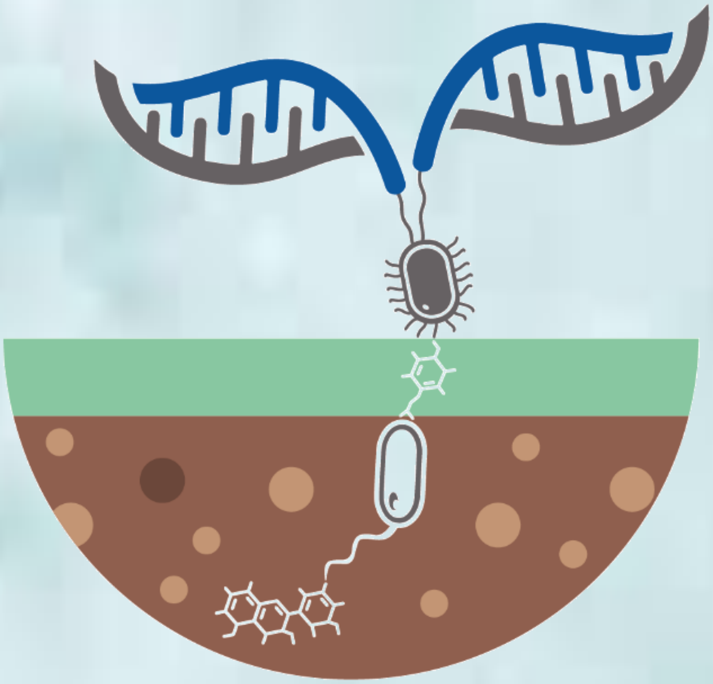

# IAS2023

## 1st International Agrienvironment Symposium

VNiVERSiDAD D SALAMANCA

Salamanca, 17th - 18th July 2023

# The 1st International Agrienvironment Symposium funding:

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**We thank María Rivas Cornejo for the administrative and technical support.**

IAS2023
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# 1st IAS2023 Organizing and Scientific Committee

**Dr. Cecilia Oliver**

**Dr. Narciso Martín Quijada**

**Dr. Esther Menéndez**

**Dr. Pablo Albertos**

**Dr. Elvira Manjón**

# Welcome to the 1st International Agrienvironment Symposium – 1st IAS2023

Dear participants of the 1st IAS2023,

On behalf of the organizing committee, we are happy to welcome you to Salamanca to participate in the 1st International Agrienvironment Symposium. This meeting provides a good opportunity to share the latest knowledge, progress and ideas on cutting-edge applications and the latest advances in basic and applied research in agrienvironmental disciplines, biotechnology, molecular and computational biology, genomics, crop production and plant interactions with pathogens and beneficial microorganisms.

This event is organized by a team of researchers within the Unit of Excellence in Agricultural Production and Environment Agrienvironment at the University of Salamanca. The symposium is divided into 6 interdisciplinary sessions with a selected panel of 10 excellent keynote speakers and 23 some short talks selected from the submitted abstracts and 52 posters presentations.

The 1st IAS2023 welcomes 125 participants from 6 different countries and more than 15 Universities and Research Institutions. The meeting will take place in the Archbishop Fonseca College is a monument declared an Asset of Cultural Interest in 1931. It was founded by the Archbishop of Toledo, Alonso de Fonseca, and its construction began in 1519. It was originally a charitable educational institution. In this way, people without economic resources could access university studies. After the sessions of talks and posters, the participants will have the time to enjoy the beautiful city of Salamanca, with the oldest University within Spain.

We look forward to meeting you in Salamanca.

The Organizing Committee

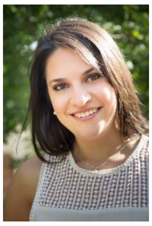
**Cecilia Oliver**

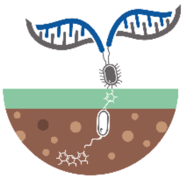
**Narciso Martín Quijada**

**Esther Menéndez**

**Pablo Albertos**

**Elvira Manjón**





IAS2023
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SALAMANCA

# Scientific Program

## July 17th 2023

- 8:30 Registration
- 9:00 Welcome to 1st IAS2023

## Session 1: Cell and molecular signalling
Chairs: Noelia Arteaga and Mónica Calvo-Polanco

- 9:30 Keynote: **Alberto Carbonell** (Instituto de Biología Molecular y Celular de Plantas, IBMCP, Spain) *Next-generation artificial small RNA-based RNAi for crop improvement*

- 10:00 Short talks selected from abstracts

  – **Sara Gómez Jiménez** (CIALE, University of Salamanca, Spain) *Nitric oxide signalling in the root stem cell niche is regulated by TGA memmbers of the bZIP family*
  – **Sonsoles Alonso** (University of Almería, Spain) *A mutation in 7-dehydrocholesterol reductase (DHCR7) gene confers brassinosteroid deficiency, dwarfism, and enhanced tolerance to salinity*
  – **Laura Huebra Montero** (CIALE, University of Salamanca, Spain) *BRABA: what's going on in BRs and ABA crosstalk?*
  – **Leila Rassizadeh** (University Jaume I, Castellón, Spain) *Transcriptomic Analysis Reveals Insights into Mechanisms behind Extracellular DNA-Induced Resistance*

- 11:00 Coffee break & poster session

## Session 2: Plant protection & sustainability
Chairs: Diego Cruz and Javier Sánchez-Martín

- 12:00 Keynote: **Francesco Asnicar** (University of Trento, Italy) *Expanding metagenomic taxonomic profiling exploiting uncharacterized species with MetaPhlAn 4*
- 12:30 Keynote: **David Turrà** (University of Napoli Federico II, Italy) Mechanisms of host sensing in *Fusarium oxysporum*-plant interactions

- 13:00 Short talks selected from abstracts

  – **Alberto Pedrero Méndez** (CIALE, University of Salamanca, Spain) *Trichoderma and its benefits to plants: biostimulation and indirect biocontrol activities*
  – **Ezequiel Peral Aranega** (University of Salamanca, Spain) *The European Spruce Bark Beetle (Ips typographus) harbours a changing bacteriome with the metabolic capacity to aid the host*
  – **Melani Mariscal** (University of Córdoba, Spain) *Cytosolic pH as a new fungal virulence signal*

- 14:00 Cocktail lunch

## Session 3: One Health and Climate Change part I

Chairs: Ignacio García Estévez and Rocio Vicentefranqueira

- 16:00 Keynote: **Elsa Brandão** (University of Porto, Portugal) *TASTE: A Flavorful Tool to Promote Healthy Food Consumption*

- 16:30 Short talks selected from abstracts

  – **Miguel Ayuso Calles** (University of Salamanca, Spain) *Effect of Rhizobium volatilome on lettuce growth under salt stress*
  – **Tiago Lopes** (University of Aveiro, Portugal) *A Multifactorial Approach to Untangle Graphene Oxide (GO) Nanosheets Effects on Plants: Plant Growth-Promoting Bacteria Inoculation, Bacterial Survival, and Drought*
  – **Ángela Martínez Gómez** (University of Castilla-La Mancha, Toledo, Spain) *Grape pomace biochar is effective for root-knot nematode control*
  – **Alexandra Díez Méndez** (Catholic University Santa Teresa de Jesús de Ávila, Spain) *Preliminary screening of bacteria isolated from a fire-affected forest soil in Avila: implications for ecosystem recovery*
  – **Lidia Garzón-García** (University of Salamanca, Spain) *Flavonoids as neuroprotector molecules: Evaluation in a transgenic strain of the model organism Caenorhabditis elegans*

- 18:00 Free time
- 19:30 Guided tour in Salamanca
- 21:00 Gala dinner (for those registered to attend)

### July 18th 2023

## Session 4: One Health and Climate Change part II

Chairs: Lorena Carro and Marta Marcos

- 9:00 Keynote: **María Reguera Blázquez** (Autonomous University of Madrid, Spain) *Environmental factors controlling seed quality of emergent crops within the context of climate change*
- 9:30 Keynote: **Letricia Barbosa Pereira** (University of Santiago de Compostela, Spain) *Upcycling food waste into functional ingredients and biobased films*

- 10:00 Short talks selected from abstracts

  – **Mónica Jesus** (REQUIMTE, University of Porto, Portugal) *Unvelling the impact of food additives on flavor of healthy foods: the rolein the polyphenol-oral constituents interactions*
  – **María Sánchez Bermúdez** (CBGP-UPM and INIA-CSIC, Madrid, Spain) *An integrative analysis of root adaptation to multiple environmental soil conditions in Brassica napus*
  – **Esperanza Miñambres Fuentes** (CIALE, University of Salamanca, Spain) *Fungi volatiles as a tool for plant tolerance against abiotic stress*
  – **María Oyón Ardoiz** (University of Salamanca, Spain) *Addition of*

*Torulaspora delbrueckii mannoproteins to red wine: effect on the phenolic composition, color and astringency*

- 11:00 Coffee break & poster session

## Session 5: Microbiome in agrosystems

Chairs: Zaki Saati and Maria Illescas

- 12:00 Keynote: **Cláudia SL Vicente** (MED-University of Évora, Portugal) *Protecting our crops – sustainable approaches for the control of plant parasitic nematodes*
- 12:30 Keynote: **Lorena Gomez-Aparicio** (IRNAS-CSIC, Spain) *Understanding global change impacts on the microbiome of Mediterranean agroforestry systems*
- 13:00 Short talks selected from abstracts

  – **Cristina Frade** (IRNASA-CSIC, Salamanca, Spain) *Dehesa: management practices and their influence on soil microbiological diversity*
  – **Irene Sanz-Puente** (University of Cantabria-CSIC, Spain) *Core bacteria endophytes are vertically transmitted to the plant offspring generation through the germ line*
  – **Benjamin Zwirzitz** (University of Natural Resources and Life Sciences, Vienna, Austria) *Sourdough – A model system for microbiome research*
  – **Luisa Liu-Xu** (University Jaume I, Castellón, Spain) *Traditional genotypes, a richer source of potential fungal endophytes for tomato improvement*

- 14:00 Cocktail lunch

## Session 6: Agricultural food security

Chairs: José David Flores Félix and Inmaculada Sánchez-Vicente

- 16:00 Keynote: **Markus Stetter** (Institute for Plant Sciences. University of Cologne, Germany) *Not quite finished crops – The ecology and genetic control of incomplete domestication*
- 16:30 Keynote: **Raquel Iglesias Fernández** (Centro de Biotecnología y Genómica de Plantas UPM – INIA, Spain) *The Quinoa4Med initiative: Promoting quinoa as a climate-smart crop for enhancing income generation in the Mediterranean marginal land*
- 17:00 Short talks selected from abstracts

  – **Victoria Widrig** (CIALE, University of Salamanca, Spain) *Kinase-fused Resistance Proteins as Novel Regulators of Race-Specific Resistance in Cereals*
  – **Muhammad Khashi u Rahman** (CIALE, University of Salamanca, Spain) *Exploration of the significance of non-leguminous crops in intercropping systems*
  – **Diana Matos** (University of Aveiro, Portugal) *Potential of Bacteria from Arid Environments to Improve Plant Drought Tolerance: Inoculating Maize with Plant Growth-Promoting Bacteria*

- 18:00 Poster & short talk prizes. Concluding remarks.

IAS2023
17th - 18th JULY
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# Session 1: Cell and molecular signalling

Chairs: Noelia Arteaga and Mónica Calvo-Polanco

# Next-Generation Artificial Small RNA-Based RNAi For Crop Improvement

Alberto Carbonell[a]*

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Genetic engineering tools should allow the reprogramming of plant genomes for environmental adaptation and, ultimately, for increased productivity. Current tools based on RNA interference (RNAi) typically function through the delivery to plant cells of double-stranded RNA (dsRNA) for the repression (or silencing) of cognate target RNA. More modern, highly specific RNAi-based tools for plants are based on artificial small RNAs (art-sRNAs) and have been optimized mainly for high efficacy and transgenic use but are still not ready for fine-tuning plant gene expression or for GMO-free application.

Here, I will present our efforts to develop next-generation art-sRNA-based RNAi (art-sRNAi) for crop improvement. Our main goals are i) optimizing art-sRNA efficacy, fine-tunability and systemicity for absolute control of induced RNAi and ii) the development of efficient GMO-free art-sRNAi through the cost-efficient production and topical delivery of art-sRNA precursors to plants, or by expressing art-sRNAs from viral vectors or from CRISPR/Cas-edited endogenous sRNA genes. I believe this enhanced art-sRNAi technology will ultimately accelerate the generation of highly productive crops in line with international laws.

## Funding

This work was supported by grants or fellowships from MCU, AEI and FEDER: PID2021-122186OB-I00, RTI2018-095118-A-I00 and RYC-2017-21648.

## Acknowledgements

We thank Javier Forment (IBMCP) for helping in the bioinformatic analyses and the greenhouse staff of IBMCP for the maintenance of plants.

Oral S1

# NITRIC OXIDE SIGNALLING IN THE ROOT STEM CELL NICHE IS REGULATED BY TGA MEMBERS OF THE bZIP FAMILY

*S. Gómez-Jimenez[a], M.G. Fernández-Espinosa[a], A. Sánchez-Corrionero[a,b], C. Mata-Pérez[a] and O. Lorenzo[a]
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[a] Department of Botany and Plant Physiology, Institute for Agribiotechnology Research (CIALE), University of Salamanca, Spain
[b] Universidad Politécnica de Madrid

Nitric Oxide (NO) is a gasotransmitter molecule playing significant roles in several plant biological processes (Manrique-Gil et al., 2021; Mata-Pérez et al., 2023; Sanchez-Corrionero et al., 2022). Therefore, NO is required for determining root architecture and meristem organization in the root apical meristem (RAM) (Fernández-Marcos et al., 2012; Sanz et al., 2014). However, little is known about the molecular targets of NO during root stem cell niche (SCN) homeostasis.

Root development is a tightly regulated process that relies on the coordination among the different cell types located into the SCN. Furthermore, TGA transcription factors have been identified as important regulators of SCN maintenance, where PERIANTHIA (PAN/TGA8) has been described as a master regulator of quiescent centre (QC) cell functioning (de Luis Balaguer et al., 2017).

Plants have developed mechanisms for NO sensing, mainly converging in changes at the gene expression level and/or the post-translational modification of proteins, driving to phenotypic effects. Here we show that TGA transcription factors contribute to the NO-mediated regulation of root SCN through phenotypical and transcriptomic approaches. First, our results indicate that NO significantly impacts on primary root length and, secondly, -through transcriptomic and bioinformatics approaches- we observed that these TGA transcription factors are responsible for the NO sensing in the SCN. Accordingly, the molecular data support the interaction among the different TGA members and the inhibition of the TGA transcriptional activity in the presence of NO. These findings highlight a molecular framework where NO modulates the function of TGA transcription factors which could be therefore considered as key regulators of root SCN development and maintenance.

## References

*de Luis Balaguer MA, et al (2017) PNAS, 114: E7632.*
*Fernández-Marcos, et al (2012) Plant Signaling and Behavior, 7:196.*
*Sanz L, et al. (2014) Plant Physiology, 166: 1972-84.*
*Manrique-Gil I et al. (2020) Journal of Experimental Botany.*
*Mata-Pérez, C et al. (2023) Frontiers in Plant Science, 1294:14.*
*Sanchez-Corrionero, A (2022) Journal of Experimental Botany, 22:erac508.*

## Funding

This work is financed by the projects PID2020-119731RB-I00, from the Ministerio de Ciencia e Innovación (MICIN), SA137P20 from Junta de Castilla y León and Unidad de Excelencia CLU-2018-04 co-funded by the P.O. FEDER of Castilla y León 2014–2020 Spain.

## Acknowledgements

We thank the RED2018-102397-T and RED2022-134072 networks for stimulating discussions. S. Gómez-Jiménez is funded by FPU (FPU20/02612).

# A mutation in *7-dehydrocholesterol reductase (DHCR7)* gene confers brassinosteroid deficiency, dwarfism, and enhanced tolerance to salinity

Sonsoles Alonso*, Keshav K. Gautam, Gustavo Cebrián, Cecilia Martínez and Manuel Jamilena

Department of Biology and Geology, Agrifood Campus of International Excellence (CeiA3) and Research Center in Agri-food Biotechnology (CIAIMBITAL). University of Almería. 04120 Almería. Spain.

The *dwfcp* mutant was selected from an EMS mutant platform of *Cucurbita pepo*. The mutant displayed a dwarf phenotype with shrinked and dark-green leaf, shortened internode and petiole, reduced fertility and de-etiolation phenotype in the dark. The causal mutation of the phenotype was found by BSA-sequencing in *Cp4.1LG17g04540*, the squash orthologue of Arabidopsis *DWF5*, encoding for 7 dehydrocholesterol reductase (DHCR7) enzyme of the brassinosteroid (BR) biosynthesis pathway. The mutation reduced the expression of *CpDWF5* in all analyzed tissues. The dwarf phenotype could be partially rescued by the exogenous application of 2,4-epibrassinolide, and the endogenous BRs content was significantly lower in the mutant than in WT. Since BRs have a key role in abiotic stress response, we analyzed the effect of *dwfcp* on salt stress tolerance during germination and early stages of seedling growth. We found that i) the delay in germination time under salt stress was lower in the mutant than in the WT, ii) despite its dwarf condition, the radicle of *dwfcp* grew at the same rate as WT under salinity conditions, and iii) the salt treatment during an etiolation test reduced the length and biomass of both roots and leaves, but this reduction was lower in the mutant than in WT. This enhanced salt tolerance of *dwfcp* indicates that BRs play a negative role in salt stress response in *C. pepo*.

