechnology to plant breeding continues to face significant legal constraints in Europe. Unlike countries such as Argentina, Brazil, Canada and the United States, the European Union maintains a regulatory framework that virtually precludes the cultivation of genetically modified organisms and still lacks specific legislation for New Genomic Techniques.

This presentation will provide an integrated overview of these topics, highlighting the strategic role of plant biotechnology in the future of sustainable agriculture and scientific innovation.

Keywords: cloning, *in vitro* culture, New Genomic Techniques, plant breeding, sustainability

Funding: This work was carried out at the R&D Unit Centre for Functional Ecology - Science for People and the Planet (UIDB/04004/2020 and UIDB/04004/2020), financed by FCT/MCTES through national funds (PIDDAC). DOI: 10.54499/UIDB/04004/2020 and 10.54499/UIDP/04004/.

Section 1 – Plant Biotechnology

Invited Communication

Regulatory mechanisms of somatic embryogenesis mediated by secreted molecules: the olive tree as a case study

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Somatic embryogenesis (SE) constitutes an *in vitro* propagation method in which somatic cells undergo dedifferentiation and molecular reprogramming, giving rise to structures analogous to zygotic embryos. Plants that develop from these embryos are therefore clones of the original plant, exhibiting genetic characteristics identical to those of the initial tissues. This technique has been widely applied for the large-scale propagation of several agronomically important species, offering advantages over traditional propagation methods based on the adventitious rooting of microcuttings or semi-hardwood cuttings. In addition to being a recognised tool for plant regeneration, SE enables the production of plants with improved agronomic traits, such as enhanced tolerance to biotic and abiotic stresses. Despite its potential, SE is still not routinely applied in species that exhibit recalcitrance to the formation of somatic embryos. This is the case of the olive tree (*Olea europaea* L.), which demonstrates difficulties in inducing SE from adult tissues. The regulation of SE involves complex interactions between endogenous factors and environmental signals. Analysing these molecular mechanisms is essential to understand the differences in embryogenic efficiency among species and cultivars, and to optimise regeneration protocols. Recent studies on olive carried out by the research groups of the Plant Breeding and Biotechnology Laboratory and the Molecular Biology Laboratory at the University of Évora have revealed proteomic and metabolomic results that play a central role in the regulation of SE. Analyses performed on embryogenic tissues with distinct efficiencies for somatic embryo regeneration, as well as on proteins, extracellular vesicles and metabolites secreted into the culture medium, have demonstrated molecular differences associated with embryogenic efficiency and with the regulation of the SE process.

Funding: The authors would like to thank MED for the support given through the exploratory project ID 10_2021 ("Unravelling olive somatic embryogenesis signalling—a focus on the extracellular bioactive molecules") and to the Portuguese Foundation for Science and Technology (FCT) for the PhD grant UI/BD/153509/2022 (https://doi.org/10.54499/UI/BD/153509/2022). Acknowledgements also to the R&D unit MED – Mediterranean Institute for Agriculture, Environment and Development (https://doi.org/10.54499/UID/05183/2025) and the Associate Laboratory CHANGE – Global Change and Sustainability Institute (https://doi.org/10.54499/LA/P/0121/2020).

Invited Communication

Genome Editing as a tool to protect plants from a changing environment: tackling stress responses in rice

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Climate change poses a serious threat to global food security, making the breeding of climate-resilient crops imperative. However, developing these varieties through conventional methods is a time-consuming process. Over the last decade, genome editing techniques, such as CRISPR/Cas9, have demonstrated great potential to accelerate the breeding of stress-adapted, high-yielding varieties.

Rice, a global staple and a traditionally important crop in Portugal, is highly sensitive to abiotic stresses, particularly water scarcity and heat. Current cultivation methods, which rely on flooded fields, are becoming unsustainable due to erratic rainfall patterns. Our group aims to identify key genes involved in rice stress responses, specifically those related to water scarcity and heat. Using genome editing, we are developing rice lines targeting these genes to functionally characterize their role in stress adaptation.

We are specifically characterizing genes involved in the Ubiquitin-Proteasome System (UPS)[1], focusing on E3-ubiquitin ligases. We selected and validated (via RT-qPCR) 16 genes showing differential expression under stress. One specific gene, *RiP4g*, is up-regulated by drought and Abscisic acid (ABA), a hormone known to mediate drought responses. To characterize *RiP4g*, we developed both knock-out (CRISPR/Cas9) and overexpression transgenic rice lines. Data regarding their response to drought will be presented.

Since water scarcity is initially sensed by roots, we are now focusing on drought-responsive, root-specific E3-ubiquitin ligases. We are generating additional edited lines to elucidate their functions, some of which are currently being evaluated for root plasticity and interactions with soil microorganisms under drought conditions. The potential of these genome editing techniques to develop climate-ready varieties will be discussed.

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1. Melo FM, Oliveira MM, Saibo NJM, Lourenço TF (2021). *Frontiers in Plant Science*, 12, 640193.

Funding: This work was carried out at the Green-it Research Unit (UID/04551/2025, UID/PRR/04551/2025) and was funded by the PrOryza Project (Fundação LaCaixa Promove – Projetos Piloto 2024, PL24-0035) and AgroServ (Horizon Europe, Grant Agreement No. 101058020). Additional support was provided by FCT through PhD fellowships awarded to Sofia Duarte (UI/BD/154554/2022) and Maria Beatriz Vieira (2024.00518.BD).

Invited Communication

Plant biotechnology and nutrigenomics: activation of defence mechanisms through induced resistance

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Nutrition science has evolved in the recent years towards precision nutrition, driven by advances in molecular biology, genomics and biotechnology. In this context, new disciplines are emerging, such as nutrigenomics, which focuses on understanding how genes interact with nutrients and how these interactions can influence health, development and response to nutritional treatments.

In the field of agriculture and biostimulant development, the study of nutrigenomics examines how genes are naturally expressed in plants and how their expression is influenced by nutrition, bioactive compounds, and other stimuli. The level of expression acts as a regulator that defines whether a trait is expressed intensely, moderately, or barely perceptibly in a plant. Transcriptomic analyses (by RT-qPCR or RNAseq) allow us to quantify gene expression levels in plant tissue at a specific point in time.