# BRABA: what's going on in BRs and ABA crosstalk?

Laura Huebra-Montero*, Jorge Rubio-Heras, Óscar Lorenzo and Pablo Albertos [1].
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Early growth after seed germination is crucial for the proper development of an adult plant and is strictly regulated by a complex hormonal balance. The phytohormone abscisic acid (ABA) is responsible for inhibiting seed germination and postgerminative growth but promoting seed maturation and seed dormancy [1]. Among the components that mediated ABA responses, the Class A of bZIP transcription factors (TFs), where ABA INSENSITIVE 5 (ABI5) is included, plays a critical role in modulating seed germination and early seedling growth [2].

Brassinosteroids (BRs) are polyhydroxy steroidal phytohormones that acts as a key signal to regulate multiple physiological processes in plants, such as germination [3], cell elongation, cell division, skotomorphogenesis and plant responses to biotic and abiotic stresses. The bHLH-like type TFs BRI1-EMS-SUPPRESSOR1 (BES1) and BRASSINAZOLE RESISTANT1 (BZR1) are master regulators of BRs responses and modulate the expression of many target genes [4]. They act as a hub that connect BRs signalling with different signalling pathways, such as ABA, gibberellins and light [5].

Our research analyzes in more detail the antagonistic relationship between ABA and BRs, focusing on the molecular interaction mechanisms happening between the bHLH-like TFs BES1 and BZR1 and the class A of bZIP TFs during postgerminative growth and development.

Firstly, phenotypical approaches indicate differential ABA sensitivity of the BRs regulated TFs. Moreover, *in silico* studies of the promoters of the class A of bZIP TFs, showed that they harbor different combinations of *cis*-elements where BES1 and BZR1 have high DNA binding affinity. This may be of great relevance for analyzing changes in gene expression directed by BES1 and BZR1 under ABA responses. Therefore, we intent to expand the present knowledge of the interplay between ABA and BRs signaling pathways and the relevance of this interaction in the control of postgerminative growth.

## References

[1] Finkelstein, R. *The Arabidopsis Book* 11, e0166. (2013).
[2] Albertos, P et al. *Nature Communications*, 6, 1-10 (2015).
[3] Steber, CM and McCourt, P. *Plant Physiology 125*, 763–769 (2001).
[4] Kono, A and Yin, Y. *Frontiers in Plant Science, 11*:617162. (2020)
[5] Hu, Y and Yu, D. *The Plant Cell*, 4394-4408, 26(11) (2014).

## Funding

P. A. María Zambrano Grant, NextGenerationEU.
L. H-M. PhD Fellowship University of Salamanca.
J. R-H. Scholarship Programa Clave, JCyL.
O. L. research: Spanish Ministry of Science and Innovation (PID2020-119731RB-I00), JCyL (SA137P20), Escalera de Excelencia (CLU-2018-04) co-funded by P.O. FEDER CyL 2014-2020.

# Transcriptomic Analysis Reveals Insights into Mechanisms behind Extracellular DNA-Induced Resistance

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In recent years, extracellular DNA (exDNA) has gained significant attention as a danger signal triggering defense signaling. Our previous results demonstrated that *Arabidopsis thaliana* responds to the application of exDNA in a species-specific and dosage-dependent manner. We showed that exDNA treatment triggers general defense responses, such as $Ca^{2+}$ signaling, $H_2O_2$ production, and activation of MAPK signaling cascade, and ultimately induces plant resistance against the major agricultural pathogens and pests with different natures (1). Here, to elucidate the defensive pathways involved in exDNA-induced resistance we performed a transcriptomic analysis in exDNA-treated and control Arabidopsis plants, both with and without thrips (*Frankliniella occidentalis*) infestation. Gene ontology (GO) enrichment analysis highlighted clear differences across treatments and exposure to the pest for specific classes of GOs. Our results revealed that in the absence of challenge, genes associated with fatty acid biosynthesis were upregulated in exDNA-treated plants. The biosynthesis of fatty acid is an important biochemical pathway that contributes to the production of defense-related compounds such as jasmonates during stress conditions (2). After thrips infestation, the exDNA-treated group showed significant enrichment in genes associated with diverse defensive secondary metabolites, including glucosinolates and phenylpropanoids. We observed that exDNA-treated plants show an enhanced defense response in the presence of the challenge, compared to the control group. Notably, the chloroplast-related GO groups (structure and photosynthesis) exhibited a similar pattern of gene enrichment in both control and exDNA-treated plants. These observations were confirmed through morphological and physiological experiments. We suggest that exDNA triggers a defense response without hindering normal growth conditions of the plant. To further investigate, the utilization of knock-out mutants in different signaling pathways, such as mutants impaired in jasmonic acid (JA) and glucosinolate biosynthesis pathways, will provide insights into the interplay between defense pathways and the underlying mechanism of exDNA-induced resistance in plants.

## References

1) Rassizadeh, L., Cervero, R., Flors, V., & Gamir, J. (2021). Extracellular DNA as an elicitor of broad-spectrum resistance in Arabidopsis thaliana. Plant Science, 312, 111036. doi:10.1016/j.plantsci.2021.111036

2) Wasternack, C., & Hause, B. (2013). Jasmonates: Biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Annals of Botany, 111, 1021–1058.

## Funding

This work was funded by the Spanish Government through the grant RTI2018-094350-B-C33 from the Ministerio de Ciencia e Innovación. By the University Jaume I through the Plan de Promoción de la investigación with the grants UJI-A2019-05 and UJI-B2019-26 and the fellowship CDEIGENT/2018/015 from Generalitat Valenciana.

## Acknowledgments

We thank the Scientific Instrumentation Service (SCIC) of the Universitat Jaume I for their technical support.

## Session 2: Plant protection & sustainability

Chairs: Diego Cruz and Javier Sánchez-Martín

# Expanding metagenomic taxonomic profiling exploiting uncharacterized species with MetaPhlAn 4

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## ABSTRACT

Accurate taxonomic profiling of microbial communities is a fundamental analysis step for understanding both the ecological and functional roles of the many microbial species sampled from a given environment. MetaPhlAn 4, is the new improved and expanded version that accurately taxonomic profiles not only in microbiome communities from the many human body sites but also from other environments, such as soil, rumen, and mouse. By integrating genomic data from both metagenomic assemblies and microbial isolates, MetaPhlAn 4 enables comprehensive taxonomic profiling of microbial communities, accurately quantifies organisms without cultured isolates, and provides a higher-resolution understanding of the composition of microbial communities. MetaPhlAn 4 leverages a curated collection of more than 1 million prokaryotic reference and metagenome-assembled genomes to define unique marker genes for over 30 thousand different species-level genome bins, including previously unidentified taxa. Extensive evaluations have demonstrated the ability of MetaPhlAn 4 to explain a significantly higher percentage of reads from diverse microbial communities, particularly in less-characterized environments like the rumen microbiome. Moreover, the application of MetaPhlAn 4 to datasets of mouse, soil, and ocean microbiome environments, highlighted the capability to accurately profile taxonomic composition across different ecosystems. The integration of 22,718 metagenome-assembled genomes from the mouse microbiome environment enables MetaPhlAn 4 to unravel dietary-related changes in the host microbiome and the identification of strong and reproducible microbial biomarkers that were previously uncharacterized. In conclusion, MetaPhlAn 4 provides a comprehensive solution for accurate taxonomic profiling across a wide range of microbiome environments. Improving and expanding microbial community profiling contribute to advancing our understanding of host-microbiome interactions, environmental processes, and the intricate dynamics of microbial ecosystems.

**Keynote S2**

# Mechanisms of host sensing in *Fusarium oxysporum*-plant interactions

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[a] Department of Agriculture, University of Napoli Federico II, Italy

## ABSTRACT

The fungal cell wall, a dynamic structure providing cell type-specific morphology, protects cells from stresses, and functions as a physio-chemical rheostat for the transmission of extracellular signals through a large set of cell-wall-anchored proteins. *Fusarium oxysporum* f. sp. *lycopersici* has been previously reported to gain access into the host plant via chemotropic growth and later to colonize root tissues by exploiting both apoplastic and symplastic routes. In both cases hyphae are supposed to readapt their shape and size to be able to fit into the sub-micrometric spaces that the fungus encounters along its way to the xylem. Which external signals are involved in *F. oxysporum* sensing of host tissue cells and how they modulate hyphal penetration is largely unknown. Here, to gain fundamental insights on the physical and mechanical cues driving fungal tropic behaviours and pathogenicity of plant roots we used reverse genetics approaches, fluorescence microscopy and microfabrication of biomimetic plant platforms. By using chemically inert patterns, we found that *F. oxysporum* hyphae are able to both follow ridges and to adapt to small-sized channels through thigmodifferentiation of extremely thin filaments that resemble those growing in between host plant epidermal cells. We further show that fungal hyphae can penetrate and grow for several microns into sub-micrometric pores as those normally found in the plant apoplast. Importantly, the compressive stress generated by forced growth in nanoenvironments leads *F. oxysporum* surface penetration suggesting that compression itself and mechanosensing might drive the colonization process in the soil pathogenic fungus *F. oxysporum*.

# *Trichoderma* and its benefits to plants: biostimulation and indirect biocontrol activities

Alberto Pedrero-Méndez*, María Illescas, Julio Ascaso-Pérez, Margarita Sanabria-Rojas, David Mendoza-Salido, Alejandro J. Alcañiz, Victoria Widrig, Rocio Olmo, Narciso M. Quijada, Javier Sánchez-Martín, M. Belén Rubio, Enrique Monte and Rosa Hermosa

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*Trichoderma* is a filamentous fungal genus with more than four-hundred fifty recognised species. Some strains are worldwide commercialized as biological control agents against agriculture important phytopathogens. Moreover, *Trichoderma* spp. are able to colonize plant rhizosphere and root endosphere causing beneficial effects such as plant growth promotion and increase of defences against biotic and abiotic stresses [1]. Here, we summarize evidences related to *Trichoderma* biostimulation and indirect biocontrol activities: i) when applied to wheat seeds, modifies bacterial and fungal communities in bulk soil, rhizosphere, and root endosphere of plants grown in a field trial, and increases in the rhizosphere the abundance of plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi [2]; ii) when applied to plant substrate, it can protect olive plants against a highly virulent *Verticillium dahliae* defoliating pathotype by preventing the access of this pathogen to root vascular bundles [3]; iii) controls *Sclerotium cepivorum* in onion crops under tropical conditions, by activation of salicylic acid-dependent defence priming [4]; iv) enhances tolerance against water stress through the activation of ROS antioxidant machinery in wheat plants [5]; and v) using a tomato split-root system reduces number of galls and adult nematodes inside the roots.

## References

1. Woo et al., 2023. *Nat. Rev. Microbiol.* 21: 312-326.
2. Illescas et al., 2020. *Front. Plant Sci.* 11: 575861.
3. Carrero-Carrón et al., 2018. *Plant Pathol.* 67: 1758-1767.
4. Rivera-Méndez et al., 2020. *Biol. Control* 141: 104145.
5. Pedrero-Méndez et al., 2021 *J. Fungi* 7: 1087.

## Funding

This research was supported by grants co-financed by the European Regional Development Fund (FEDER) and the governments of Spain (MCIN/AEI PID-2021-126575OB-I00) and Castile and Leon (SA094P20, Escalera de Excelencia CLU-2018-04 and IR2020-1-USAL05).

## Acknowledgements

A.P.-M., M.I., J.A.-P. and M. S.-R. acknowledge the support of their predoctoral contracts by Regional Government of Castile and Leon.

# The European Spruce Bark Beetle (*Ips typographus*) harbours a changing bacteriome with the metabolic capacity to aid the host.

Ezequiel Peral-Aranega[1,2]*, Zaki Saati-Santamaría[1,2,3], Miguel Ayuso-Calles[1,2], Martin Kostovčík[3], Tereza Veselská[3], Karel Švec[3], Raúl Rivas[1,2,4], Miroslav Kolařik[3], and Paula García-Fraile[1,2,3,4]

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The European spruce bark beetle (*Ips typographus*) is the most destructive pest of spruce woodlands in Europe and a serious threat to these ecosystems since, due to Climate Change, population outbreaks are more frequent [1,2]. Previous research have shown the importance of fungi in *I. typographus* ecology, but as for the bacteriome, we are still to unveil its composition and relevance [2,3].

We explored the bacteriome in different life stages: larvae, pupae, teneral adult, and adult by running a metabarcoding analysis of the V5-V6 hypervariable region of the 16S rRNA gene and by identifying our collection of isolates. We observed that the bacteriome seems to evolve across the different stages, being more diverse when larvae, but relatively consistent in pupae and teneral adults. Once they become adults, diversity is like that of larvae. Furthermore, our results suggest that the core bacteriome include taxa belonging to the *Erwiniaceae* family, the *Pseudoxanthomonas* and *Pseudomonas* genera, and an undescribed genus within the *Enterobactereaceae* family. Concretely, isolates identification suggest that *Erwinia typographi* (*sensu lato*), *Pseudomonas bohemica*, and *Pseudomonas typographi* are ubiquitously present in the beetle.

Regarding the bacteriome metabolic potential, all strains shown the capacity to hydrolyse one or more complex polysaccharides, which may provide of an extra carbon source to the beetle. In this sense, isolates might also provide nitrogen as we observed that most of them could grow when uric acid or allantoin were the N sources available in the medium, or when no source of N was added. Finally, many strains showed antagonism to *Ips* entomopathogenic fungi, which could assist to the beetle defence.

*I. typographus* bacteriome changes across its life stages, although some taxa seem to remain. Isolates belonging to frequently found taxa, and other found in a lower frequency, e.g., *Staphylococcus*, *Acinetobacter*, *Curtobacterium, Bacillus,* and *Streptomyces*, have potential to aid the beetle by providing nutrients or antagonizing pathogens.

## References

1. Biedermann PHW, Müller J, Grégoire JC, Gruppe A, Hagge J, Hammerbacher A, et al. Bark Beetle Population Dynamics in the Anthropocene: Challenges and Solutions. Trends Ecol Evol. 2019;34:914–924. https://doi.org/10.1016/j.tree.2019.06.002
2. García-Fraile P. Roles of bacteria in the bark beetle holobiont – how do they shape this forest pest? Ann Appl Biol. 2018;172:111–125. https://doi.org/10.1111/aab.12406
3. Six DL. Bark beetle-fungus symbioses. Insect symbiosis. 2003;1:97-114. https://doi.org/10.1201/9780203009918.

## Funding

This research was cofounded by Czech Science Foundation (GAČR), grant number GAČR-Senior 19-09072S and the Regional Government of Castile and Leon (Escalera de Excelencia CLU-2018-04), co-funded by the Operational Program of the European Regional Development Fund for Castile and Leon 2014–2020. E.P.-A. received a grant co-financed by the Regional Government of Castille and Leon and the FEDER funds. Z.S-S also received a grant co-financed by the European NextGenerationEU, Spanish "Plan de Recuperación, Transformación y Resiliencia", Spanish Ministry of Universities, and University of Salamanca ("Ayudas para la recualificación del sistema universitario español 2021-2022").

Oral S2

# Cytosolic pH as a new fungal virulence signal

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In fungi, pH acts a key player in development and virulence. We previously found that ambient pH governs plant infection in the vascular wilt pathogen *Fusarium oxysporum* by reprogramming phosphorylation levels of mitogen-activated protein kinases (MAPKs) (1). Using the ratiometric GFP-based pH sensor pHluorin (2), we found that *F. oxysporum* responds to extracellular alkalinization and acidification with a transitory shift in cytosolic pH ($pH_c$)(3). How pHc control is linked to MAPK signaling is currently unknown. The essential plasma membrane $H^+$-ATPase Pma1 is the major regulator of pHc homeostasis in fungi. Pharmacological inhibition of Pma1 with Diethylstilbestrol (DES) triggers a rapid and sustained decrease of $pH_c$ accompanied by rapid and transitory changes in MAPK phosphorylation (3). Here we used a combination of reverse genetics and live-cell imaging to investigate the role of the $H^+$-ATPase Pma1 in pHc homeostasis. A gene knockout of *F. oxysporum* casein kinase 1, which acts a negative regulator of Pma1, resulted in a marked increase of Pma1 activity accompanied by extracellular acidification and altered MAPK phosphorylation levels (4). Interestingly, the *ck1*Δ mutants exhibited reduced growth and were impaired in invasive hyphal growth through cellophane membranes and in pathogenicity on tomato plants (4). Live-cell imaging of fluorescent Pma1-Clover revealed that this plasma membrane-localized $H^+$-ATPase is excluded from the hyphal tip, suggesting a possible role in regulation of polarized growth. Overall, our results shed light on the regulation of $pH_c$ and its role in development and virulence of fungal pathogens.

## References

1. Masachis S, Segorbe D, Turrà D, Leon-Ruiz M, Fürst U, El Ghalid M, et al. A fungal pathogen secretes plant alkalinizing peptides to increase infection. Nature microbiology. 2016;1(6):16043.
2. Fernandes TR, Serrano A, Di Pietro A. In vivo monitoring of cytosolic pH using the ratiometric pH sensor pHluorin. Methods Mol Biol. 2022;2391:99-107.
3. Fernandes TR, Mariscal M, Serrano A, Segorbe D, Fernandez-Acero T, Martin H, et al. Cytosolic pH Controls Fungal MAPK Signaling and Pathogenicity. mBio. 2023;14(2):e0028523.
4. Mariscal M, Miguel-Rojas C, Hera C, Fernandes TR, Di Pietro A. *Fusarium oxysporum* casein kinase 1, a negative regulator of the plasma membrane H+-ATPase Pma1, is required for development and pathogenicity. Journal of Fungi. 2022;8(12):1300

## Funding

This work was supported by grants from the Spanish Ministry of Science and Innovation (MICINN, grant PID2019-108045RB-I00) and Junta de Andalucía (P20_00179) to A.D.P.; M.M. was supported by PhD fellowship BES-2017-082775 from MICINN. T.R.F. was supported by the Marie Curie ITN FUNGIBRAIN (FP7-PEOPLE-ITN-607963).

## Acknowledgements

We thank Mª Dolores Alcaide Caballero for valuable technical assistance. We are grateful to Dr. Rafael Fernández Muñoz, IHSM "La Mayora", UMA-CSIC, Malaga, Spain for kindly providing seeds of tomato cultivar Moneymaker.

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## Session 3: One Health and Climate Change part I

Chairs: Ignacio García Estévez and Rocio Vicentefranqueira

# TASTE: A Flavorful Tool to Promote Healthy Food Consumption

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Non-communicable diseases, such as diabetes and cardiovascular disease, are reaching alarming rates. These diseases are related to unhealthy diets due to the consumption of highly processed foods (high added sugar/salts) and low amounts of fruit and vegetables(1).

So, the ultimate challenge is to switch worldwide consumers to healthy and sustainable diets, increasing the intake of plant-based food rich in several bioactive compounds with potential health benefits, such as polyphenols (PP).

Taste has become one of the most crucial factors in consumers' decision-making process. Regarding plant-based products, one of their main obstacles is the perception of astringency and bitterness induced by PP in high intensity. In contrast, both taste properties could be desirable in certain products when they are present at balanced levels (e.g., red wine and beer). Astringency is a tactile sensation of dryness and roughness felt in the oral cavity, while bitterness is a taste(2). The food industry has already some strategies to balance astringency and bitterness such as the addition of high amounts of sugar or artificial sweeteners or physical methods for the removal of the astringent/bitter (but also healthy) compounds. However, this results in a loss of nutritional value and the elimination of possible health benefits, while bringing concerns about food allergies and safety. So, efficient astringency and bitterness modulation towards pleasantness and health improvement, while maintaining minimal processing, is one of the main challenges of the food industry.

In recent years, polysaccharides (PS) have been the most promising approach to answering this challenge, reducing PP interaction with salivary proteins (SP) and, therefore modulating the astringency and bitterness perception(3). Aligned with a sustainable and economical approach, different sources of PS have been tested, including the use of several agrofood by-products. Indeed, PS from grape skins and fruit peels have been showing their potential ability to disrupt PP interactions with SP, and be used as astringent modulators.

**References**

1.Tresserra-Rimbau A, Arranz S, Vallverdu-Queralt A. New Insights into the Benefits of Polyphenols in Chronic Diseases. Oxidative Medicine and Cellular Longevity. 2017;2017:1432071.
2.Soares S, Brandão E, Guerreiro C, Soares S, Mateus N, de Freitas V. Tannins in Food: Insights into the Molecular Perception of Astringency and Bitter Taste. Molecules. 2020;25(11):2590.
3.Brandão E, Fernandes A, Guerreiro C, Coimbra MA, Mateus N, de Freitas V, et al. The effect of pectic polysaccharides from grape skins on salivary protein – procyanidin interactions. Carbohydr Polym. 2020;236:116044.

**Funding:** This work was financially supported through the project LAQV/REQUIMTE (UIDB/50006/2020 and UIDP/50006/2020) through national founds.
**Acknowledgements:** Elsa Brandão thanks the research contract (CEECIND/01598/2018), and Carlos Guerreiro and Mónica Jesus thank their doctoral fellowships from Fundação para a Ciência e Tecnologia (SFRH/BD/148549/2019) and Associated Laboratory for Sustainable Chemistry, Clean processes and Technologies LAQV (UI/BD/151275/2021), respectively. The authors also thank for financial support supported by AgriFood XXI I&D&I project (NORTE-01-0145-FEDER-000041) co-financed by European Regional Development Fund (ERDF), through the NORTE 2020 (Programa Operacional Regional do Norte 2014/2020).

# Effect of *Rhizobium* volatilome on lettuce growth under salt stress

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Soil salinity is a major issue affecting agricultural productivity worldwide, by reducing crop yields and quality. Given this continuing problem, the use of plant growth-promoting rhizobacteria (PGPR) can be a sustainable alternative, as they exhibit different direct and indirect plant growth promotion mechanisms (Ayuso-Calles et al., 2021). These include the production of volatile organic compounds (VOCs), which despite being one of the least studied mechanisms, has been reported to trigger a response in plant-bacteria interactions (Cappellari and Banchio, 2020). The main objective of this work was to characterize the volatiles emitted by a new bacterial strain from the genus *Rhizobium* and their effect on lettuce growth.

First, VOCs produced by this strain were determined by HS-SPME-GC-MS, including volatiles of interest such as α-pinene or 2-methyl-1-butanol. Differences in the volatilome profile were observed between normal and salinity conditions.

Subsequently, the effect of the volatiles emitted by the bacteria on lettuce seedlings growth under both conditions was analyzed in vitro. Results showed improvements in aerial and root development of plants, due only to VOCs exposure.

Finally, genomic DNA was extracted, sequenced by Illumina technique, and analyzed by RAST, antiSMASH or TYGS tools. Genome analysis allowed us to identify this strain as a possible new species within the genus *Rhizobium*, by ANIb and dDDH method. As well as the presence of annotated genetic machinery of interest in its interaction with the plant and the environment, such as terpenes or osmolytes (betaine or trehalose) synthesis pathways. In conclusion, VOCs production is a PGPR mechanism of interest which plays an important role in mediating plant resistance to abiotic stress.

## References

Ayuso-Calles, M., Flores-Félix, J.D., Rivas, R., 2021. Overview of the role of rhizobacteria in plant salt stress tolerance. Agronomy 11, 1–23. https://doi.org/10.3390/agronomy11091759

Cappellari, L. del R., Banchio, E., 2020. Microbial Volatile Organic Compounds Produced by Bacillus amyloliquefaciens GB03 Ameliorate the Effects of Salt Stress in Mentha piperita Principally Through Acetoin Emission. J. Plant Growth Regul. 39, 764–775. https://doi.org/10.1007/s00344-019-10020-3.

## Funding

This research was funded by the grant VA2I/463AC06 from Salamanca Provincial Government.

## Acknowledgements

M.A.C thanks the predoctoral grant from the University of Salamanca- Banco Santander. Authors thank Agribiotechnology Research (CIALE) Regional Government of Castile and Leon (Escalera de Excelencia CLU-2018-04) and co-funded by the Operational Program of the European Regional Development Fund for Castile and Leon 2014–2020.

# A Multifactorial Approach to Untangle Graphene Oxide (GO) Nanosheets Effects on Plants: Plant Growth-Promoting Bacteria Inoculation, Bacterial Survival, and Drought

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Drought is a limiting factor for agricultural productivity. Climate change threatens to expand the areas of the globe subjected to drought, as well as to increase the severity and duration of water shortage. Plant growth-promoting bacteria (PGPB) are widely studied and applied as biostimulants to increase plant production and to enhance tolerance to abiotic and biotic constraints. Besides PGPB, studies on the potential of nanoparticles to increase plant productivity and tolerance are also thriving. This possibility can be hampered by the toxicity of nanoparticles both for bacteria and plants, described in many studies mostly performed in laboratory conditions. However, contradictory results are often found between laboratory and field tests and the effect of nanoparticles towards bacterial cells and communities in soils are scarce and opportunities to increase plant productivity sustainably can be lost. The combined application of nanoparticles and PGPB can be one of these opportunities, but are poorly explored and it is important to unravel the potentialities of their application as a way to potentiate food production. In this study, *Rhizobium* sp. E20-8 and graphene oxide (GO) nanosheets were applied on container-grown maize seedlings in watered and drought conditions. Bacterial survival, seedling growth (dry weight), and biochemical endpoints (photosynthetic pigments, soluble and insoluble carbohydrates, proline, lipid peroxidation, protein, electron transport system, and superoxide dismutase) were evaluated. Results showed that simultaneous exposure to GO and *Rhizobium* sp. E20-8 was able to alleviate the stress induced by drought on maize seedlings through osmotic and antioxidant protection and mitigation of GO effects on the plant's biochemistry by *Rhizobium* sp. E20-8. These results constitute a new lead on the development of biostimulant formulations to improve plant performance and increase food production sustainably in water-limited conditions.

## References

Vurukonda, S.S.K.P.; Vardharajula, S.; Shrivastava, M.; SkZ, A. Enhancement of Drought Stress Tolerance in Crops by Plant Growth Promoting Rhizobacteria. Microbiol. Res. 2016, 184, 13–24. https://doi.org/10.1016/j.micres.2015.12.003

Perreault, F.; de Faria, A.F.; Elimelech, M. Environmental Applications of Graphene-Based Nanomaterials. Chem. Soc. Rev. 2015, 44, 5861–5896. https://doi.org/10.1039/C5CS00021A

Cheng, F.; Liu, Y.-F.; Lu, G.-Y.; Zhang, X.-K.; Xie, L.-L.; Yuan, C.-F.; Xu, B.-B. Graphene Oxide Modulates Root Growth of Brassica napus L. and Regulates ABA and IAA Concentration. J. Plant Physiol. 2016, 193, 57–63. https://doi.org/10.1016/j.jplph.2016.02.011

Cardoso, P.; Alves, A.; Silveira, P.; Sá, C.; Fidalgo, C.; Freitas, R.; Figueira, E. Bacteria from Nodules of Wild Legume Species: Phylogenetic Diversity, Plant Growth Promotion Abilities and Osmotolerance. Sci. Total Environ. 2018, 645, 1094–1102. https://doi.org/10.1016/j.scitotenv.2018.06.399

**Funding:** This research was funded by FCT through the financial support to the H2O value project (PTDC/NAN-MAT/30513/2017) also supported by FCT/MEC through national funds, and the co-funding by the FEDER, within the PT2020 Partnership agreement and Compete 2020 (CENTRO-01-0145-FEDER-030513). This research was also funded by FCT/MCTES through the financial support to CESAM (UIDP/50017/2020 +UIDB/50017/2020), through national funds.

# Grape pomace biochar is effective for root-knot nematode control

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Root-knot nematodes are obligate endoparasites that need to establish and feed inside the plant's roots to complete its life cycle. These nematodes represent a major threat to agriculture and economy worldwide. Due to increasing restrictions on pesticide use, it is essential to develop alternative management strategies. In this respect, wine-making residues are quite abundant in Mediterranean regions, which can be transformed into biochar, with different uses in agriculture.

We obtained and characterised two different biochars (BC350, BC700) derived from the pyrolysis of red grape pomace at two different temperatures (350 °C or 700 °C). Both biochars had higher concentrations of C and plant macro- and micronutrients (K, Ca, P, Mg or Fe) when compared to the raw material. These elements were largely present in a water-soluble form and its concentration increased at higher pyrolysis temperature. Both biochars presented a highly porous structure filled with crystals of different morphology and composed of elements such as Ca, K, Mg, P, Al or Si, as revealed by scanning electron microscopy coupled with energy dispersive X-ray spectroscopy. Biochar-derived aqueous extracts were phytotoxic to tomato seedlings at high concentrations. However, no effect was observed on the nematode's infective stage larvae (J2) or during the plant-nematode interaction on *in vitro* assays.

When tomato plants were grown in pots with washed biochar (for 24 hours with distilled water; BC350W) at a 0.75% (w/w) application rate and inoculated with *Meloidogyne javanica* juveniles, we observed a reduction of their infective and reproductive traits (42% reduction of the number of egg masses/plant and a 29% reduction of the number of eggs/plant) when compared to the untreated control plants. Future experiments will help identify the mechanism of action responsible for this effect, as well as its efficacy against other pathogens or different types of stress.

**Funding** This work was supported by the Spanish Government (PID2019-105924RB-I00 MCIN/AEI/10.13039/501100011033; RED2018-102407-T) to CE, and (AGL2014-53771-R; AGL2017-87591-R) to LEH, and by the Castilla-La Mancha Government (SBPLY/17/180501/000287 and SBPLY/21/180501/000033) to CE. AM-G was recipient of a PhD grant from Fundación Tatiana Pérez de Guzmán el Bueno. EU FEDER funds complemented all the grants.

**Acknowledgements** We thank Bodega S.A.T Coloman (Pedro Muñoz, Ciudad Real, Spain)

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# Preliminary screening of bacteria isolated from a fire-affected forest soil in Avila: implications for ecosystem recovery.

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Forest fires, often caused by human activities, have profound implications for ecosystems, altering physico-chemical factors and being a challenges for life survival, highlighting the need to understand the recovery processes in fire-affected areas. In 2021, Avila province experienced one of the most devastating fires in its history, with 12,000 hectares being destructed. In this study, we explore the isolation of extreme-resistant bacteria surviving the forest fire temperatures, with the aim of conducting a preliminary screening of their abilities to recover burnt environments. Sampling of ashes was conducted near Sotalbo village and isolation over specific media resulting in the recovery of 29 bacterial strains. Identification of the isolates by sequencing the 16S rRNA gene of the strains shown that predominant phyla were *Bacillota, Actinomycetota and Pseudomonadota* phylum. These strains were tested for their ability to grow under extreme conditions, including temperatures of 45°C, 55°C, and 70°C, as well as anaerobic conditions. The screaning revealed that 75.8% of the bacterial strains exhibited growth at 45°C, while this percentage decreased to 59% at 55°C, and to 44.8% at 70ºC. In terms of anaerobic conditions, 86.5% of the bacterial strains demonstrated a successful growth. Based on the screening results, the three most promising bacterial strains were selected for further assays simulating the recovery of burned soil. The assays were survilled for 60 days, evaluating several parameters, and revealed that strain A,2.3 increase recovery and plant development of the evaluated soils suggesting its potential for burned soil rehabilitation.

The findings highlight the notable capacity of some resistant bacteria isolated from fire-affected soil to withstand high temperatures and facilitate soil recovery. Further investigations are necessary to unravel their specific mechanisms of resilience and understanding the contribution of these bacteria to soil regeneration and nutrient cycling will be crucial for developing effective strategies for ecosystem restoration in fire-affected areas.

## References

Certini, G., Moya, D., Lucas-Borja, M. E., & Mastrolonardo, G. (2021). The impact of fire on soil-dwelling biota: A review. *Forest Ecology and Management*, *488*, 118989.

Knelman, J. E., Schmidt, S. K., Garayburu-Caruso, V., Kumar, S., & Graham, E. B. (2019). Multiple, compounding disturbances in a forest ecosystem: fire increases susceptibility of soil edaphic properties, bacterial community structure, and function to change with extreme precipitation event. *Soil Systems,* 3(2), 40.

Barreiro, A., & Díaz-Raviña, M. (2021). Fire impacts on soil microorganisms: Mass, activity, and diversity. *Current Opinion in Environmental Science & Health,* 22, 100264.

Bardgett, R. D., & Caruso, T. (2020). Soil microbial community responses to climate extremes: resistance, resilience and transitions to alternative states. *Philosophical Transactions of the Royal Society B,* 375(1794), 20190112.

Carro, L., Castro, J. F., Razmilic, V., Nouioui, I., Pan, C., Igual, J. M., ... & Klenk, H. P. (2019). Uncovering the potential of novel micromonosporae isolated from an extreme hyper-arid Atacama Desert soil. *Scientific reports,* 9(1), 4678.