In this work, we applied these techniques to obtain information on what is happening at the molecular level after the application of a biostimulant with pathogen resistance-inducing function (PROCROP ISR, Alltech). The results show that exogenous treatment promotes specific and broad-spectrum immune responses by stimulating the expression of chitinases (PR-3, PR-4), glucanases (PR-2), phytoalexin production (PAL) and the activation of hormonal pathways such as jasmonic or salicylic acid, contributing to greater resistance to pathogens and abiotic stress.

Nutrigenomics allows us to determine the mode of action of products in an objective, quantifiable and precise manner, as well as to discover other biological pathways involved, which could become new targets for the improvement and development of biostimulants.

Funding: This work is financially supported by IDEAGRO SL.

Assessing genetic diversity in Portuguese populations of the Southwest Iberian endemic *Juniperus navicularis*

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Juniperus navicularis is a dioecious shrub found mostly in mainland Portugal, being a southwest Iberian Peninsula endemism[1]. This species is important for ensuring the stability of the sandy soil where it grows, as well as for regulating the water and nutrient cycle. Furthermore, *J. navicularis* is a source of food and shelter for several species[2]. However, it has been classified as Near Threatened (NT)[3] and its populations have been declining[1] due to anthropogenic pressure. The irregular seed production and the absence of seminal germination in nature limit the regenerative capacity of populations to only vegetative propagation[4]. This fact restricts the genetic diversity of the species and hinders the development of more effective management measures.

This work arises to investigate the genetic diversity of *J. navicularis* in Portugal, integrated into the Zimbral for Life project[5]. Thus, the main objective will be to verify the genetic diversity of the species across the country and within populations; secondarily, it will be verified whether variations in ploidy occur and what the range of vegetative propagation is. For this purpose, the work will be divided into 2 stages: 1) 10 individuals will be sampled from 5 different populations, 2 populations representing the extreme distribution in Portugal and 3 will be found in intermediate regions, which will allow the analysis of interpopulation variation at the national level and ploidy variation; 2) plant material will be collected from 50 individuals from 3 populations, so that their internal diversity can be estimated, in addition to allowing the determination of the reach of vegetative propagation. Genome variability will be analyzed using molecular markers (Simple Sequence Repeats, SSRs) developed from available information on different *Juniperus* species.

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Improving clonal propagation of *Arbutus unedo*: Insights from *ex vitro* rooting trials

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The strawberry tree (*Arbutus unedo* L.) is a small evergreen species native to the Mediterranean basin valued for its ecological resilience and growing agro-economic relevance. It is highly tolerant to drought and exhibits strong post-fire regeneration, making it suitable for forestry, land restoration, and diversified agronomic systems such as orchard cultivation and agroforestry. However, its low rooting capacity when propagated via cuttings limits the success of traditional vegetative propagation. At the same time, seed-based propagation not only generates substantial genetic variability but also produces seedlings that grow slowly and take several years to reach reproductive maturity, thus reducing their practical utility for commercial production. As a result, micropropagation has emerged as a promising strategy for the large-scale propagation of genetically uniform, high-performance clonal material. Optimised *in vitro* culture protocols may therefore enhance propagation efficiency, support the preservation of genotypes, and further the sustainable expansion of *A. unedo*. In this study, an *ex vitro* rooting trial was undertaken using plantlets previously established *in vitro* on DKW[1] and WPM[2] basal medium supplemented with cytokinin. Microshoots originating from WPM exhibited a markedly higher capacity for *ex vitro* root induction, producing more developed root systems and achieving greater survival during acclimatisation. These outcomes demonstrate that the basal medium used during the multiplication stage strongly influences rooting success in *ex vitro* conditions. Overall, the results highlight the effectiveness of WPM-derived material for successful plant establishment, further supporting micropropagation as a reliable method for producing high-quality planting stock.

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Funding: This work is financially supported by Microplant Lda. and through IEFP - Instituto do Emprego e Formação Profissional.

Poster 3

Molecular analysis of PIN-mediated auxin transport during adventitious root formation in *Juglans regia*

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In vitro propagation of common walnut (*Juglans regia*) via microcuttings is an effective method for large-scale clonal multiplication of elite genotypes, but many cultivars remain recalcitrant to adventitious root formation (ARF). Improving propagation efficiency requires clarifying the molecular mechanisms underlying recalcitrance. ARF involves plant responses to wounding and to the rooting inducer, typically the auxin indole-3-butyric acid (IBA). IBA is absorbed at the stem base, transported and converted to its active form (IAA – indole acetic acid) and then redistributed to target tissues where root initiation occurs. This redistribution relies on PIN-FORMED (PIN) auxin efflux carriers, key components of polar auxin transport and previously associated with ARF in several species. The role of *PIN* genes in *J. regia* ARF was examined through an *in vitro* rooting assay using plantlets of cv. 'Chandler'. Basal region of plantlets established on medium with or without IBA were sampled at multiple time points (0 h to 7 days). Among the different *JrPIN* gene members identified in the *J. regia* genome, only four members of the *JrPIN1* subfamily displayed detectable and dynamic expression. Among these, *JrPIN1b* was the only gene that did not respond to IBA treatment; instead, its expression was significantly higher in control explants at 5 days, suggesting a response more closely associated with cutting stress rather than ARF. Both *JrPIN1a* and *JrPIN1d* showed strong induction at 24 hours in both IBA-treated and untreated explants. However, significant differences between IBA-treated and untreated samples were observed only at 3 and 5 days, respectively, possibly indicating a role in later stages of ARF induction. In contrast, *JrPIN1f* was upregulated in IBA-treated explants from 6 hours onward, supporting its involvement in ARF from the early induction phase through differentiation. Overall, these results strongly support that PIN-mediated polar auxin transport plays a central role in ARF in *J. regia*.