**Funding** The authors received no financial support.

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# Flavonoids as neuroprotector molecules: Evaluation in a transgenic strain of the model organism *Caenorhabditis elegans*

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With the increased life expectancy of the world's population, the prevalence of cognitive disorders related to ageing is also increasing [1]. Alzheimer's disease (AD), one of the most common neurodegenerative pathologies, is characterised by the accumulation of amyloid β (Aβ) deposits and hyperphosphorylated Tau protein neurofibrillary tangles (p-Tau) as main hallmarks [2]. It has been suggested that the regular intake of foods rich in flavonoids might help to prevent or mitigate AD progression [3]. This study explores the possible role of flavonoids to protect against AD, in particular, to reduce the damage associated with p-Tau aggregates. For this, the potential of the flavan-3-ol epicatechin (EC) and the flavonol quercetin (Q) as neuroprotective molecules was studied using the transgenic strain BR5270 of the model organism *Caenorhabditis elegans*, which expresses the F3 pro-aggregation fragment of the human tau protein [4]. Synchronised nematodes were propagated at 20 °C on NGM plates seeded with *E. coli* OP50 as a food source and containing EC or Q 150 µM as treatment, or DMSO 0.1 % as control. Nematodes cultured in the presence of EC and Q increased their lifespan by 8.9 % and 7.9 % respectively, compared with the control group. Furthermore, the chemotaxis index was significantly higher in individuals treated with both flavonoids. Finally, locomotion was evaluated by counting the body bends for 30 seconds at different ages (days 1, 3, 5, 8 and 11 of adulthood), showing that from day 3 the locomotion of the control group was significantly reduced. Currently, the molecular mechanisms of actions of flavonoids are being investigated through studies of gene expression. So far, the results suggest that both, EC and Q, could delay the neurodegeneration related to p-Tau accumulation in *C. elegans*, pointing to the capacity of these flavonoids to reduce the risk of AD progression.

**References:** [1] European Commission (2020). The 2021 Ageing Report: Underlying Assumptions and Projection Methodologies. [2] Cvetković-Dožić et al (2001). Neuropathological hallmarks of Alzheimer's disease. Arch Oncol 9, 195-199. [3] Holland et al. (2020). Dietary flavonols and risk of Alzheimer dementia. Neurology Apr, 94 (16) 1749-1756. [4] Fatouros et al. (2012). Inhibition of Tau Aggregation in a Novel *Caenorhabditis elegans* Model of Tauopathy Mitigates Proteotoxicity. Hum. Mol. Genet. 21, 3587–3603.

**Acknowledgements**: The funding received through the projects PID2019-106167RB-I00, financed by MCIN/AEI/ 10.13039/501100011033, and SA093P20, financed by Junta de Castilla y León, is acknowledged.

# Session 4: One Health and Climate Change part II

Chairs: Lorena Carro and Marta Marcos

Keynote S4

# Environmental factors controlling seed quality of emergent crops within the context of climate change

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Current climate scenarios project drought episodes increasing in frequency, duration, and intensity accompanied by heat waves, especially affecting the Mediterranean region. Thus, water stress and elevated temperatures have become substantial threats to global food security (Kopittke et al., 2019). To mitigate their impact on agriculture, different strategies have been proposed, including the diversification of agriculture utilizing abiotic stress-resilient crops with improved water-use efficiency and heat tolerance which could greatly contribute to coping with agronomical losses associated with environmental constraints. In this regard, quinoa (Chenopodium quinoa Willd.) represents a promising crop, given its tolerance to several abiotic stresses, including water deficit (Hinojosa et al. 2018). It is an ancient crop from the Andean Altiplano that has undergone a great expansion worldwide in the last decades due to the remarkable nutritional quality of its seeds, including a high protein content, a balanced amino acid profile, and a high antioxidant capacity. However, very few studies have explored how different environmental factors (including water stress or elevated temperatures) can affect the nutritional quality of quinoa seeds. Considering that the genetic diversity of quinoa confers this plant with the potential of growing under a wide range of environments, including unfavorable soil and climatic conditions (Khaled et al., 2021), this work analyzes the adaptation of quinoa plants to different Mediterranean environments evaluating the impact on the nutritional properties of the seeds. A multidisciplinary multi-omic approach combined with plant physiological analyses were used in different quinoa cultivars grown in the field and in the greenhouse aiming at unraveling the molecular, regulatory, and signaling pathways that regulate source-sink (leave-seed) interactions resulting in changes in the nutritional properties of quinoa seeds. The overall goal is to shed light on the environmental impact on the nutritional value of this emergent crop, still underutilized, under new climate scenarios with special attention to water deficit environments of the Mediterranean basin.

## References

Kopittke, P. M., Menzies, N. W., Wang, P., McKenna, B. A., & Lombi, E. (2019). Soil and the intensification of agriculture for global food security. Environment International, 132. https://doi.org/10.1016/J.ENVINT.2019.105078
Hinojosa, L., González, J. A., Barrios-Masias, F. H., Fuentes, F., & Murphy, K. M. (2018). plants Quinoa Abiotic Stress Responses: A Review. https://doi.org/10.3390/plants7040106
Khaled, S., Khalil, G. A.-N., Hussein, A. H. A., Darwish, K. M., & Ibrahim, O. M. (2021). Impact of Irrigation Deficit on Yield, and Water Productivity of Quinoa. Journal of the Advances in Agricultural Researches, 26(3), 120–128. https://doi.org/10.21608/JALEXU.2021.188848

## Funding

The authors gratefully acknowledge the financial support received from the Ministerio de Ciencia e Innovación (MICINN, Spain) and the Agencia Estatal de Investigación (PID2019–105748RA-I00 AEI/10.13039/501100011033), the FPI UAM Fellowship Programme 2019 (to SG-R), and the Ramón y Cajal Programme 2019 (to MR, Ref. RYC2019-028148-I).

## Acknowledgments

The authors greatly thank Susana Vilariño (Algosur, Spain) for her support helping to conceive and plan the experiments and for providing, together with Dr. Sven Jacobsen (Quinoa Quality, Denmark), the quinoa seeds used in this study.

# Upcycling food waste into functional ingredients and biobased films

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According to the UN, in 2019, an estimated 931 million tons of food, 17% of total food available to consumers, was wasted across three sectors: household (61%), food service (26%), and retail (13%) [1]. To "ensure sustainable consumption and production patterns" by 2030, SDG 12.3 aims to "halve per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including post-harvest losses" [2].

Agro-industrial by-products are sources of nutrients, such as minerals, fatty acids, vitamins, proteins, fiber, and other components of interest as volatile compounds or polyphenols with high antioxidant capacity. Therefore, these by-products have great potential to be used as functional food ingredients with beneficial effects on human health. Some components, such as polyphenols, can be used as food additives (antioxidants and preservatives) to develop active packaging and thus extend food shelf-life. Furthermore, other components, such as pectin and proteins, are suitable for developing edible films and coatings.

This presentation will examine recent key research findings conducted on the recovery of several by-products from different food sectors in the framework of three international research projects.

Different recovering approaches for high-value compounds, using innovative extraction and green technologies, their chemical characterization and bioactivities, the safety assessment, and the stabilization of these compounds before the final applications on functional food development or/and food packaging materials will be discussed.

This presentation will highlight the main results obtained with some examples of food by-products applications and open new perspectives of research and discussion on their valorization within the framework of a circular economy that is crucial due to economic and environmental reasons.

## References

1. United Nations Environmental Programme (2021). Food Waste Index Report 2021.
2. https://www.un.org/sustainabledevelopment/sustainable-development-goals/

## Funding

1. ValICET project is supported by the PRIMA program (Section 2 Funded Projects 2020, Agri-Food Value Chain) and funded in Spain by the Spanish Ministry of Science and Innovation and the State Research Agency project PCI2021-121965 (MCIN/AEI/10.13039/501100011033 and by the European Union "NextGenerationEU"/PRTR).
2. VIPACFood project (selected by ERA-NET ARIMNet2 call 2016 (Coordination of Agricultural Research In the Mediterranean) of the European Union through the 7th Framework Programme and funding by the Spanish National Institute for Agricultural and Food Research and Technology (INIA) ref. APCIN2016-0006-00-00.
3. COVALFOOD project has received funding from the European Union's Seventh Framework programmefor research and innovation under the Marie Skłodowska-Curie grant agreement No. 609420-2020 researchers: Train to Move (T2M).

## Acknowledgements

L. Barbosa-Pereira is grateful to the Spanish Ministry of Science and Innovation for her "Ramón y Cajal" Grant (Agreement No. RYC2021-033505-I).

# Unveiling the impact of food additives on flavor of healthy foods: the role in the polyphenol-oral constituents interactions

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Food additives are added to food products during processing to improve taste, appearance, texture, and shelf life, among others. Polyphenols are responsible for key organoleptic properties like colour and taste [1], and with several health benefits. One of the sensory attribute for which they are responsible is named astringency, and it is charactarized by a dry, puckering sensation and a feeling of roughness in the mouth [2]. The most accepted mechanism for this sensation is the precipitation of salivary proteins, however the interaction of the polyphenols with other oral constituents such as tongue epithelial cells and the mucosal pellicle have been also reported [3]. Previous research has shown that some compounds commonly used as food additives (with different purposes), have been able to restrain some of the referred interactions inside the oral cavity [4]. However, if this effect is effective with different classes of polyphenol astringent molecules and the mechanism beyond this effect is so far unkown. So, in this work, the effect of different food additives found in food and beverages (sodium carbonate, sodium bicarbonate, and ammonium bicarbonate) on polyphenol-oral constituent interactions was studied using a cell-based model of the oral epithelium. The results obtained for a green tea extract rich in flavanols indicated that the three additives seem to reduce the polyphenol interaction with salivary proteins and the oral epithelium. For sodium carbonate the pH shift appears to be a key factor in the mechanism by which this additive affects the interaction, as inhibition is stronger at pH values around 10 than at pH close to 7. Furthermore, with tannic acid (hydrolysable tannins) a different pattern was observed, suggesting that the modulation effect relies on the family of polyphenols present in the food matrix.

## References

1. Cheynier, V., *Phenolic compounds: from plants to foods*, Phytochem. Rev., **2012**, 11(2): p. 153-177.
2. Huang, R. and C. Xu, *An overview of the perception and mitigation of astringency associated with phenolic compounds*, Compr. Rev. Food Sci. Food Saf., **2021**, 20(1): p. 1036-1074.
3. Soares, S., et al., *Development of a new cell-based oral model to study the interaction of oral constituents with food polyphenols*, J. Agric. Food Chem., **2019**, 67(46): p. 12833-12843.
4. Ramos-Pineda, A., et al., *Influence of chemical species on polyphenol–protein interactions related to wine astringency*, J Journal of agricultural food chemistry, **2019**, 68(10): p. 2948-2954.

**Funding:** This work was financially supported through the project LAQV/REQUIMTE (UIDB/50006/2020 and UIDP/50006/2020) through national founds.
**Acknowledgements:** Mónica Jesus thanks the Doctoral fellowship UI/BD/151275/2021 to Associated Laboratory for Sustainable Chemistry, Clean processes and Technologies LAQV and to Fundação para a Ciência e Tecnologia (FCT). The authors thank the Science and Technology Foundation (FCT) for researcher contracts CEECIND/01598/2018, CEECIND/01265/2018. The authors also thank for financial support supported by AgriFood XXI I&D&I project (NORTE-01-0145-FEDER-000041) co-financed by European Regional Development Fund (ERDF), through the NORTE 2020 (Programa Operacional Regional do Norte 2014/2020).

**Oral S4**

# An integrative analysis of root adaptation to multiple environmental soil conditions in *Brassica napus*

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Climate change is a global threat for agriculture and has a strong impact on nutritional security worldwide (1). The increases in the average global temperature and the frecuency of other climatic stresses have a negative impact in crop yield (2). Roots play an essential role in plant growth and adaptation under different climatic environments. Better understanding of the root responses to abiotic stress combination in crops is required to improve crop tolerance to climate change. With this aim we are studying the phenotypic variation and root/shoot responses to the combination of warming temperatures and nutrient deficiencies on root growth and development in a *Brassica napus* collection. We have observed significant variation on root architecture in response to nutritional stress between cultivars, including alteration on root traits related to root depth, width, distribution, and the number and distribution of secondary roots. We performed statistical analysis, including a principal component analysis and hierarchical clustering, to indentify cultivars with differential responses. We selected two cultivars, Westar and Wesway, that showed diferential responses among the different stress treatments in root depth and lateral root density, for further analysis. Westar showed a differential response to the combination of both stresses, including higher expression of the nitrogen transporter *BnNRT2* than Wesway. Finally, we have performed an RNA-sequencing analysis of roots and shoots of both cultivars grown under warming temperature and nitrogen deficiency. We are now performing analyses of the differentially expressed genes to identify the main gene regulatory networks underlying this responses. These and further results will be discussed.

## References

1. Arora, N. K. (2019). Environmental Sustainability, 2(2), 95-96.
2. Malhi, G. S., Kaur, M., & Kaushik, P. (2021). Sustainability, 13(3), 1318.

## Funding

This work was funded by grant PID2020-113479RB-I00 from Ministerio de Ciencia e Innovación and by the "Severo Ochoa Program for Centres of Excellence in R&D" (SEV-2016-0672, 2017-2021) from the Agencia Estatal de Investigación of Spain granted to the CBGP.

**Oral S4**

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# FUNGI VOLATILES AS A TOOL FOR PLANT TOLERANCE AGAINST ABIOTIC STRESS

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Volatile organic compounds (VOCs) are important molecules in the communication between plant and fungi, that affect different processes like the proliferation of lateral roots, plant growth, water and nutrient acquisition or defence against different environmental stresses (Calvo-Polanco *et al*., 2019, Ditengou, *et al*. 2015, Venneman, *et al*., 2020). Within our current research, we aim to determine the main molecular mechanisms of VOCs in the plant responses to osmotic stress. We are testing how VOCs of *Laccaria bicolor*, *Hebeloma cylindrosporum* (ectomycorrhizal fungi typically found in symbiosis with tree species) and *Serindipita indica* (symbiont endophyte inducing stress tolerance in plants) affect plant development and lateral root (LR) formation in Arabidopsis lines impaired in the auxin and cytokinin pathways under osmotic and control conditions. Moreover, reporter Arabidopsis lines of the auxin and cytokinin pathways fused to ß-GUS (*SKP2B::GUS, ARR1::GUS*) were used to quantify the effects of fungal VOCs in shoots and roots using western blot assays. Our first results indicate that fungal VOCs affect primary root length of the different Arabidopsis lines only when subjected to osmotic stress. In addition, fungal VOCs increased the number of LR, especially in the cytokinin mutant *arr1-4*, while they were inhibited at the two auxin insensitive mutants, *axr1-3* and *aux1-7*. Western blot assays using anti ß-GUS indicated that the presence of VOCs produces a significant increase in the induction of *ARR1* and *SKP2B*. Secondly, we have identified 3-octanone as one of the main VOCs emmitted in our 3 fungal species and have tested its role in seed germination and plant development. These results could be relevant in the current scenario of climate change in which the search of new mechanisms takes special interest for plant survival and its proper development.

## References

Calvo-Polanco *et al.* (2019), J.Exp. Bot.
Ditengou *et al.* (2015), Nat. Commun.
Venneman *et al.* (2020). Front. Plant Sci.

## Funding

This work is supported by the Excellence Research Unit AGRIENVIRONMENT of the University of Salamanca, SA137P20 from Junta de Castilla y León and Escalera de Excelencia CLU-2O18-04 co-funded by the P.O. FEDER of Castilla y León 2014–2020 Spain (to O.L.). E. Miñambres is beneficiary of FPU predoctoral fellow (FPU21/03849).

# Addition of *Torulaspora delbrueckii* mannoproteins to red wine: effect on the phenolic composition, color and astringency

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Global climate change is having a great impact on vine phenology, which can ultimately lead to changes in the accumulation of sugar, acids and phenolic compounds in the grapes, resulting in the production of wines that can present altered color and unbalanced astringency [1]. The addition to wine of mannoproteins (MPs) could be useful to stabilize wine color and soften astringency [2,3]. However, not all MPs seem to have these positive effects and some authors have shown that MPs do not contribute to the improvement of wine sensory properties [4].

MPs are glycoproteins located in the yeast cell wall which are highly substituted with mannose residues. In a previous study carried out in our laboratory, MPs from *Torulaspora delbrueckii* were, among the MPs derived from other oenological yeast, the most effective ones for the modulation of wine color [2].

Therefore, the objective of this study was to deepen into the effects of *T. delbrueckii* MPs on wine organoleptic properties (*i.e.* color and astringency). MPs were extracted from the yeast by three treatments: induced autolysis, enzymatic digestion and alkaline extraction. The protein and polysaccharidic moieties of the MPs were characterized by SDS-PAGE, Lowry method, HRSEC-RID and HPLC-DAD-MS. Then, the MPs were added to a Tempranillo red wine and changes in color and phenolic composition were analyzed by tristimulus colorimetry and HPLC-DAD-MS, respectively. Wine astringency was also evaluated by a sensory panel.

The obtained results indicate that the MPs showed important differences in their size and monosaccharide composition. Furthermore, the addition to wine of these MPs led to changes in the phenolic composition. The addition of the MPs obtained by yeast autolysis and enzymatic digestion led to a decrease of wine astringency, although the first ones also had negative effects on wine color.

## References

[1] Droulia, F., & Charalampopoulos, *Atmosphere, 12*, 495, 2021
[2] Oyón-Ardoiz, M., Manjón, E., Escribano-Bailón, M. T., & García-Estévez, I. *LWT-Food Science and Technology, 172*, 114219, 2022
[3] Manjón, E., Brás, N. F., García-Estévez, I., & Escribano-Bailón, M. T. *J. Agric. Food Chem., 68*, 13459-13468, 2020
[4] Guadalupe, Z., & Ayestarán, B. *J. Agric. Food Chem., 56*, 9022-9029, 2008

## Funding

This research was financially supported by Grant PID2021-127126OB-C21 funded by MCIN/AEI/10.13039/501100011033 and by “ERDF A way of making Europe”. M. Oyón-Ardoiz thanks Junta de Castilla y León, cofunded by Consejería de Educación and Fondo Social Europeo Plus (FSE+), her pre-doctoral contract. Thanks are also due to Junta de Castilla y León-FEDER Programme for the Strategic Research Programs for Units of Excellence (Escalera de Excelencia CLU-2O18-04).

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# Session 5: Microbiome in agrosystems

Chairs: Zaki Saati and Maria Illescas

Keynote S5

# Protecting our crops – sustainable approaches for the control of plant-parasitic nematodes

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Plant-parasitic nematodes (PPN) are a threat to global agriculture with losses estimated at 100 billion USD per year (Savary et al., 2019). Ranking 3rd as the most serious PPNs in agriculture, root-lesion nematodes (RLNs), *Pratylenchus* spp., are known by their worldwide distribution and broad host range (Jones et al., 2013). *Pratylenchus penetrans* is one of the most damaging RLN with over 400 hosts affecting economically important agronomic and horticultural crops (Castillo and Volvas, 2017). In Europe, *P. penetrans* has been detected in potato cultures and ornamental plants in Portugal and in association olive trees in Spain, Italy, and Turkey. This RLN is classified as an A1 quarantine pest in South America, while in EU, is a regulated A2 non-quarantine pest. Effective control measurements for RLN, and specifically for *P. penetrans*, are limited due to the lack of natural resistant varieties. The impact of *P. penetrans* alone and in interaction with other plant pathogens (e.g., bacteria and fungi) in such cultures emphasises an urgent demand for the development of new and sustainable control approaches as alternative to common agriculture chemicals. In this sense, the molecular mechanisms of *P. penetrans* parasitism are being investigated for the development of alternative and more specific control strategies (Vieira et al., 2019; Vicente et al., 2019).

## References

Savary et al. (2019). Nature Ecol Evol. DOI: 10.1038/s41559-018-0793-y
Jones et al. (2013). Mol Plant Pathol. 14:946-961. DOI: 10.1111/mpp.12057
Castillo and Volvas (2007). Leiden: Brill. p. 529. DOI: 10.1111/j.1365-3059.2009.02097.x
Vieira et al. (2019) Front Plant Sci 10:971. DOI: 10.3389/fpls.2019.00971
Vicente et al. (2019) PLoS ONE 14: e0212450. DOI: 10.1371/journal.pone.0212540

## Funding

This work is funded by National Funds through *Fundação para a Ciência e Tecnologia* (FCT, Lisbon, Portugal) to CSLV by 2018.00040.CEECIND and projects PratyOmics (FCT, PTDC/ASP-PLA/0197/2020) + UIDB/05183/2020 to MED.

Keynote S5

# Understanding global change impacts on the microbiome of Mediterranean agroforestry systems

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Mediterranean agroforestry systems dominated by evergreen oaks are High Nature Value Systems with a long history of agricultural, pastoral and silvicultural uses. The long-term sustainability of these valuable systems is now threatened by the loss of tree health driven by global change factors including climate change and exotic pathogens of the genus *Phytophthora*. Because plant health is intimately linked to the characteristics of its microbiome, analyzing how global change factors alter the characteristics of microbial communities is of high relevance for a realistic assessment of tree resistance to increasing abiotic and biotic stressors. In this talk, I will first show recent advances on our understanding of the impacts of a drier and warmer climate on the soil microbiome of *Quercus suber* forests. Results suggest that drought and warming might have large negative effects on the abundance of soil bacteria, fungi and *Phytophthora* communities, with many losers and just a few winners within their taxonomic composition. However, the net consequence of these changes for tree performance might not be negative, given the large benefits inherent to a lower abundance of aggressive *Phytophthora* pathogens. In the second part of the talk, I will present the results of recent projects aim to disentangle the role of the soil microbiome as a modulator of tree health in *Quercus ilex* dehesas invaded by the exotic pathogen *Phytophthora cinnamomi* and affected by severe problems of tree decline. We will dig into the role of beneficial fungi (ectomycorrhizal and mycoparasitic fungi) and bacterial networks as potential components of suppressive soils in asymptomatic oaks and discuss how this knowledge might contribute to the design of management actions aim to achieve healthier soils and trees in these unique but threatened systems.

**Oral S5**

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# *Dehesa*: management practices and their influence on soil microbiological diversity.

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Extensive agro-silvopastoral systems provide important ecosystem services. However, because most of these systems, such as *dehesa*, are seriously limited by their low profitability they are subjected to two opposing trends: abandonment or ecological intensification through pasture improvement and/or livestock management. The soil microbiome plays a key role in nutrient cycling, fertility and carbon sequestration. Therefore, alterations in the soil microbiome can lead to significant changes in crop and livestock production. In this work, we studied soils of nine dehesas located at Badajoz, Cáceres and Salamanca. We analysed the effects of five different agricultural and livestock management practices on taxonomic richness (α diversity) and composition (β diversity) of soil bacteria and fungi, which in turn were related to plant richness and composition. The results indicated that management technique, soil pH and aridity explained 19%-25% of soil fungal and bacterial taxonomic richness, respectively. Old legume sowing (Lv) and abandonment (Ab) resulted in more diverse bacterial populations than livestock management: rotational (Ro) and traditional (Ct) grazing. In addition, young legume sowing (Lj) presented higher bacterial richness than Ro. However, in terms of fungal diversity, only Ro was higher than Lj. The composition of both soil populations was affected by the treatment and soil pH, but aridity had no significant influence. A correlation among the taxonomic composition of plants, fungi and bacteria was also found. In view of these results, we anticipate that selection of appropriate management practices will be decisive to obtain a healthy soil in *dehesa* systems.

**Key words:** sequencing, microbiology, soil, dehesa, management

## References

Bhattacharyya, S. S., & Furtak, K. (2023). Soil–Plant–Microbe Interactions Determine Soil Biological Fertility by Altering Rhizospheric Nutrient Cycling and Biocrust Formation. In *Sustainability (Switzerland)* (Vol. 15, Issue 1). MDPI

Fierer, N. (2017). Embracing the unknown: Disentangling the complexities of the soil microbiome. In *Nature Reviews Microbiology* (Vol. 15, Issue 10, pp. 579–590). Nature Publishing Group.

Hartmann, M., & Six, J. (2023). Soil structure and microbiome functions in agroecosystems. In *Nature Reviews Earth and Environment* (Vol. 4, Issue 1, pp. 4–18). Springer Nature.

Jansson, J. K., & Hofmockel, K. S. (2018). The soil microbiome — from metagenomics to metaphenomics. In *Current Opinion in Microbiology* (Vol. 43, pp. 162–168). Elsevier Ltd.

Sollen-Norrlin, M., Ghaley, B. B., & Rintoul, N. L. J. (2020). Agroforestry benefits and challenges for adoption in Europe and beyond. In *Sustainability* (Switzerland) (Vol. 12, Issue 17). MDPI.

**Funding**: This work was funded through the project "PID2019-108313RB-C33" of the Spanish Research Agency and the project "CLU-2019-05 – Unidad de Excelencia IRNASA-CSIC" funded by the Junta de Castilla y León and cofunded by the European Union (FEDER "Europa impulsa nuestro crecimiento").

## Acknowledgements

The speaker would like to thank Virgina Gascón for her help with the PLFAs analysis and to colleagues from the University of Extremadura for their efforts in the study of plant diversity and carbon fluxes. And, of course, to my supervisors for their support in the development of my doctoral thesis.

**Oral S5**

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# Core bacteria endophytes are vertically transmitted to the plant offspring generation through the germ line.

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Plant-associated microorganisms are known to impact plant health and disease. Endophytic microbiota is intimately associated with host plants and can dwell different plant tissues, including the seeds. Seed endophytes have a particular interest as they have the potential to be vertically transmitted. This route is of paramount importance for the establishment and evolution of long-term stable bacteria host-associated interactions that can persist over generations. Hundreds of seed-bacteria associations have been recently uncovered; however, most seem to be transient or unspecific. Several studies also characterized transmission of seed-borne bacteria from seeds to seedlings, but limited knowledge is available on the succession of seed endophytes through plant generations. Transmission of microorganisms from plant tissues to seeds has been also already reported, however, transfer of certain bacteria from seeds through plant generations has not been experimentally confirmed. In order to address this issue, we first identified a **C**ore **E**ndophytic **G**enus (CEG) inhabiting the endosperms of different wheat cultivars from different worldwide locations. by cultivation-dependent and -independent methods. CEG is part of the core wheat microbiome, as it is present in most varieties and tissues, regardless of their geographical location. Interestingly, its abundance gradually increases from root to spike wheat developmental stages in the field. This vertical enrichment suggests transfer of these endophytic bacteria through the seeds. To test this hypothesis, a CEG isolate was GUS-labelled with a plasmid encoding the enzyme β-glucuronidase. Inoculation of seeds and flowers of wheat and *Lolium* with the labelled isolate demonstrated its ability to naturally colonize the next generations of cereal plants, confirming bacterial vertical inheritance to through plant generations.

# Sourdough – A model system for microbiome research

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The advent of DNA sequencing technologies allowed us to catalogue microbial communities of entire ecosystems on different scales. This led to a new understanding of the importance of microbial communities, or microbiomes, in the environment. Microbiomes are key drivers of global biogeochemical processes and ecosystem functioning and are known to be highly sensitive to environmental change. Researchers now try to predict microbial community dynamics and their effect on the environment, or vice versa, using computational models based on sequencing data. However, the environment is complex and difficult to control, so converting the empirical data and correlations that were generated in environmental studies, into an actual mechanistic understanding of what exactly is happening remains challenging. In modern biology, model organisms (e.g. *E. coli*, *S. cerevisiae*, *A. thaliana*, etc.) are studied in great depth in controlled experiments to investigate specific biological processes. However, the available model microbial organisms are studied in pure cultures and therefore are not suitable to investigate ecological and evolutionary theories on the formation and dynamics of microbial communities. Thus, we need to establish model microbial communities that we can control and manipulate for microbiome research purposes. Sourdoughs are populated by microorganisms, especially yeasts and lactic acid bacteria, which are responsible for its characteristic properties. Notably, the microbial community of sourdoughs is less complex than communities from natural environments and they develop in a short manner of time, within days to months. This rapid development facilitates predictable access to many replicated samples over relatively short time periods, enabling the exploration of various experimental questions. Thus, we propose to use sourdough as a potential model system for microbiome ecology and evolution research and showcase a few ongoing experiments on that topic that we are currently performing in our lab.

**References** n.a.
**Funding** n.a.
**Acknowledgements** n.a.

**Oral S5**

IAS2023
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# Traditional genotypes, a richer source of potential fungal endophytes for tomato improvement

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The plant microbiome has gained importance since it has been reported to influence on plant growth, health, and resistance to biotic and abiotic stresses[1]. The microbiome composition seems to be determined by several factors, including plant genotype[2]. In this regard, wild plant species and traditional cultivars, which have undergone less manipulation than commercial cultivars, may hold an unexplored microbial potential for agronomic application[3,4]. The objective of this study was to explore the fungal endophytic microbiota of several tomato genotypes. Four traditional varieties from the Mediterranean region (ADX-2, TH-30, ISR-10, MO-10) were examined alongside two commercial cultivars (Ailsa Craig, Moneymaker). The microbiome study was done by performing Illumina sequencing, targeting ITS region. The subsequent bioinformatics analysis revealed distinctions among genotypes, demonstrating that traditional varieties harbored a more diverse microbial composition compared to their commercial counterparts. This diversity suggests that traditional varieties encompass a wider array of microorganisms that could help against plant stresses. Consequently, several fungal endophytes were isolated from traditional varieties for further investigation. Additionally, here we presented the application of one of the isolates that has not been previously characterized. We evaluated its influence on different tomato genotypes under greenhouse conditions, and proved its potentially beneficial effects as endophyte. Thus, this study established a link between six tomato plant genotypes and their endophytic microbiomes, while also investigating the potential impact of a novel fungal isolate on tomato plants. This serves as confirmation that traditional tomato varieties might represent a valuable source of novel fungal endophytes with the potential to enhance crop resilience against stressful conditions.

## References

1. Trivedi P., Batista B.D., Bazany K.E., Singh B.K. (2022) Plant-microbiome interactions under a changing world: responses, consequences and perspectives. New Phytol. 234(6):1951-1959. doi: 10.1111/nph.18016
2. Compant S.,Samad A., Faist H., Sessitsch A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application, Journal of Advanced Research, Volume 19, Pages 29-37. ISSN 2090-1232. doi:10.1016/j.jare.2019.03.004.
3. Pérez-Jaramillo J., Carrión V., Bosse M. et al. (2017). Linking rhizosphere microbiome composition of wild and domesticated Phaseolus vulgaris to genotypic and root phenotypic traits. ISME J 11, 2244–2257. doi:10.1038/ismej.2017.85
4. Sun, X., Kosman, E. and Sharon, A. (2020) ‘Stem endophytic mycobiota in wild and domesticated wheat: Structural differences and hidden resources for wheat improvement’, Journal of Fungi, 6(3), pp. 1–19. doi:10.3390/jof6030180.

## Funding

Spanish Ministry of Science and Innovation project grant AGL2017-85987-C3-1-R, PID2021-123600OR-C43 Project funded by MCIN/AEI/ 10.13039/501100011033, Spanish Ministry of Universities predoctoral fellowship FPU18/02891, Generalitat Valenciana project grant GV/2019/028, Universitat Jaume I project grant UJI-A2019-19 and UJI-B2022-30.

## Acknowledgements

The authors are grateful to the Integrated Microbiome resources and especially to Andre Comeau from the Department of Pharmacology in Dalhousie University (Canada).

# Session 6: Agricultural food security

José David Flores Félix and Inmaculada Sánchez-Vicente

**Keynote S6**

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# Not quite finished crops - The ecology and genetic control of incomplete domestication

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Plant domestication was the foundation for modern human societies and has taken place in many species almost simultaneously in different regions of the world. The intense selection often changed characteristic traits that adapted plants to agroecological systems and human preferences. The process of domestication not only generated repeated trait changes within and across taxa, but also similar molecular patterns. Yet some crops show only few of those trait changes, despite long cultivation histories and similar domestication centers. These species represent ideal models to study how repeatable evolution is and what determines its outcome. The seed as the reproductive and harvestable organ is of particular importance for grain crops. Grain amaranth is a pseudo-cereal from the Americas that has been domesticated three times, but only few domestication traits changed each in each crop species. One trait that did change was the seed color. While all wild amaranths have dark seeds, domesticated amaranths have predominantly pale white seeds. We study the genomic pattern of repeated, but incomplete domestication and the genetic control of seed color change in grain amaranth. To assess the ecological function of seed pigments, we measured germination properties in a mapping population and find that pale seeds germinate faster than dark seeds. Differential gene expression analysis in diverse accessions with contrasting seed pigmentation revealed downregulation of almost all flavonoid biosynthesis pathway genes in pale seeds. Genomic and molecular analysis suggests a transposable element insertion into a regulator of this pigment pathway as the causal mutation for the seed color change during amaranth domestication. In our model of repeated incomplete domestication, we reconstruct the evolutionary and functional history of plant adaptation to understand the complex interaction between the genetic control of traits and their ecological function in new environments.

## References

Stetter, M. G., Vidal-Villarejo, M., & Schmid, K. J. (2020). Parallel seed color adaptation during multiple domestication attempts of an ancient new world grain. *Molecular Biology and Evolution*, *37*(5), 1407-1419.

## Funding

We acknowledge funding by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany´s Excellence Strategy – EXC-2048/1 – Project ID 390686111 and grant STE 2654/4 to MGS by the DFG.

# The Quinoa4Med initiative: Promoting quinoa as a climate-smart crop for enhancing income generation in the Mediterranean marginal land

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The United Nations General Assembly (UNGA) declared 2013 as the "International Year of Quinoa" to promote *Chenopodium quinoa* Willd as a means of contributing to food security. *C. quinoa* is an Andean crop that can grow on a wide range of marginal agricultural soils[1]. Given its tolerance to multiple abiotic stresses and high nutritional properties, quinoa offers a promising crop to face the global climate change scenario and growing food insecurity in developing countries[2]. Quinoa4Med (Q4M) unites scientific partners and socio-economic actors from five Mediterranean countries to develop quinoa genetic resources that can thrive in challenging climates and promote its cultivation through an integrated approach. Aligned with national and international priorities, the Q4M project showcases the potential of quinoa farming across twelve demonstration sites, each representing different cropping systems and facing diverse climatic conditions. Leveraging data from past projects and combining complementary initiatives, the Q4M goal is to identify stable and adaptable quinoa varieties and introduce them into multiple agricultural systems.

Our work in the frame of the Q4M project is aimed at generating a database for seed germination and dormancy behaviour in 250 quinoa varieties, and their response to abiotic stress (saline, heat, and abscisic acid sensitivity). Additionally, we have set up RNA purification protocols in seeds for subsequent RNA-seq analysis and define reference stable genes suitable for expression analysis by qPCR during seed germination in control conditions and in the presence of abiotic stresses. All will be subsequently used for determining molecular markers of quinoa seed vigor.