Funding: This work is financially supported by national funds through FCT under the project UID/05183/2025

Poster 4

Selection of reference genes for gene expression analysis during olive tree (*Olea europaea* L.) adventitious rooting

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Adventitious roots (AR), which develop from non-root tissues, may arise during normal growth or be induced by stress, including mechanical stress associated with tissue culture. Their formation plays a central role in the vegetative propagation of many horticultural and forest species. In olive tree (*Olea europaea* L.), several cultivars show low AR formation efficiency, limiting propagation and the availability of plant material for orchard establishment. AR formation is controlled by a complex regulatory network influenced by multiple factors, with leaves contributing actively to signaling and hormonal regulation, making them a relevant tissue for studying early molecular events associated with rooting capacity. To investigate the molecular mechanisms in leaves that regulate AR formation, reliable reference genes are essential for accurate analysis of key genes through reverse transcription quantitative real-time PCR (RT-qPCR). In this study, ten candidate reference genes (*CYTOX5*, *NTCR*, *KINASE*, *NADH*, *SDIR*, *RER1a*, *ZF19*, *OUB*, *ACTIN*, and *EF1a*) were evaluated in a rooting experiment. *In vitro*-grown plantlets of cv. 'Galega vulgar' were treated with indole-3-butyric acid (IBA) and transferred to rooting medium (experimental details in Velada et al.[1]), and control plantlets were maintained without IBA. Leaves were sampled at different time points. RNA extraction, cDNA synthesis and RT-qPCR followed protocols described by Velada et al.[1,2]. Expression stability was assessed using *geNorm*, NormFinder, and BestKeeper according to Campos et al.[3]. Stability assessment revealed *NADH*, *OUB*, and *SDIR* as the most stable reference genes across all algorithms. *geNorm* identified *NADH* and *OUB* as the best-performing pair, and the pairwise variation $V_3/_4$ (< 0.15) indicated that adding *SDIR* is recommended to ensure reliable normalization. These three genes were therefore selected as the most suitable reference genes for expression studies in olive leaves during adventitious root formation.

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Poster 5

In Vitro Micrografting of *Prunus dulcis* (Mill.) D.A. Webb: Optimizing Grafting Device and Rooting Medium

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Micrografting combines the advantages of *in vitro* propagation and conventional grafting, providing uniform propagation material in a phytosanitary state[1]. In this study, the micrografting process for almond tree was optimized through a comparative evaluation of four grafting devices (parafilm, agarose, tape and double-layer aluminium foil) and different auxin exposure conditions to improve rooting and acclimatization success of micrografts. A first assay, using wild almond as rootstock and Canhota and Casa Nova varieties as scions, demonstrated that double-layer aluminium foil is the most effective grafting device, providing superior micrografting junction parameters, namely the healing and the success rates. In terms of micrograft growth, agarose showed the best results with the higher growth index and the lower number of shoots in the rootstock. However, the success rate of agarose was negatively impacted by the presence of *callus* in the junction. Double-layer aluminium foil was then used in a second assay in which Canhota and Ferraduel varieties were micrografted into a commercial rootstock (Garnem®) and acute and prolonged exposure to auxin were tested. Acute exposure to auxin was found to be beneficial to the success of rooting and acclimatization of almond micrografted plants, with 100% acclimatized plants after eight weeks *ex vitro*. Our results highlight the potential to extend the applications of the micrografting methodology to other fruit species, which could open up new avenues for research in the field.

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Poster 6

Isolation and identification of endophytic microbial communities from long-term *in vitro* plant cultures

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Endophytes, microorganisms that colonize internal plant tissues, have been increasingly associated with growth promotion in *in vitro* tissue culture systems of various woody species. Their recurrent appearance in long-term *in vitro* cultures, without causing visible negative effects on plant development, suggests a potential role in enhancing plant performance and growth. In this study, long-term *in vitro* cultures of three important tree crops — olive (*Olea europaea* L.), walnut (*Juglans regia* L.), and carob (*Ceratonia siliqua* L.) — were examined for the isolation of endophytic bacteria that could be further evaluated as plant growth-promoting bacteria (PGPB).

In vitro-grown plantlets of olive cv. 'Galega vulgar' cl. 1441, walnut hybrid 'Paradox' cl. Vlach (*J. regia × J. hindsii*), and carob var. 'Cavi' were used to isolate facultative endophytic communities. Bacterial inocula were collected from the culture medium in which each culture was established and plated on a range of culture media selected according to the nutritional profiles of each plant system. Isolates were repeatedly subcultured to confirm purity prior to identification through molecular tools.

Following the establishment of pure cultures, genomic DNA was extracted from three endophytic bacterial strains, one from each plant species. The 16S rRNA gene was amplified using primers Bact27F and Bact1492R-y under the conditions described by Chelius and Triplett[1], and sequencing of the amplicons enabled the determination of the taxonomic identity of each isolate.

The genus/species identification, combined with the ability of these bacteria to grow under liquid medium, and their persistence in long-term *in vitro* cultures without causing detrimental effects, supports their potential as PGPB candidates for further evaluation in different plant species and biological systems.

To complement this study, an additionally microbiome analysis will be done through metagenome sequencing aiming to characterize the diversity of bacteria communities on long-term *in vitro* cultures.

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Poster 7

A Portuguese grapevine breeding program for improved resistance to *Plasmopara viticola* and *Erysiphe necator*

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Downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*) are the major diseases affecting grapevine (*Vitis vinifera* L.), one of the most widely cultivated crops with high economic importance worldwide. Current disease management strategies strongly rely on chemical control, requiring intensive fungicide applications, which increase production costs and cause significant environmental impacts. The present work describes a breeding program established by the company PLANSEL Lda. (Montemor-o-Novo, Portugal), based on controlled crosses between genotypes carrying well-characterized resistance loci (kindly provided by the Julius Kühn-Institut, Germany) and traditional Portuguese cultivars (selected based on their importance in the viticulture sector). The application of molecular markers, particularly Simple Sequence Repeats (SSRs, also known as microsatellites), for the selection of F1 hybrid plants carrying resistance *loci* is a central component of this strategy. Their use reduces both costs and the required cultivated area, since plants can be analyzed after germination, still under greenhouse conditions. F1 hybrid plants (known as PIWI) were initially selected based on their response to inoculation with both pathogens. The marker-assisted selection (MAS) was performed targeting *loci* associated with resistance to powdery mildew (Ren3 and Ren9) and downy mildew (Run1, RPV1, and RPV3.1). The selection of the most promising genotypes to proceed with backcrosses or new crosses aimed at resistance *loci* pyramiding is crucial for the success of a breeding program. From 350 plants analyzed, nearly 60% exhibited at least one resistance *locus*. The MAS analysis enabled the selection of 40 genotypes, which have already been established under field conditions for subsequent phenotyping.