In summary, Q4M bridges the gap between the agronomic and molecular aspects of quinoa, providing valuable insights into its genetics and physiological mechanisms that contribute to its adaptation and resilience in challenging environments.

## References

[1] Alandia, G., J. P. Rodriguez, S. E. Jacobsen, D. Bazile, and B. Condori. 2020. ‘Global Expansion of Quinoa and Challenges for the Andean Region’. *Global Food Security* 26:100429. DOI: 10.1016/j.gfs.2020.100429

[2] Gordillo-Bastidas, E., DA Díaz-Rizzolo, E. Roura, T. Massanés, and R. Gomis. 2016. ‘Quinoa (*Chenopodium Quinoa* Willd), from Nutritional Value to Potential Health Benefits: An Integrative Review’. *Journal of Nutrition & Food Sciences* 06:8. DOI: 10.4172/2155-9600.1000497

## Funding

1. Quinoa as a climate-smart crop diversification option for higher income generation from marginal lands in the Mediterranean (Quinoa4Med). PRIMA-EU program 2021 (Section 2) and *Proyecto de Colaboración Internacional (*ref.: PCI2022-132988). RIF: IP-UPM group, and WP3-leader.
2. La quínoa como una nueva alternativa para el éxito en condiciones agrícolas marginales”- Banco Santander- UPM (IP.: RIF. 2021).

**@Quinoa4Med / Quinoa4Med (Facebook)**

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# Kinase-fused Resistance Proteins as Novel Regulators of Race-Specific Resistance in Cereals

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The recognition of pathogen effectors by intracellular nucleotide-binding leucine-rich repeat receptors (NLRs) is a vital branch of the plant immune system. While much of current research focuses on pathogen perception and molecular activation of NLRs, recent work in cereals shows that around 40% of race-specific resistance (*R*) genes do not encode NLR immune receptors. We identified two of these *R* genes, *Pm4b* and *WTK4*, encoding an ER-anchored chimeric protein complex composed of a serine/threonine kinase and multiple C2 domains and transmembrane regions and a protein with two kinase domains in tandem, respectively[1,2].

Kinase-fused proteins-mediated resistance resembles NLR-mediated resistance: cytosolic localization and specific recognition of pathogen effectors. However, resistance is initiated prior to effector delivery into the cytoplasm and kinase-fused proteins show strong homology to pattern-recognition receptors (PRR), responsible of providing basal immunity to entire classes of pathogens. We will present our recent identification of receptor-like cytoplasmic kinases (RLCKs) - the executors of signaling downstream upon PRR activation- as interactors of kinase-fused resistance proteins, prompting us to speculate about a new mechanism of race-specific activation of the plant immune system where PRR/co-receptor/RLCK complexes activate kinase-fused proteins leading to resistance. Moreover, we have conducted a mutagenesis-based approach to find mildew genetic components controlling avirulence on *Pm4b* and *WTK4* resistance genes. For *AvrPm4b*, we have identified a gene encoding a protein with an effector-like part fused to an EBNA-3B antigen domain, an unusual mildew effector. Interestingly, it increases the auto-phosphorylation of both *Pm4b* isoforms.

Our work reveals a fascinating example how novel molecular mechanisms of plant immunity has evolved in the hexaploid bread wheat. The molecular identification of *Pm4* and *WTK4* expands our understanding of both receptor architecture and the mechanisms of race-specific activation of the plant immune system, offering the possibility to explore these newly discovered gene families for disease resistance breeding.

## References

[1]**Sánchez-Martín** et al. 2021. *Nature Plants* 7(3):327-341. DOI: doi.org/10.1038/s41477-021-00869-2
[2]**Gaurav** et al. 2022. *Nature Biotechnology*. 40 (3):422-431. DOI: doi.org/10.1038/s41587-021-01058-4

## Funding

This project was financially supported by the University of Zurich, Swiss National Science Foundation grants 310030_204165 and 310030B_182833 to B.K. JSM is recipient of the grant “Ramon y Cajal” Fellowship RYC2021-032699-I funded by MCIN/AEI/10.13039/501100011033 and by the “European Union NextGenerationEU/ PRTR”. VW and JSM acknowledge the support of the Junta de Castilla y León through the projects "Escalera de Excelencia CLU-2018-04, and CL-EI-2021-04 support to the internationalization of AGRIENVIRONMENT - Unidad Producción Agrícola y Medioambiente" of the University of Salamanca, both co-financed by the European Regional Development Fund (ERDF "Europe drives our growth").

# Exploration of the significance of non-leguminous crops in intercropping systems

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Leguminous crops are generally the first option for intercropping because they offer several agronomic and environmental benefits including fixing soil nitrogen[1], enhancing nutrient cycling[2], improving soil fertility[3], and promoting soil biodiversity[4]. As such, non-leguminous crops for intercropping are generally overlooked, leaving a substantial knowledge gap in their significance in crop production systems. In this meta-analysis, we screened a total of 3415 research articles to determine the impact of intercropping with non-leguminous crops ($\text{IC}_{\text{nl}}$) on soil physicochemical properties, bacterial and fungal biomass, and α-diversity in agroecosystems. We used moderating variables such as experiment duration (i.e., long, medium, and short), type (i.e., field and pot), and soil pH (i.e., alkaline and acidic) for change in soil physicochemical properties and also the sample type (i.e., rhizosphere and bulk) for microbial biomass and α-diversity to understand the $\text{IC}_{\text{nl}}$ impact in more depth. We found that $\text{IC}_{\text{nl}}$ greatly improves soil physiochemical properties, and promotes soil bacterial and fungal α-diversity, crop plant biomass, and crop yield. The soil physicochemical properties and plant growth-promoting effects were more evident in long-term field experiments in alkaline soils, whereas increased microbial diversity was found in rhizosphere samples of short-term field experiments in alkaline soils. These results indicate $\text{IC}_{\text{nl}}$ in field crop production in alkaline soils could improve soil biochemical health and highlight the significance of the inclusion of non-leguminous crops in field intercropping systems.

## References

1. Soumare, A., Diedhiou, A.G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S. and Kouisni, L., 2020. Exploiting biological nitrogen fixation: a route towards a sustainable agriculture. *Plants*, *9*(8), p.1011
2. Moreau, D., Bardgett, R.D., Finlay, R.D., Jones, D.L. and Philippot, L., 2019. A plant perspective on nitrogen cycling in the rhizosphere. *Functional Ecology*, *33*(4), pp.540-552
3. Ananthi, T., Amanullah, M.M. and Al-Tawaha, A.R.M.S., 2017. A review on maize-legume intercropping for enhancing the productivity and soil fertility for sustainable agriculture in India. *Advances in environmental biology*, *11*(5), pp.49-64
4. Meena, B.L., Fagodiya, R.K., Prajapat, K., Dotaniya, M.L., Kaledhonkar, M.J., Sharma, P.C., Meena, R.S., Mitran, T. and Kumar, S., 2018. Legume green manuring: an option for soil sustainability. *Legumes for soil health and sustainable management*, pp.387-408

## Funding

This research was funded by European Commission under the Marie Skłodowska-Curie program with grant number 101034371, the Regional Government of Castile and Leon (Escalera de Excelencia CLU-2018-04), co-funded by the Operational Program of the European Regional Development Fund for Castile and Leon 2014–2020. ZSS acknowledge a Grant co-financed by the European NextGenerationEU, Spanish "Plan de Recuperación, Transformación y Resiliencia",Spanish Ministry of Universities, and University of Salamanca ("Ayudas para la recualificación del sistema universitario español 2021-2022").

Oral S6

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# Potential of Bacteria from Arid Environments to Improve Plant Drought Tolerance: Inoculating Maize with Plant Growth-Promoting Bacteria

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Extreme weather events, such as drought, pose considerable challenges to crops, impacting food security. Agriculture is the most affected sector by drought, it sustains 82% of all drought impacts. Plants respond to drought with a series of protective adaptations, either morphological, molecular, physiological or biochemical.

Besides inducing plant growth and crop productivity plant growth-promoting bacteria (PGPB) were also described to enhance plant tolerance to drought by regulating water use efficiency, improving root growth, and boosting antioxidant defenses, thus playing a crucial role in this process.

This study aimed to promote plant growth and enhance crop tolerance to drought by inoculating maize plants with osmotolerant bacterial strains. Bacteria were isolated in the root system of plants from an arid climate (Cape Verde). Strains were isolated, their osmotolerance evaluated and plant growth-promoting abilities under normal and drought stress conditions determined. The strains with the best abilities were used to inoculate a drought-tolerant maize cultivar grown in control and drought conditions, and their influence on plant growth, physiology and biochemistry was investigated.

Some strains evidenced the ability to enhance the growth and osmotolerance of maize. Eight strains promoted seedling emergence under normal and osmotic stress conditions, while two strains significantly increased root weight and length of drought-stressed plants. Moreover, thirteen strains significantly increased the proline content in stressed plants.

The ability of these bacteria to produce phytohormones, siderophores, and osmolytes, as well as to induce the antioxidant response and solubilize phosphate, further enhances plant drought tolerance. Inoculating crops with osmotolerant bacteria has the potential to protect plants from water scarcity, improve water management efficiency, minimize yield losses, and contribute to food security.

## References

FAO. The Impact of Disasters and Crises on Agriculture and Food Security: 2021. In *Impact of Disasters and Crises on Agriculture and Food Security*; FAO: Rome, Italy, 2021; Volume 2021

Ayub, M.; Ashraf, M.Y.; Kausar, A.; Saleem, S.; Anwar, S.; Altay, V.; Ozturk, M. Growth and Physio-Biochemical Responses of Maize (Zea mays L.) to Drought and Heat Stresses. *Plant Biosyst.* 2021, 155, 535–542

Ashry, N.M.; Alaidaroos, B.A.; Mohamed, S.A.; Badr, O.A.M.; El-Saadony, M.T.; Esmael, A. Utilization of Drought-Tolerant Bacterial Strains Isolated from Harsh Soils as a Plant Growth-Promoting Rhizobacteria (PGPR): Utilization of Drought-Tolerant Bacterial Strains. *Saudi J. Biol. Sci.* 2022, 29, 1760–1769

Ullah, A.; Nisar, M.; Ali, H.; Hazrat, A.; Hayat, K.; Keerio, A.A.; Ihsan, M.; Laiq, M.; Ullah, S.; Fahad, S.; et al. Drought Tolerance Improvement in Plants: An Endophytic Bacterial Approach. *Appl. Microbiol. Biotechnol.* 2019, 103, 7385–7397

## Funding

This research was also funded by FCT/MCTES through the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020), through national funds.

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# Poster presentations

Poster 1

# Identification and characterization of substances involved in the biocontrol of wine spoilage microorganisms

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Wine spoilage by yeasts has increased in recent years as a result of the higher microbial susceptibility of wines, partially due to the lower doses of preservatives demanded by consumers. *Brettanomyces bruxellensis* is a well-known yeast species in wine industry because of the great economic losses it causes. The objective of this work was to perform a methodical study to find biocontrol microorganisms against yeast *Brettanomyces*.

By sequencing the 16S rRNA gene, *Bacillus* strains isolated from the wine environment with inhibitory activities against wine spoilage microorganisms were identified, resulting in *B. velezensis*. The cell-free supernatants (CFS) of bacterium cultures exhibited strong inhibitory activities against *B. bruxellensis* in assays *in vitro*. Reverse-phase liquid chromatography mass spectrometry analysis revealed that several antimicrobial compounds were contained in the CFS. Three lipopeptides bacillomycin, fengycin A (C16, C17, C18) and iturin (C13, C15) were identified. In adition, the polyketides macrolactin (A and B) and bacillaene were found.

Transcriptomic analyzes showed that in the stationary phase growth only the lipopeptide bacillomycin and dipeptide antibiotic bacilysin biosynthetic pathways are working, suggesting that theses compounds could have a more relevant role in the *B. velezensis* antagonistic activity against *Brettanomyces*.

Differential interference contrast and fluorescent microscopy revele loss of cell wall integrity, increased plasma membrane permeability and cell lysis when *B. bruxellesis* cells are treated with CFS. However, which specific compounds leaf these changes remains to be elucidated.

## References

Bartowsky, E. J. (2009). Bacterial spoilage of wine and approaches to minimize it. *Letters in Applied Microbiology*, *48*(2), 149–156. https://doi.org/10.1111/j.1472-765X.2008.02505.x

Berbegal, C., Garofalo, C., Russo, P., Pati, S., Capozzi, V., & Spano, G. (2017). Use of autochthonous yeasts and bacteria in order to control brettanomyces bruxellensis in wine. *Fermentation*, *3*(4), 1–11. https://doi.org/10.3390/fermentation3040065

Portugal, C., Sáenz, Y., Rojo-Bezares, B., Zarazaga, M., Torres, C., Cacho, J., & Ruiz-Larrea, F. (2014). Brettanomyces susceptibility to antimicrobial agents used in winemaking: In vitro and practical approaches. *European Food Research and Technology*, *238*(4), 641–652. https://doi.org/10.1007/s00217-013-2143-2

Fazle Rabbee, M., & Baek, K. H. (2020). Antimicrobial Activities of Lipopeptides and Polyketides of *Bacillus velezensis* for Agricultural Applications. In *Molecules (Basel, Switzerland)* (Vol. 25, Issue 21). https://doi.org/10.3390/molecules25214973

**Funding** Spanish Ministry of Science and Innovation, AEI and European Union funds, grant RTC-2017-6136-2

**Acknowledgements** Martha thanks the Universidad de Salamanca-Banco Santander for the scholarship received to carry out doctoral studies.

Poster 2

# Responses of *Trichoderma*-treated wheat plants subjected to drought and drought recovery

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*Trichoderma* is a cosmopolitan ascomycete fungal genus including species with relevance in agriculture due to their activity as biocontrol agents against phytopathogens. *Trichoderma* species are capable of colonizing plant rhizosphere, establishing symbiotic relationships with the host and improving plant responses to biotic and abiotic stresses (Woo et al., 2023). Drought represents a major stress limiting crop production worldwide, and climate change is boosting the adverse effects caused by long periods of absence of water (Pedrero-Méndez et al., 2021).

In a greenhouse assay, we have evaluated the ability of two *Trichoderma* strains (*T. asperellum* T25, isolated from soil; and *T. simmonsii* T137, isolated from root endosphere of healthy wheat plants grown in a field assay under non-irrigation conditions) to increase the tolerance of wheat plants to water stress (WS) and their drought recovery capacity. Fungi were applied to the growth substrate of wheat (Basilio variety) plants and four irrigation conditions, and their corresponding controls, were considered: optimal irrigation (OI), WS, OI+OI and WS+OI. WS consisted in a complete removal of irrigation for 9 days when plants were 2 weeks old, and after this period, plants were allowed to be recovered by rehydration twice for 72 h. Plant material was collected at two different times and physiological (fresh and dry weight, relative water content) and biochemical (ROS content; antioxidant enzymatic activities) parameters were analysed.

Results showed that both *Trichoderma* strains were able to increase the tolerance of Basilio wheat plants to WS and to improve their ability of recovering by irrigation after a WS period. These beneficial abilities being related to a decrease of oxidative levels and an increase of antioxidant enzymatic activities in *Trichoderma*-treated plants.

## References

Pedrero-Méndez A, Insuasti HC, Neagu T, Illescas M, Rubio MB, Monte E and Hermosa R (2021). Why is the correct selection of *Trichoderma* strains important? The case of wheat endophytic strains of *T. harzianum* and *T. simmonsii*. *Journal of Fungi* 7, 1087.

Woo S, Hermosa R, Lorito M and Monte E (2023). *Trichoderma*: a multipurpose, plant-beneficial microorganism for eco-sustainable agriculture. *Nature Reviews Microbiology* 21, 312–326.

## Funding

Research was supported by grants co-financed by the European Regional Development Fund (FEDER) and the governments of Spain (MCIN/AEI PID-2021-126575OB-I00) and Castile and Leon (SA094P20, Escalera de Excelencia CLU-2018-04 and IR2020-1-USAL05).

## Acknowledgements

J.A.-P., A.P.-M. and M.I. acknowledge the support of their predoctoral contracts by Regional Government of Castile and Leon.

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# Enhancing Plant Disease Control with HBP2: A Natural Alternative

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Plants synthesize antimicrobial compounds to defend themselves against herbivores and pathogenic microorganisms. While synthetic chemistry is widely used for pesticide discovery, the search for safe alternatives against pathogens remains crucial due to accessibility and affordability concerns (Scalschi et al., 2018; Li et al., 2021). The apoplast is the area where many molecules involved in the defense against pathogens are found (Farvardin et al., 2020). Since the first phases of infection take place in the apoplastic environment, we have focused the search for active molecules on it. In this study, we investigated the antimicrobial properties of an apoplastic protein, HBP2, extracted from tomato plants treated with 1-Methyl tryptophan (1-MT) against *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) (Camanes et al., 2015, Llorens et al., 2020). Our in vitro findings indicated that increasing HBP2 concentration reduced the bacterial growth rate, making it a potential alternative to combat *Pst*. In order to check wheter it acts on a wide range of pathogens, it has been tested on several plant pathogens as for example *Xanthomonas vesicatoria*, *Clavicacter michiganensis michiganensis* and *Botrytis cinerea*. Additionally, HBP2-treated tomato plants, upon infection with *Pst*, showed fewer disease symptoms and a lower bacterial population count compared to control plants. Interestingly, HBP2 stimulates the production of 12-oxo-phytodienoic acid (OPDA), which acts as a mobile signal to trigger induced systemic resistance (ISR) in plants. This systemic response, induced by HBP2, enhanced the overall resistance of HBP2-treated tomato plants to *Pst* by inhibiting bacterial growth.Similar preventive effects were observed in *Arabidopsis thaliana*, indicating the potential of HBP2 in controlling plant diseases across different species.

In conclusion, the discovery of HBP2 together with its antibacterial properties and its ability to induce ISR represents a significant advancement in plant disease control. This natural alternative offers a safe, accessible, and affordable solution to combat plant pathogens, potentially enhancing crop protection and ensuring future food security.

## References :

Scalschi, L., Llorens, E., González-Hernández, A. I., Valcárcel, M., Gamir, J., García-Agustín, P., Vicedo, B., & Camañes, G. (2018). 1-Methyltryptophan Modifies Apoplast Content in Tomato Plants Improving Resistance against *Pseudomonas syringae*. *Journal of Frontiers in Microbiology*, 9, 2056. doi:10.3389/fmicb.2018.02056

Li, J., Hu, S., Jian, W., et al. (2021). Plant antimicrobial peptides: structures, functions, and applications. *Botanical Studies*, 62, 5. doi:10.1186/s40529-021-00312-x

Farvardin, A., González-Hernández, A. I., Llorens, E., García-Agustín, P., Scalschi, L., & Vicedo, B. (2020). The Apoplast: A Key Player in Plant Survival. *Antioxidants*, 9(7), 604. https://doi.org/10.3390/antiox9070604

Camañes, G., Scalschi, L., Vicedo, B., González-Bosch, C. and García-Agustín, P. (2015), An untargeted global metabolomic analysis reveals the biochemical changes underlying basal resistance and priming in *Solanum lycopersicum*, and identifies 1-methyltryptophan as a metabolite involved in plant responses to *Botrytis cinerea* and *Pseudomonas syringae*. *Plant J.* 84: 125-139. https://doi.org/10.1111/tpj.12964.

Llorens, E., González-Hernández, A. I., Scalschi, L., Fernández-Crespo, E., Camañes, G., Vicedo, B., & García-Agustín, P. (2020). Priming mediated stress and cross-stress tolerance in plants: Concepts and opportunities. pp. 1-20. https://doi.org/10.1016/B978-0-12-817892-8.00001-5

**Funding**: Grant PID2021-123600OR-C4 funded by MCIN/AEI/ 10.13039/501100011033 and by FEDER A way of making Europe.

**Acknowledgements**: We thank Serveis Centrals d’Intrumentació Científica (SCIC) from Universitat Jaume I (UJI, Castellón, Spain).

**Poster 4**

IAS2023
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# Neuroprotective role of epicatechin in Alzheimer's disease. Study in *C. elegans*.

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Although the incidence of cognitive disorders, such as Alzheimer's disease (AD), is increasing, efficient treatments are lacking (1). Epidemiological studies have shown that a regular intake of flavonoids could prevent the progression of AD (2). Epicatechin (EC), belonging to the class of flavan-3-ols, is one of the most abundant flavonoids in the diet. In order to study the potential neuroprotector of the EC and the subjacent mechanisms, different transgenic models of *C. elegans* have been employed, such as the strain CL4176, which expresses the human $A\beta_{1-42}$ peptide in muscle cells, associated with a paralysis phenotype (3), and the strain CL2355, which expresses $AB_{1-42}$ in neurons giving rise to a deficient chemotaxis behavior (4). Studies of paralysis, chemotaxis index (CI), and gene expression by RT-qPCR were carried out in these mutant strains cultivated in presence or not of EC 150 µM. The results showed that the treatment with EC was able to reduce the paralysis associated with the accumulation of Aβ peptide, induced by temperature up-shift, in CL4176 worms. Besides, this flavonoid also significantly increased the chemosensory response to the odorant attractant in the CL2355 strain compared with untreated worms. RT-qPCR analysis revealed that EC modulates the gene expression of intracellular signaling cascades such as the autophagy-related pathways. Thus, treatment with EC increased the expression of *cpr-5*, an ortholog of human cathepsin B that promotes cellular proteolysis. Furthermore, worms treated with the flavan-3-ol showed a significant decrease in the expression of *egp-8*, a gene related to the phosphoinositide 3-kinase (PI3K) complex. It was observed that the autophagosome reduction by PI3K complex delayed Aβ-induced paralysis in CL4176 worms (5). In conclusion, EC decreases the production of Aβ in different transgenic models of the nematode, suggesting that it can potentially reduce AD progression. Although more studies are needed, it seems that this flavan-3-ol could act as a modulator of autophagy-related signal pathways.

## References

**(1)** Bukhari (2022). Antioxidants., 11(3):554.
**(2)** Caruana et al. (2016). Front nutr., 12(3):31.
**(3)** Link (2006) Exp. Gerontol., 41:1007-1013
**(4)** Dosanjh et al. (2010). Alzheimer’s Dis., 19:681–90
**(5)** Lin (2022). Front. Aging Neurosci. 14:885145.

## Funding

The funding received through the projects PID2019-106167RB-I00, financed by MCIN/AEI/ 10.13039/501100011033, and SA093P20, financed by Junta de Castilla y León, is acknowledged.

# Studying the role of microRNAs in heat stress response in *Arabidopsis* using innovative *in vitro* conditions

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The expected increase of average temperatures and frequency of extreme heat events due to global warming are a real risk to crop productivity. Knowing how plants respond to heat stress is essential to address this challenge. However, most experiments designed to study plant adaptation to heat stress apply conditions that are very different to natural field conditions, since they used the same high temperatures for both shoots and roots. In the field, the soil acts as a buffer, forming a decreasing temperature gradient that protects underground parts of plants. Our group has engineered a novel device, the TGRooZ, that allows to grow plants mimicking natural conditions, as it keeps roots in the dark and in a decreasing temperature gradient. *Arabidopsis* grown in high temperatures using this system presented a more efficient root system compared to plants grown in homogeneous high temperature. In addition, their shoots were bigger, although they were exposed to the same air temperature[1], suggesting a communication from the well-developed roots towards the heated aerial part. Since microRNAs are known players in abiotic stresses, and they can act as mobile gene regulators[2], we decided to study their role in the response to heat in TGRooZ conditions. We conducted RNAseq and smallRNAseq analyses, comparing plants grown in 22° and 32ºC, in both cases using TGRooZ. We have focused on members of the miR395 and miR399 families that accumulate differentially in response to heat and had been previously proved to move from shoot to root and to be involved in starvation responses to different nutrients[3]. Transcriptomic data complements our observations, finding targets of these miRNAs differentially expressed, together with differentially enriched categories related to heat stress and nutrient assimilation among others. Future work will aim at understanding in more detail the relationship between heat and nutrient deficiency plants' responses.

**References:** [1]González-García *et al*., Plant Communications, 2022. DOI: 10.1016/j.xplc.2022.100514.

[2]Pagliarani & Gambino, International Journal of Molecular Sciences, 2019. DOI: 10.3390/ijms20174306.

[3]Fujii *et al*., Current Biology, 2005. DOI: 10.1016/j.cub.2005.10.016.

**Funding:** Research was supported by PID2020-113479RB-I00 grant by MCIN/AEI/10.13039/501100011033/ to J.C.P and by the "Severo Ochoa Program for Centres of Excellence in R&D" from the AEI of Spain (grant SEV-2016-0672 (2017-2021) to the CBGP. VBG is supported by a predoctoral fellowship FPU20/07453 from the Ministry of Universities of Spain. ALE is supported by a predoctoral fellowship FPI "PRE2021-098261" from MCIN/AEI /10.13039/501100011033 and "FSE invierte en tu futuro".

IAS2023
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# Study of the role of commercial mannoproteins on the color stability of oxidized rosé wine

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Rosé wine consumption has increased greatly in the last years, reaching over 10% of the global market in wines. The colour of rosé, like that of red wines, is due to the anthocyanins extracted from red grapes skins and to the derived pigments formed through reactions involving other must and wine components. Because of the lack of other polyphenols, antocyanins in rosé wines are quite exposed to oxidative reactions leading to an irreparable loss of the bright rosé colour. Oenological mannoproteins (MPs) have been described for their positive effect on the color stabilization in red wines [1], and it would be of interest to explore their effect on the color stability of rosé wines.
The aim of this work is to study the effect of the addition of different commercial oenological mannoproteins on the pigment evolution of a rosé wine after induced oxidation.
To do this, three commercial mannoproteins (issued from yeast) were characterized by SDS-PAGE, HPLC-RID [2], Lowry method [3] and HPLC-MS [4]. Rosé wines were made using grapes from *V. vinifera cv* Tempranillo. The mannoproteins used presented a wide range of average molecular weights, between 60.3 kDa and 125.3 kDa and their protein content was ranging from 10 to 35% and presented notable differences in their monosaccharide composition. Each individual mannoprotein was added to the rosé wine that was treated with an oxidizing agent. The pigment evolution of the oxidized sample-wines in presence and in absence of the mannoproteins was analyzed by HPLC-MS [5]. These results showed that different effects were observed depending on the composition of the mannoproteins. Mannoproteins with lower molecular weight, which present higher mannose content and lower protein content, could exert a protective effect against gentle oxidative conditions in rosé wines.

## References

[1] C. Alcalde-Eon, *et al., Food Res. Int.*, 2019, 126, 108650.
[2] E. Manjón, *et al., J. Agric. Food Chem*. 2020, 25; 13459 .
[3] O. H. Lowry *et al., J. Biol. Chem.* 1951, 193, 265.
[4] Y. Ruiz-García *et al., Carbohydr Polym.* 2014, 114, 102.
[5] C. Alcalde-Eon, *et al., Biochem. Syst.and Eco.,* 2014, 53, 76-80.

## Funding

This research was financially supported by Grant PID2021-127126OB-C21 funded by MCIN/AEI/10.13039/501100011033 and by “ERDF A way of making Europe”. D.M. Bosch-Crespo thanks to Junta de Castilla y León, cofunded by Consejería de Educación and Fondo Social Europeo Plus (FSE+), her pre-doctoral contract. Thanks are also due to Junta de Castilla y León-FEDER Programme for the Strategic Research Programs for Units of Excellence (Escalera de Excelencia CLU-2O18-04).

# Effect of water deficit on the grain yield and quality of wheat grown under combined elevated $CO_2$ and high temperature

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Wheat is an essential cereal in human nutrition. It provides 20% of the calories in daily intake and is an important source of proteins, carbohydrates, minerals, vitamins, and phytochemicals. In the coming decades, the global demand for wheat will increase with the growing population worldwide, whereas its productivity may decrease due to climate change. In this context, the increase in the concentration of CO2 and temperature of the atmosphere will be accompanied by more frequent, intense and long-lasting periods of drought, which, in turn, could jeopardize food security. The objective of this study was to investigate the effect of drought stress on grain yield and quality of wheat grown under combined elevated CO2 and high temperature. To achieve this goal, wheat plants were grown in controlled environment growth chambers under long-term moderate water deficit applied from the vegetative developmental stage (65% field capacity), terminal water deficit (50% field capacity) applied from the ear emergence, and well-watered conditions (100% field capacity). At crop maturity, the plant biomass and the grain yield and quality were assessed. Both water deficits decreased plant biomass, grain yield, ear and grain number, C/N ratio and Ca, Mn and Zn contents, but increased the content of proteins and K in the grain. Long-term moderate water deficit increased the root/shoot ratio and the Na content compared to well-watered conditions. Terminal water deficit reduced the harvest index and fructose content but increased the total antioxidant capacity and the content of polyphenols and S in the grain. Between water deficits, there were differences in the stem biomass, C/N ratio, total antioxidant capacity and the content of proteins, polyphenols, Na and S. This study reflects the negative impact of complex environmental conditions associated with climate change on the wheat grain yield and quality.

**Funding:** This research was funded by the Spanish Ministry of Science and Innovation (project PID2019-107154RB-100) and the Junta de Castilla y León (project CSI260P20, ERDF). N. Bueno-Ramos had a JCyL fellowship (E-37-2020-0042432).

# Synthetic communities of rot fungi and rhizobacteria with plant growth-promoting potential.

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The utilization of consortia comprising rot fungi and rhizobacteria is a promising approach for promoting plant growth[1]. Rot fungi have remarkable capabilities, breaking down complex organic matter and releasing essential nutrients into the soil[2]. This nutrient enrichment enhances plant nutrition and growth[3]. Rhizobacteria establish beneficial associations with plant roots, leading to enhanced nutrient uptake, growth-promoting substance production, and protection against pathogens[4]. The synergistic interactions between rot fungi and rhizobacteria consortia create a conducive environment for optimal plant development. These consortia not only enhance nutrient availability but also aid in soil recovery, potentially enabling bioremediation and improving overall performance. Such approaches can ultimately increase crop yields and reduce reliance on chemical fertilizers, making them an environmentally sustainable solution for plant growth promotion[5].To evaluate the incorporation of rot fungi and rhizobacteria consortia as a strategy for sustainable agriculture and high-quality crop production, an assay with *Zea mays* (maize) was conducted. The assay included 12 conditions to assess the abilities of bacteria and fungi, individually and in microbial consortia. A control group of non-inoculated soil was included, individual inoculation with *Trametes*, *Pleurotus*, *Rhizobium* and *Bacillus* strains were also included. The remaining test groups involved different combinations of fungi and bacteria, inoculated in soil-filled containers and after maize was sown. Plants were grown at controlled temperature, light intensity and photoperiod. Results confirmed that rot fungi and rhizobacteria can indeed promote plant growth. The combination of *Pleurotus* and *Rhizobium* exhibited notably auspicious results, achieving the fastest and most sustained growth throughout the assay compared to the non-inoculated control group. Thus, synthetic communities of rot fungi and rhizobacteria may enhance crop productivity, potentially contributing to meet the growing world population's food demands in a sustainable way.

## References

**1-** Carneiro, B., Cardoso, P., Figueira, E., Lopes, I., & Venâncio, C. (2023). Forward-looking on new microbial consortia: Combination of rot fungi and rhizobacteria on plant growth-promoting abilities. Applied Soil Ecology, 182, 104689.
2- Kirtzel, J., Ueberschaar, N., Deckert-Gaudig, T., Krause, K., Deckert, V., Gadd, G. M., & Kothe, E. (2020). Organic acids, siderophores, enzymes and mechanical pressure for black slate bioweathering with the basidiomycete Schizophyllum commune. Environmental microbiology, 22(4), 1535-1546.
3- Cruz, C., Cardoso, P., Santos, J., Matos, D., Sá, C., & Figueira, E. (2023). Application of Plant Growth-Promoting Bacteria from Cape Verde to Increase Maize Tolerance to Salinity. Antioxidants, 12(2), 488.
4- Cardoso, P., Alves, A., Silveira, P., Sá, C., Fidalgo, C., Freitas, R., & Figueira, E. (2018). Bacteria from nodules of wild legume species: Phylogenetic diversity, plant growth promotion abilities and osmotolerance. Science of the Total Environment, 645, 1094-1102.
5- PANDA, A., Ankita, D. A. S. H., & MISHRA, B. B. (2021). Potassium mobilization and plant growth promotion by soil bacteria isolated from different agroclimatic zones of Odisha, India. Acta agriculturae Slovenica, 117(3), 1-14.

**Funding and acknowledgements**: This work is financed by national funds through FCT – Foundation for Science and Technology I.P., under the MIRACLE project (2022.03612.PTDC). This research was also funded by FCT/MCTES through the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020), through national funds.

IAS2023
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# Analysis of resistomes in raw materials, final products and food industry environments by whole metagenome sequencing

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Food processing environments can act as a reservoir for antimicrobial resistant microorganisms, and the spread of antimicrobial resistance genes (ARGs) throughout the food production process is a growing concern that requires further study. With the main objective of determining the dispersion of ARG in different food production systems, a resistome analysis (ARGs pool) was carried out in 113 food processing plants (3 vegetables, 6 fish, 19 meat or meat products and 76 cheese factories). A total of 384 samples of raw materials were collected, 1044 from environments (food contact surfaces and non food contact surfaces) and 451 from final products, from which DNA was extracted for subsequent sequencing using Illumina 150PE technology, obtaining around 7 .5 Gbp per sample. Reads and assembled contigs were aligned against the ResFinder database to detect ARGs.

Meat industries showed higher ARG abundance and richness and lower ARG diversity than other types of industries, while environmental samples showed higher ARG abundance, richness and diversity than raw materials and final products. The ARGs associated with tetracyclines were the most abundant, in general, while those associated with beta-lactams and aminoglycosides were the most abundant in meat and cheese industry environments, being *tet(L)*, *tet(M)*, *blaOXA-211*-like and *tet(K)* the main ARGs found.