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Poster 8

Proteomic analysis of Portuguese *Vitis vinifera* L. cultivars to identify the molecular mechanisms involved in drought tolerance

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Grapevine (*Vitis vinifera* L.) is a species of great historical and economic importance. When exposed to environmental stresses, it can adjust gene expression, leading to changes in the proteome that drive metabolic, physiological, biochemical, and morphological adaptations. Studying these proteome-level changes is essential to understand the mechanisms underlying stress tolerance, as proteomic analyses allow the identification of stress-responsive proteins and the characterization of their roles in key metabolic pathways. Beyond fundamental knowledge, proteomics offers valuable tools for biotechnological applications in viticulture, supporting the discovery of molecular markers associated with drought resilience and informing breeding, selection, and management strategies to improve vineyard sustainability under changing climatic conditions.

While various proteomic studies exist on grapevine under drought stress, knowledge remains highly limited concerning traditional Portuguese cultivars grown under field conditions. This work aims to address this gap by characterizing the protein profile of leaves of the cultivars 'Touriga Franca', 'Touriga Nacional', and 'Tinta Caiada' from the ampelographic field of Esporão's vineyards, where they are subjected to different water regimes (under deficit irrigation and non-irrigated vines).

To this end, proteins will be extracted using a methanol/chloroform precipitation method, separated by Two-Dimensional Electrophoresis (2-DE), and protein spots differentially expressed will be analysed using Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-ESI-MS/MS) to identify the corresponding proteins. Proteins of interest identified in this analysis will be selected for validation through the quantification of the expression of their encoding genes by RT-qPCR. This approach is expected to identify proteins and genes associated with water stress response mechanisms, thus contributing to the identification of potential molecular markers for drought tolerance.

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Poster 9

Metabolomics applied to the identification of somatic embryogenesis biomarkers in olive (*Olea europaea* L.)

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The olive tree (*Olea europaea* L.) is a major agronomic species across the Mediterranean basin, yet several elite cultivars exhibit strong recalcitrance to adventitious root formation when propagated through semi-hardwood cuttings—the method commonly used in nurseries. This limitation highlights the need for alternative propagation strategies. Somatic embryogenesis (SE), an *in vitro* regeneration process that enables the formation of embryos from somatic cells, offers a promising solution because it bypasses the requirement for adventitious rooting. Understanding the molecular mechanisms governing SE efficiency will support the development and optimization of propagation protocols for elite olive cultivars. This work aims to identify key metabolites that characterize highly efficient embryogenic lines compared with low-efficiency lines, which may be proposed as SE-associated biomarkers. Special emphasis will be placed on metabolites secreted into the liquid culture medium. The role of biomolecules released into the culture medium—including polysaccharides, amino acids, growth regulators, vitamins, proteins, lipophilic molecules, and secondary metabolites—has been highlighted as modulating the embryogenic response[1-3]. Characterization of the exometabolome will be conducted using HPLC and mass spectrometry, enabling the identification of differentially accumulated metabolites in high and low-competent embryogenic lines. Embryogenic lines were previously established through the protocol of Pires et al[4,5], and metabolomic analysis was performed according to a protocol developed by the same authors[6]. Metabolic pathways identified will be validated using a transcriptomic approach through the analysis of key enzyme-encoding genes and their associated epigenetic regulation. This study will contribute to a deeper understanding of the molecular and metabolic mechanisms underlying SE in the olive tree and will provide tools to optimize regeneration protocols.

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Poster 10

Biotechnological improvement of semi-domesticated and orphan crops of the *Solanaceae* family

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The aim of this work was to identify the most effective combinations and concentrations of growth stimulants for callus induction, shoot regeneration of species: *Solanum aculeatissimum*, *S. aviculare*, *S. betaceum*, *S. muricatum, S. quitoense, S. sesiliflorum*. The analysis was conducted in PubMed, using the keywords: callus, regeneration + name of species. The list of 76 articles was prepared. The parameters used for analysis: type of explant; the best concentrations & combinations of stimulants for callus & shoot initiation; the effectiveness of stimulants (%). The data was analysed in Flourish application, Sankey diagram was prepared. The best explants for callus formation were leaves & hypocotyls, for shoot regeneration - leaves. The highest percent of callus initiation was: 100 (*S. aculeatissimum*, 1 mg/L NAA), (*S. betaceum*, 5 mg/L 2,4-D). The best stimulants for shoot regeneration were BAP, BAP+NAA and KIN+NAA. The highest percentage of shoot regeneration was for *S. betaceum* – 100% (5 mg/L NAA), *S. aviculare* - 90% (0.5 mg/L BAP), *S. muricatum* - 93.33% (4 - 5 mg/L BAP). The following results can be used during planning of experiments dedicated to gene editing and transformation of *Solanaceae* crops.

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Poster 11

Plant tissue culture *in vitro*: A long journey with lingering challenges

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In recent years, plant tissue culture has become a crucial component of the modern bioeconomy. From a commercial perspective, plant micropropagation remains one of its most valuable applications. Plants exhibit remarkable developmental plasticity; however, many species still remain recalcitrant in tissue culture. While the term recalcitrant is commonly used to describe plants with poor *in vitro* regeneration capacity, from a biological point of view it suggests that the minimal culture requirements for this species were unmet. Despite evidence that the Skoog-Miller exogenous hormonal balance theory and Murashige–Skoog medium were species-limited in applicability, generations of plant biotechnologists applied these tools indiscriminately. This led to systemic propagation of ineffective protocols, publication of misleading standards, and a culture of scientific inertia—costing both time and resources. The field must now move beyond historical dogma toward data-driven, species-specific innovation based on multiple endogenous auxin biosynthesis pathways, epigenetic re-programing of competent cell and further modern biotechnologies which are evolving. We proposed possible solutions for plant biotechnology which allow to significantly improve effectiveness of it[1]. The proposed solutions have been applied for optimization of plants growth *in vitro* including seeds formation[2] and for plant regeneration from single cell system leaf and root protoplasts[3].

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Section 2 – Environmental Biotechnology

Invited Communication

Biopolymers and bioactives: A natural partnership

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Plant secondary metabolites encompass large families of compounds, characterized by biological properties related to plant protection and ecological interactions. From simpler to more complex molecules, more or less toxic, bioactive compounds from plants are used for many purposes in several fields. Plant extracts such as essential oils have been used since ancient times for medicinal and cosmetic applications, as nowadays their benefits are also being explored in animal nutrition, food preservation, environmental remediation and agricultural applications[1].

As a food application, the AlBread project aims to develop an acorn flour-based bread functionalized with essential oils, aiming to provide a functional food with the benefits of the oil´s antioxidant and antifungal properties, including possible health benefits and taking into account the valorisation of the Montado ecosystem. The project Lignin for Hair aimed at a cosmetic application, using lignin extracted from acacia wood for the development of lignin-based air conditioners, free of conventional conditioning agents.

Agricultural and forestry biomass rich in phenolic compounds and hydrocarbons can also be processed into suitable materials for environmental applications. Defatted olive oil pomace can be used to remove methylene blue from water, while lignin and tannins can be used to replace phenol on phenolic foams usually used as insulating materials in construction. Lignin can also be incorporated into synthetic or natural polymers, for the preparation of composites able to avoid the adhesion of microorganisms into surfaces, while also providing antioxidant activity[2–4]. This talk highlights only a few examples of the endless solutions provided by plants and their compounds, from biopolymers to bioactive molecules. In the end, shedding light on how it is possible to sustainably obtain a whole variety of functionalized and advanced biomaterials from an ecosystem.

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Field-deployable molecular tools and machine learning for environmental monitoring of animal tuberculosis

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Animal tuberculosis (TB), caused mainly by *Mycobacterium bovis*, affects public health, livestock production, and wildlife. As in other European Union countries, TB remains endemic in cattle and wild ungulate populations in Portugal[1]. Traditional control methods, focused on testing and culling strategies targeting cattle alone, are insufficient in complex ecological scenarios where the bacterium circulates among multiple species and environments[2-4]. While direct contact remains the most recognized transmission route, the role of indirect transmission via environmental contamination is still poorly understood [5]. This knowledge gap limits the effectiveness of targeted interventions and hinders TB control efforts, emphasizing the need for predictive, environmental surveillance tools. In recent years, portable molecular technologies have emerged that show great promise for direct application in the field, especially in low-resource settings. Loop-Mediated Isothermal Amplification (LAMP) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) combination are powerful molecular tools integrating rapid isothermal amplification with CRISPR detection for point-of-care diagnostics[6]. This combination offers high sensitivity, specificity, and reading by fluorescence, colorimetry, or other lateral assays[7,8]. On the other hand, paper-based platforms and portable devices with lyophilized reagents have demonstrated feasibility for epidemiological surveillance *in situ*, with reduced response times[9]. Their implementation requires primer optimization, amplicon contamination management, and clinical validation to ensure robustness and reproducibility, suitable for field use by non-specialized technical personnel. This project aims to develop and validate a portable molecular platform, integrating LAMP and CRISPR-Cas12a, for the detection and differentiation of members of the *Mycobacterium tuberculosis* complex (MTBC) in environmental samples. This LAMP-CRISPR system will enable the discrimination of *M. bovis* from other members of the MTBC. With this diagnostic tool, we will analize environmental substrates in TB hotspots and control areas. After, we will perform ecological niche modeling to predict environmental suitability and TB transmission risk areas in Portugal.

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Section 3 – Microbial Biotechnology

Invited Communication

Are fermented foods a contemporary healthy and sustainable panacea?

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Food systems are major drivers of climate change, which threatens food security and food safety. Transition to sustainable and healthy diets is no longer optional, but mandatory.

Fermented foods (FF) have been considered a promising component of healthy and sustainable diets, offering numerous benefits for health and environment, but they are not a universal panacea. They offer unique opportunities to enhance health while supporting the Sustainable Development Goals (SDGs). But are FF truly sustainable choices?

FF are microbial foods and beverages, which result from fermentation processes performed by microorganisms that transform raw materials into foods with enhanced and distinctive sensory, nutritional and functional properties. They have been part of human diets worldwide for millennia. In ancient times, fermentation offered a robust strategy for food preservation by lowering pH, reducing water activity and producing antimicrobial molecules. Nowadays, FF are also known for their improved organoleptic properties and nutritional value. More recently, an increased interest in FF has been driven by hypotheses about their capacity to deliver live microbes and fermentation-derived metabolites with potential benefits for human health, including modulation of the gut microbiome, enhancement of bioavailability of nutrients, and generation of bioactive compounds. FF have been generally recognized as healthy and safe. But is there enough scientific evidence that support the promotion of the health benefits of FF?

Considering sustainability global challenges, such as food waste and climate change, fermentation is a key strategy to preserve surplus vegetables, or optimise milk production, for example. Nevertheless, the production of other FF, such as fermented meats, highlights the need for strategies to mitigate their environmental impact, while retaining their benefits.

On the edge of tradition and innovation, FF may support the transition towards more sustainable food systems, paving the way for future planetary diets that simultaneously address public health issues and environment-friendly policies.

Invited Communication

Algae production in Portugal: General remarks

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Portugal is in the road map of Algae in Europe and is considered one of the biggest players. In fact, the highest and the oldest microalgae industrial facility in algae are in Portugal. Concerning the expertise and science driven, Portugal is also considered an excellent example with extremely good science research groups as well as Human Resources.

Algae potential is huge. From sustainable food to marine restoration and bioplastics, algae offer innovative solutions that benefit both people and planet.

Algae can be produced in Open or in Closed Systems such as photobioreactors and raceways, respectively, or even in heterotrophic pathway without light and in presence of a sugar source, by fermentation.

The production mode is season and strain dependent. Several challenges are imposed to the (micro)algae industrial producers like the regulamentation aspects, certifications, the market and business development as well as the production costs that are still prohibited and the scale up that needs to be profit in the end.

Dissecting root exudate chemistry and quorum sensing in biofilm formation

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Plant-associated microbiomes are key determinants of plant performance[1], yet the mechanisms by which root exudates and bacterial quorum sensing (QS) shape root microbiome assembly remain poorly understood. QS is a key communication system used by bacteria, known to be involved in several essential steps for successful root colonization[2]. Root exudates, comprising sugars, amino acids, organic acids, phenolics, and volatiles, are released into the rhizosphere, where they act as carbon sources and chemical signals, shaping the microbial communities[3]. However, their chemical composition is very dynamic and depends on plant species, developmental stage and environmental stress[4]. Therefore, our main goal is to dissect the role of QS and root exudate composition in biofilm formation and bacterial community assembly using a legume-rhizobium symbiotic model.

Here, we developed a functionally representative synthetic community (SynCom) for *Trifolium subterraneum* by integrating culture-dependent and culture-independent approaches. Approximately 300 bacterial strains were isolated from the rhizoplane of plants grown in three distinct soils, and amplicon-based metagenomic data from rhizosphere, rhizoplane, and endosphere compartments at 15 and 30 days were used to select taxa capturing the dominant diversity and functional potential of the rhizoplane microbiome. In parallel, we are characterizing the composition of root exudates by LC-QTOF-MS under contrasting environmental conditions to assess how plant-derived metabolites influence bacterial community assembly, biofilm formation, and QS-mediated communication. Linking exudate profiles with the behavior and structure of the SynCom will enable controlled testing of how specific compounds and QS signals potentially modulate root colonization and legume-rhizobium symbiosis. This work will establish a tractable SynCom-based model for subterranean clover that can be used to dissect the role of root exudate chemistry and bacterial communication in root microbiome assembly, providing mechanistic insight into plant-microbiome interactions in legume systems.

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Poster 14

Functional profiling of the gut rare biosphere: insights into metabolic potential and antimicrobial resistance

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Rare biosphere, *i.e* low-abundance microbial taxa, is traditionally overlooked in microbiome research. However, recent studies suggest these taxa represent a disproportionally active part of microbiomes, with outsized functional roles in microbial community dynamics[1], including stress adaptation, metabolic versatility, and acting as gene reservoirs[2]. Thus, rare taxa are hypothesized to significantly contribute to microbial genetic diversity and ecological resilience. The pig gastrointestinal tract shares over 90% of microbiome functions with human's, cementing pigs as animal models for the gut system[3, 4]. Further, swine production is responsible for over 34 % of global meat supply, emphasizing the need to assure pig's overall health[5]. The pig gut microbiome is a complex ecosystem shaped by diet, farming practices, and antibiotic exposure. Understanding the functional potential of rare members is critical for addressing AMR dissemination, optimizing animal health, and enhancing food safety under a One Health lens. By uncovering the taxonomic diversity and functional potential of rare gut microbes, this project aims to uncover keystone species with important ecological functions in the pig's gut and shed light on previously overlooked contributors to metabolic adaptability and antimicrobial resistance. For these purposes, three pig cohorts have been defined, rare species will be retrieved through an amplicon sequencing strategy combined with culturomics, followed by functional role prediction, characterization of metabolic and resistance traits, and integrative analysis combining these results with breeding and management practices. Mapping metabolic potential of rare species will allow better understanding of microbial ecology, enabling transposition of knowledge to the human gut, while generating useful information for food production systems, antimicrobial stewardship and biosecurity.

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Poster 15

The Hidden Players: Investigating the Role of the Gut Mycobiome in Antimicrobial Resistance Emergence

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Cattle is an important source of global staple foods, including beef, milk and dairy derivatives, with profound economic, cultural and environmental impacts. However, more than half of globally used antibiotics are administered in livestock husbandry systems, leading to AMR emergence in WHO critical and high priority bacteria[1]. Some of these bacteria (*e.g. Escherichia coli*, *Enterococcus* spp.) are commensals of the bovine gastro-intestinal tract (GIT) and may harbour in their genomes major determinants of antimicrobial resistance[2]. Fungi coexist with bacteria in the gut as a minority, with *c.a.* 10^6 fungal cells to 10^{10} bacterial cells per gram of rumen contents[3]. Antibiosis relationships between fungi and bacteria have been reported both in the rumen and *in vitro*[4]. Transcriptomic efforts and metabolite investigation remains short, however promising studies in co-cultivated GIT bacteria and fungi suggest upregulation of bacterial drug efflux pumps[5] while fungal secondary metabolism may be a reservoir of bioactive compounds and putative bacteriocins[3].

Here we hypothesize that the mycobiome may influence the selection of resistant bacterial resistomes within the cattle's GIT, with special attention to commensals with zoonotic potential. To address this hypothesis, the following objectives where established: (1) characterization of the bacteriome and mycobiome of selected Portuguese cattle breeds; (2) resistome profiling of commensal *E. coli* isolates; (3) assessment of selective pressure of fungal metabolites against selected bacterial isolates; and lastly, (4) to explore correlations among fungal taxa, bacterial resistomes and host metadata. The innovation of this work is tied to potential new insights into AMR selection in commensal bacteria of zoonotic relevance under the uncommon perspective of the gut mycobiome as an endogenous, non-therapeutic driver.

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Poster 16

Microbial dynamics during poultry manure composting enriched with silicate rock

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Composting is a simple and sustainable technique for producing nutrient-rich organic amendments that can be further enriched with low-cost materials such as silicate rock powder. These enriched inputs may offer additional agronomic benefits for crops like rice, which readily incorporates silicon into its tissues. This study evaluated the dynamics of microbial communities during composting as indicators of microbial quality and process evolution. The methodology focused on monitoring temporal shifts in the compost microbiota, examining changes in the abundance of bacterial groups across the composting stages, and comparing community development between treatments with and without magnesium silicate. Throughout the process, the compost underwent typical maturation patterns, including a reduction in the Carbon/Nitrogen ratio and increased nutrient availability, pH, and stabilizing compounds. Microbial succession followed trends commonly reported in composting environments. The class *Bacilli* dominated both treatments, underscoring its central role in organic matter degradation, while the presence of the contamination-associated class *Clostridia* declined toward the end of composting, indicating improved microbiological stability. The addition of silicate rock powder did not modify the overall structure of the bacterial community, suggesting that the composting microbiota is resilient to this type of enrichment. These findings enhance the understanding of microbial dynamics in composting systems supplemented with silicate rock materials and support their use without disrupting key microbial processes essential for compost stabilization.

Genomic surveillance reveals clonal adaptation and silent spread of *E. coli* in cattle herds

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Antimicrobial resistance (AMR) is recognized as one of the most significant threats to public health in the 21st century. In 2019, it was estimated that 1.27 million deaths were caused by AMR, with projections pointing to 10 million by 2050.[1,2] The AMR problematic affects clinical, agricultural and environmental settings, thus demanding an integrated One Health surveillance approach[3]. *Escherichia coli* is a commensal of mammals' gastrointestinal tract and a relevant model organism for the understanding of AMR. In this study, we aimed to understand *E. coli* epidemiology in cattle farms. Fecal samples from cattle (n=282) representing 12 herds managed under extensive husbandry were processed for *E. coli* isolation. Isolates (n=282) were tested using phenotypic, genotypic and genomic approaches. Phenotypic resistance was low, with tetracycline (10.6%) and ampicillin (3.5%) being the most common. Notably, resistance to European Medicines Agency Category A drugs was detected, despite bans on their use in animals in the European Union. There were 15 distinct phenotypic resistance profiles, with six isolates (2.1%) being multidrug-resistant and 15 (5.3%) identified as Shiga toxin-producing *E. coli*. Despite the low phenotypic resistance rates, whole-genome sequencing (n=108 isolates) revealed the presence of genes associated to resistance to various antibiotic classes, as well as a high diversity of virulence genes. Phylogenetic analysis showed high similarity between isolates of the same herd but also among different herds. This study highlights adaptation of specific clones to cattle and epidemiological links between and within herds, suggesting increased transmission potential of specific *E. coli* lineages. Evidence for latent genome-encoded AMR and virulence determinants calls for active surveillance to monitor public and animal health trends and to prevent future dissemination.

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Section 4 – Biomedical Biotechnology

Invited Communication

Microalgae as sources of bioactive compounds: applications in skeletal biology

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Bone erosive pathologies such as osteoporosis are increasing with the ageing of the world population. There is strong evidence that shows the involvement of the immune system with the development of these conditions, however, no immunomodulatory drugs have been developed to prevent bone loss. In the search for next-generation treatments for these conditions, natural compounds are emerging as promising candidates for the discovery of novel drugs. Microalgae are attracting significant interest, due to the combination of their ability to synthesize bioactive compounds, technologically advanced cultivation technologies and ease of use for genetic engineering[1]. Here we explore the osteogenic and anti-osteoclastogenic effects of extracts of the microalgae *Tetraselmis striata* CTP4 and *Skeletonema costatum* and evaluate their osteoactive potential. Using zebrafish as a model for bone formation and regeneration, we demonstrated the ability of extracts to promote osteogenesis both in vivo and in vitro[2], as well as a reduction in the incidence of skeletal malformations. *S. costatum* has also shown the ability to inhibit the recruitment of osteoclastic progenitors and delay their differentiation into mature osteoclasts in vivo[3]. The transcriptome of fin blastemas in early stage of regeneration revealed a downregulation of genes involved in inflammation, T cell activation and antigen presentation, suggesting that the extract exerts its effects through immunomodulatory mechanisms. To assess the therapeutic potential, we tested *S. costatum* extract in a RANKL-induced osteoporosis model in medaka and a macrophage cell line. The extract effectively prevented bone loss in fish and inhibited osteoclastic differentiation of murine macrophages. These studies contribute to understanding the potential of microalgae as sources of osteoactive compounds with therapeutic potential, evidencing their osteoprotective potential through modulation of the immune system and mechanisms of skeletal formation.

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Invited Communication

Innovative strategies to optimize biopharmaceutical processing and formulation

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Biopharmaceuticals play an increasingly significant role in the health sector, contributing to the treatment and prophylaxis of numerous hard-to-treat diseases. Among them, protein- and nucleic acid-based therapeutics have driven major advances in modern medicine. Despite their growing clinical relevance, the manufacturing of biopharmaceuticals involves complex upstream and downstream steps that still pose substantial challenges and greatly influence overall production costs.[1] The downstream processing remains the main challenge and, despite the efforts made in the last decades to improve the already used strategies, they still contribute to more than 80% of the total production cost. In parallel, the formulation of these biological products to ensure stability and preserve therapeutic efficacy represents another critical bottleneck.[2]

To address these limitations and promote the development of cost-effective, sustainable purification strategies and stable formulations, alternative solvent systems, such as bio-based ionic liquids and glycine-betaine-based deep eutectic solvents, have been explored. Several studies have demonstrated the potential of these solvents to enhance the downstream processing of biopharmaceuticals, including RNA, by improving purity and recovery yields, as well as to support the design of more robust and stable biopharmaceutical formulations.

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Invited Communication

Biotechnology, therapeutic translation and entrepreneurship: An integrated perspective on neuroscience research

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The evolution of biotechnology in recent decades has become essential for bringing scientific discoveries closer to real therapeutic solutions. This presentation aims to explore how an academic background in Biotechnology and Biomedical Sciences can naturally converge towards innovation, the development of new therapeutic approaches, and the translation into medical applicability.

After several years of fundamental research focused on understanding the cellular and molecular mechanisms involved in Parkinson's disease (PD), the creation of a therapeutic solution with potential clinical impact emerged, leading to the establishment of a start-up dedicated to translating scientific knowledge into a valuable product. The start-up NeuroSoV is primarily dedicated to research and development (R&D) of molecules with therapeutic potential for PD and other neurodegenerative diseases, using preclinical validation models, both *in vitro* and *in vivo*. At this stage, the main goal is to use these models to validate the effectiveness of a new drug candidate in preventing PD progression. For this purpose, the use of animal experimentation is essential in this validation process, as it provides more robust biological and behavioural results and responses.

It is important to highlight the essential role of scientific entrepreneurship as a driver of social and economic impact, emphasizing the importance of recognizing opportunities at the right moment. For students of biotechnology and related fields, this illustrates the relevance of integrating scientific curiosity with vision, because the intersection between science, technology, and entrepreneurship currently represents a fertile ground for generating innovation and real social impact.

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Poster 18

Deep eutectic solvents based on analogues of glycine-betaine as a new class of excipients for enhanced RNA stabilization

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RNA has a revolutionary impact in the biopharmaceutical field on the treatment and prophylaxis of a range of hard-to-treat diseases, either as a therapeutic agent or the target itself. However, several challenges still persist in RNA bioprocessing technologies and have hindered the clinical progress of RNA-based therapeutics. One of the main bottlenecks is related with RNA stability during its long term storage, given the high susceptibility of RNA to degradation due to its intrinsic labile nature[1].

Deep eutectic solvents (DES), an emerging class of "designer solvents", are gaining attention as efficient excipients for biomolecules and may be composed of natural and low-toxic compounds, thus offering innovative and potentially more biocompatible approaches in this field. In this context, the main goal of this study is to address the potential of several biobased DES to act as excipients for the stabilization of RNA, even under stress conditions, such as when exposed to ribonucleases. A set of several analogues of glycine-betaine-based DES was designed for this purpose, due to the biocompatible and natural origin of glycine-betaine, its relatively low cost and high availability[2], as well as its application in health-related formulations.

The structural integrity and stability of RNA in the selected DES was assessed by agarose gel electrophoresis, circular dichroism (CD) spectroscopic measurements and UV/Vis spectroscopy. Overall, this work highlights the potential of DES as biocompatible solvents for improving the stability of RNA biopharmaceuticals.

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Influence of Appetitive Food Visualization on Salivary Biomarkers: Modulation by Age and Time of Day

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Exposure to visual food stimuli influences preferences and consumption decisions and triggers neurophysiological mechanisms that prepare the body for ingestion, including anticipatory changes in salivation. While increases in saliva volume are well established, recent evidence shows that saliva composition can also change when food is anticipated through smell or visualization. Our team previously demonstrated that food images affect the salivary proteome; however, observing an image may not elicit the same response as viewing real food, which allows sensory evaluation. Individual factors such as age, sex, eating habits, physiological state, emotional context, and time of day may further modulate these anticipatory responses and are also known to influence saliva composition.

This study investigated how visual exposure to a real chocolate cake affects salivary biochemical composition and whether these effects vary according to time of day and/or age. Saliva was collected before and during exposure to the cake (4 minutes each) in adults tested in the morning (N = 21) and afternoon (N = 21), and in children tested in the morning (N = 19). Participants reported the sensations elicited by viewing the cake and later tasted it. Salivary flow rate, total protein concentration, and α-amylase activity were measured.

Distinct response patterns emerged between children and adults. In children, changes in salivary secretion were more closely linked to perceived sweetness, whereas in adults they related more to self-reported hunger or thirst. In children, the feeling of mouthwatering correlated positively with the sweetness perceived when tasting the cake. Exposure to the cake increased salivary flow rate, while total protein concentration decreased. α-Amylase activity also increased during anticipation, with a tendency for a stronger response in males.

Overall, these findings show that viewing appetitive food modulates saliva composition, and that the magnitude and nature of this response depend on the sensations evoked and on the individual's age.

Ethical statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Commission of the University of Évora for human studies (no.GD/29823/2022).

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Modulatory effect of a NADPH oxidase inhibitor on the expression of α-synuclein and β-amyloid in an *in vivo* model of Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder of the central nervous system[1]. Pathologically, it is characterized by the progressive loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc) and the formation of Lewy bodies[2, 3]. These pathological changes underlie motor and non-motor symptoms, including cognitive impairment associated with Parkinson's disease with dementia (PDD)[4]. PDD involves pathological molecular mechanisms, such as oxidative stress and the aggregation of α-synuclein and β-amyloid, which contribute to cognitive decline[5]. NADPH oxidases (Nox), enzymes responsible for producing reactive oxygen species, play a central role in these mechanisms, affecting a variety of cognitive functions, with the Nox4 isoform being particularly implicated in the progression of the pathology[6]. In this context, we investigated the ability of an inhibitor of Nox isoforms 1 and 4, in an innovative ionic liquid formulation (N1(4)inh-IL), to prevent pathological protein deposition in a paraquat-induced PD animal model. To this end, the presence of α-synuclein, β-amyloid, and oligomer species (A11 positive) was analyzed in the SNpc and hippocampus. Results indicated a remarkable attenuation of protein aggregates in N1(4)inh-IL-treated animals compared to controls. These findings suggest that Nox4 inhibition through this innovative formulation attenuates pathological protein accumulation in the analyzed brain regions. Thus, these preliminary results highlight the potential of this biotechnological strategy, opening new perspectives for further studies in the context of preserving cognitive function and controlling the progression of PD.

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New strategy to prevent the progression of neurodegeneration occurring in Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the loss of motor neurons in the motor cortex, brainstem, and spinal cord, leading to muscle weakness, atrophy, and fatal respiratory failure within a few years of diagnosis[1,2]. Approximately 10% of ALS cases correspond to genetic mutations, being one of the most studied the copper-zinc-dependent protein superoxide dismutase 1 (SOD1) mutation[3]. Pathological SOD1 mutations promote the accumulation of reactive oxygen species (ROS), contributing to oxidative stress and neurodegeneration[2]. NADPH oxidases (NOX), another major source of ROS in the central nervous system, further exacerbate oxidative damage, and in ALS context, NOX's are responsible for generating superoxide anion, the substrate for the reaction catalyzed by SOD1[3]. Emerging evidence suggests that NOX inhibition may offer therapeutic potential for ALS[3]; therefore, we developed a novel NOX1 inhibitor, Cholinium-based ionic-liquid (IL) (N1inh-IL). In this study, we have used a murine motor neuron cell line NSC-34 expressing the hSOD1 gene under the control of a doxycycline-inducible promoter and characterized its differentiation profile and susceptibility to toxin-induced and NOX1 inhibitor cytotoxicity. The results shows that the retinoic acid promoted effective differentiation in all the cell lines, observed by increased neurite extension and morphological changes. The cytotoxicity profiling identified the conditions to induce cell loss using rotenone and paraquat, and toxicity testing of N1inh-IL revealed that concentrations lower than 100 µM were non-toxic. Altogether, these results establish a robust characterization of the NSC-34 cellular model and demonstrate that N1inh-IL is safe at low concentrations, crucial for advancing the study of the inhibitor's efficacy in this *in vitro* model of ALS.

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