Contigs analysis showed that most ARGs were associated with bacteria from the ESKAPEE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. y *Escherichia coli*), which are critically relevant for human health. The analysis of MGE showed a high percentage of ARGs located in different MGEs, including plasmids and integrons. Although ARG levels were lower in the final product than in environments, monitoring of these is important to control the spread of ARG through the food chain, specially if they are harbored by uman pathogenic bacteria.

## Funding

This work was funded by the Horizon 2020 Research and Innovation Program of the European Union, under grant agreement No. 818368 (MASTER). NMQ is currently funded by the Horizon 2020 Research and Innovation Program of the European Union through the Marie Skłodowska-Curie Grant Agreement No 101034371.

IAS2023
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# Selection of potential probiotics for improving the digestibility of agri-food waste in Gryllus bimaculatus (Orthoptera: Gryllidae) breeding

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Orthoptera is one of the insect groups with the greatest market potential for human consumption worldwide. Among the most commercialized species is the cricket Gryllus bimaculatus, which is distributed throughout Europe. However, one of the biggest cost factors in the production of this group of insects is feeding, as mainly chicken feed is used. One of the possible applications to solve this problem is the use of agrifood by-products as a feeding substrates and probiotics to help their digestion. This study focused on the analysis of the gut microbiota of industrially produced and wild crickets and the selection of potential probiotics to improve the digestibility of agrifood by-products for the mass rearing of G. bimaculatus. Initially, wild and industrially produced G. bimaculatus were collected, the gut bacteriome of each of the samples was analyzed by 16S rRNA gene amplicon sequencing, and the cellulolytic, pectinolytic, xylanolytic and amylolytic capacity of the bacterial strains present in the sample was isolated and evaluated. As a result, significant differences were found in the intestinal bacteriome of the samples from the field with a predominance of the Firmicutes phylum and the Clostridia class and the samples from the company with a predominance of the Proteobacteria phylum and the Bacteroidia class. The strains with hydrolytic activity, which are considered good potential probiotics, mostly belong to the order Enterobacterales, including possible new species.

## References

Ahuja, K., & Bayas, S. (2020). Edible Insects Market Size by Product (Beetles, Caterpillars, Grasshoppers, Bees, Wasps, Ants, Scale Insects & Tree Bugs), By Application (Flour, Protein Bars, Snacks), Industry Analysis Report, Regional Outlook, Application Potential, Price Trends, Competitive Market Share & Forecast, 2020 – 2026. En Global Market Insights Inc. https://www.gminsights.com/industry-analysis/edible-insects-marketFunding

Bai, J., Ling, Y., Li, W. J., Wang, L., Xue, X. B., Gao, Y. Y., Li, F. F., & Li, X. J. (2022). Analysis of Intestinal Microbial Diversity of Four Species of Grasshoppers and Determination of Cellulose Digestibility. Insects, 13(5). https://doi.org/10.3390/insects13050432

Franks, K., Kooienga, E., Sanders, M., Pendarvis, K., Yang, F., Tomberlin, J. K., & Jordan, H. R. (2021). The Effect of Rhodococcus Rhodochrous Supplementation on Black Soldier Fly (Diptera: Stratiomyidae) Development, Nutrition, and Waste Conversion. Journal of Insects as Food and Feed, 7(4), 397–408.

Savio, C., Mugo-Kamiri, L., & Upfold, J. (2022). Bugs in Bugs: The Role of Probiotics and Prebiotics in Maintenance of Health in Mass-Reared Insects. Insects, 13.(4)376

## Funding:

This study was funded by H2020-MSCA-COFUND-2020, USAL4EXCELLENCE-PROOPI-369

IAS2023
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# Glucosinolates and fungal nitrilases during the Botrytis cinerea-host plant interaction: is nitric oxide taking sides?

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Nitric oxide (NO) is a gasotransmitter that plays a significant role in various plant responses, including biotic and abiotic stresses (Reviewed in Manrique *et al*., 2021). In the case of plant immune responses against fungi, NO is responsible for an extensive gene expression reprogramming (Mur *et al*., 2017). Moreover, basal defences and the hypersensitive response rely on NO (Mur *et al*., 2013). It has been hypothesized that the necrotrophic fungus *Botrytis cinerea* exploits NO production to enhance colonization (Turrión-Gómez *et al*., 2010; Turrión-Gómez and Benito, 2011). However, research into the presence and function of NO in plant-fungal interactions is limited. Therefore, in this study, we aimed to investigate the involvement of NO during the interaction of *Botrytis cinerea* and the host plant in the context of glucosinolate (GSL) metabolism and nitrilase function, using available NO homeostasis impaired lines of Arabidopsis thaliana and *B. cinerea* strains. Our results suggest that the phenolic and GSL profile varies between mutants with impaired NO homeostasis, and this variation could be further influenced by *B. cinerea* infection. We also demonstrate that NO deficient and overproducing Arabidopsis mutants may experience degradation of GSL hydrolysis products such as nitriles, which could be attributed to an impairment of nitrilase gene expression. Interestingly, *B. cinerea* has a nitrilase coding gene (*Bcin12g06180*) that is particularly induced after the addition of exogenous NO. Taken together, these findings provide novel insights into the role of NO in the metabolism of GSLs during plant-pathogen interactions.

## References

Manrique-Gil, I., Sánchez-Vicente, I., Torres-Quezada, I. and Lorenzo, O. (2021). *Journal of Experimental Botany*, 72 (3), 904–916.
Mur, A.J., Hebelstrup, K. H., &amp; Gupta, K. J. (2013). *Frontiers in Plant Science*, 4, 288.
Turrión-Gómez, J. L., Eslava, A. P., and Benito, E. P. (2010). *Fungal Genetics and Biology*, 47(5), 484-496.
Turrión-Gómez, J. L., and Benito, E. P. (2011). *Molecular Plant Pathology*, 12(6), 606-616.

## Funding and acknowledgements

This work was supported by grants PID2020-119731RB-I00 (to O.L.) from Ministry of Science and Innovation (Spain) and by Junta de Castilla y León (SA137P20 and Escalera de Excelencia CLU-2018-04) co-funded by the P.O. FEDER of Castilla y León 2014–2020 (to O.L.) and PID2019-110605RB-100 (to E. P.-B.). ITQ is currently in the postdoctoral orientation period (POP) of her PhD grant from University of Salamanca.

# WHEAT-CANOLA ROTATION ROOT-ASSOCIATED MICROBIOME: ITS DYNAMICS AND BIOTECNOLOGICAL PROPIERTIES

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Soil is one of the most important sources of microorganisms, specially providing a wide taxonomic variety of strains, which compose the soil microbiome (1). Numerous studies demonstrated that plants could select those microorganisms with the more interesting capacities (2, 3). Amongst them, plant growth promotion mechanisms and biocontrol activities are desirable capacities when bacterial strains are selected for their use as plant probiotics (2, 3). Our main aim is to study the structure, dynamics and functions of wheat-canola rotation associated microbiomes. Samples were taken from two different fields under two regimes: rainfed and irrigation. We describe the taxonomic and functional differences between both regimes. For that, 16S rDNA metabarcoding analysis was carried out to study the metataxonomic distribution of both crops and their dynamics through the crop development. After that, we obtained, identified, and screened isolates by means of PGP mechanisms (P and K solubilization, IAA-like molecules detection, hydrolytic enzymes and siderophores production) and antimicrobial activity tests against different phytopathogens (fungi and plant parasitic nematodes).

Our results indicate that genera *Pseudoarthrobacter*, *Rubrobacter* and *Craurococcus* are highly represented on irrigated field and genus *Ensifer* on rainfed field. A higher proportion of isolates from irrigated field showed positive results for all the PGP traits (23%), rather than the isolates from rainfed crop (3%). Strains showing multi-PGP traits were tested as biocontrol against both fungi and nematodes. Strains with the best results were tested in pinewood plantlets to study their biocontrol activity *in planta*. Here, we focus on 0TCS1.6A strain, a novel species (*Phyllobacterium soli* sp. nov.), with interesting results under *in vitro* and *in planta* experimental conditions. Its genome was analysed to find the metabolic pathways and secondary metabolites implicated in its biocontrol capacity. All these results confirm the huge potential of these crop rotation root-associated strains as multifunctional plant probiotic bacteria, excellent candidates to be included in bioformulations (biofertilizers and/or biopesticides).

## References

1.- Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M. C. C., Charles, T., ... & Schloter, M. (2020). Microbiome definition re-visited: old concepts and new challenges. Microbiome, 8, 1-22.

2.- Upadhyay, S. K., Rajput, V. D., Kumari, A., Espinosa-Saiz, D., Menendez, E., Minkina, T., ... & Mandzhieva, S. (2022). Plant growth-promoting rhizobacteria: A potential bio-asset for restoration of degraded soil and crop productivity with sustainable emerging techniques. Environmental Geochemistry and Health, 1-24.

3.- Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., & Nasrulhaq Boyce, A. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. Molecules, 21(5), 573.

## Funding and Acknowledgements

Authors acknowledge funds received by the Regional Government of Castilla y León, Escalera de Excelencia CLU-2018-04, and cofunded by the P.O. FEDER of Castilla y León 2014 to 2020. DES acknowledges "Programa Investigo", funded by Junta de Castilla y León and "Next Generation EU". EM acknowledges a EUROPEAN UNION'S HORIZON 2020 Marie Sklodowska-Curie Actions (Grant Agreement no. 897795). ZSS received a grant cofinanced by the European NextGenerationEU, Spanish "Plan de Recuperación, Transformación y Resiliencia," Spanish Ministry of Universities, and the University of Salamanca ("Ayudas para la recualificación del sistema universitario español 2021-2022"). CV acknowledge the Fundação para a Ciência e Tecnologia (FCT) funded projects PratyOmics (PTDC/ASP-PLA/0197/2020) and MED Strategic Project (UIDB/05183/2020).

# Effect of nitric oxide on the morphological development *of Marchantia polymorpha*

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The gasotransmitter Nitric oxide (NO) is involved in numerous processes in plant development, such as seed dormancy, germination, photomorphogenesis, mitochondrial activity, leaf expansion, root growth, stomatal closure, fruit ripening or senescence, and maintenance of the meristems. But also, NO participates during the responses to abiotic stresses, such as salinity, drought and floods. Essentially, NO-derived RNS interact with biomolecules to modify both their structure and function [2], through post-translational modifications such as the nitration of tyrosine residues, the *S*-nitrosylation of cysteines, and the nitrosation of transition metals. Despite the role of NO in plants has been studied, in *Marchantia polymorpha* is still unknown. To know the role of this molecule and if it has been conserved throughout plant evolution, the objective of our work is to study the effect of NO on *M. polymorpha,* which is considered a model plant in evolutionary studies, given its position at the base of the phylogeny of terrestrial plants and to conserve most of the genes that regulate growth and development [1]. We analyzed the morphological characterization in different developmental phases *of M. polymorpha* through pharmacological treatments with NO donors and scavengers. On the other hand, we analyzed the effect that NO exerts indirectly on the transcription of the TFs *MpABI5A* and *MpABI5B* at the beginning of development, given the regulatory role that NO has on ABI5 in *Arabidopsis thaliana* [3].

[1] Especial number about *Marchantia polymorpha*, Plant and Cell Physiology **57**, 2 (2016).
[2] Sánchez-Vicente, I. et al. *Journal of Experimental Botany*, **70** (17), 4441-4460 (2019).
[3] Albertos, P. et al. Nature Communications, 6 (1), 1-10 (2015).

## Funding

Project PID2020-119731RB-I00 (Ministry of Science and Innovation), SA137P20 (Junta de Castilla y León) -CLU-2018-04 co-funding by P.O. FEDER de Castilla y León 2014–2020 España (a O.L.).

## Acknowledgements

Group of Physiology and Hormonal Signaling in Plants, Department of Botany and Plant Physiology, Institute for Research in Agrobiotechnology (CIALE), University of Salamanca.

# Effect of types of tillage in a non-irrigated semi-arid cultivated soil of the Guadalquivir Valley under ambiental and reduced rainfall

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Climate model forecasts indicate a significant drop in annual rainfall in southern Spain by the end of the century, which would especially affect rainfed agricultural systems (IPCC 2021). Soil management has a marked influence on the use of water resources (Madejón et al., 2023). In this work, it has been evaluated whether conservation tillage techniques (no tillage and reduced tillage) increase resistance to drought in non-irrigated soils in the expected scenario of reduced rainfall compared to traditional tillage. Aspects related to the activity and diversity of soil biotic communities are studied. A rain manipulation experiment was established in rainfed soils of the Guadalquivir Valley. Agricultural production was monitored for two years (broad bean and wheat in rotation), and the main physicochemical properties, microbial activity, fungal and bacterial diversity, and soil nematodes were characterized. The experimental drought produced a decrease in the plant biomass of the broad bean crop, especially in reduced tillage, although not in its grain production. The exclusion of rain had a marginally significant effect on the organic carbon content of the superficial soils in the broad bean crop, which was slightly higher in the soils of the exclusion treatment, possibly due to an increase in the root:shoot ratio of the plants. Drought did not produce significant effects on various indices of microbial activity (soil respiration and enzymatic activities). However, tillage significantly influenced the amount of microbial biomass and the colonization patterns of broad bean plants by arbuscular mycorrhizal fungi (being higher in non-tillage), which was positively related to the levels of plant water potential. The nematode community was affected by the type of tillage, such that the maturity and structure indices were higher under the no-tillage system, reflecting the negative effect of tillage on the populations of the genera of nematodes more sensitive to disturbances.

## References

IPCC (International Panel of Climate Change), 2021. IPCC, 2021: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. t al., (eds.)]. Cambridge University Press. In Press.

Paula Madejón, Elena Fernández-Boy, Laura Morales-Salmerón, Carmen M. Navarro-Fernández, Engracia Madejón, María Teresa Domínguez. 2023. Could conservation tillage increase the resistance to drought in Mediterranean faba bean crops?. Agriculture, Ecosystems and Environment 349. 108449.

## Funding

US- 126062 (Programa Operativo FEDER, Universidad de Sevilla) and the Spanish Ministry of Science and Innovation (project PID2021–122628OB-I00 WASTE4DROUGHT)

## Acknowledgements

Patricia Puente and Cristina García de Arboleya for their help with field and lab work

# Bacterial biofortification improves production and modifies organic chemical composition of blueberry

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Blueberry production has strategic importance in Portuguese agriculture due to its possibility of spreading to inland territories and its economic relevance, devoting a large part for external markets. Thus, the production requires new solutions to obtain a more sustainable and nutritious final product where biofortification using Plant Growth Promoting Bacteria (PGPB) can be an important solution, as has been seen in other red fruits [1]. In the present work, the qualitative and quantitative effect of four bacteria-based biofortification treatments was analyzed, one using native bacteria (*Paenibacillus* sp. VMFR46), non-native bacteria (*Rhizobium laguerreae* PEPV16), lactic acid bacteria (*Lactiplantibacillus plantarum* QSE79) and a consortium of these last two. The experiment was conducted in Covilhã and showed that all treatments were capable of improving production by up to 60% (VMFR46), increasing sweetness (total soluble solids-TSS) from 11.75 to 14.55 (Brix degree). Also, maturity index (TSS/TA ratio) showed an improvement in all treatments (from 17% to 44%), without detecting differences in the firmness of the fruits. In this way, phenolic profile of the yielded fruits was analyzed by HPLC-DAD-ESI/MS. A total of 31 phenolic compounds were identified showing that delphinidin 3-*O*-galactoside (between 5000 and 1500 µg/g of dw) was the most abundant compound. In turn, 5-feruloylquinic acid, myricetin 3-O-glucoside, myricetin 3-O-pentose were only present in the fruits from control treatment, while quercetin 3-O-rutinoside, myricetin aglycone and quercetin derivative were specific from biofortified treatments. In turn, an improvement in the antioxidant capacity of the fruits against DPPH, NO and SO radicals was detected. These data reveal that biofortification employing PGP bacteria is an effective tool to improve blueberry production at a quantitative level together with a qualitative improvement through the increase of sweetness, maturity index, and phenolic compounds concentration with influence on its antioxidant capacity, obtaining more production, healthier and more attractive for market.

## References

1. Flores-Félix, J. D., Velázquez, E., García-Fraile, P., González-Andrés, F., Silva, L. R., & Rivas, R. (2018). *Rhizobium* and *Phyllobacterium* bacterial inoculants increase bioactive compounds and quality of strawberries cultivated in field conditions. Food Research International, 111, 416-422.

**Funding** This research was funded by the European Union's Horizon 2020 Programme for research and innovation under the Marie Skłodowska-Curie grant agreement No 101003373. The authors are grateful to the Foundation for Science and Technology (FCT), the Ministry of Science, Technology and Higher Education (MCTES), the European Social Fund (EFS) and the European Union (EU) for the PhD fellowship of Ana C. Gonçalves (2020.04947.BD). Luís R. Silva was supported by project CENTRO-04-3559-FSE-000162, Fundo Social Europeu (FSE).

**Acknowledgements** : We would like to thank to BeiraBerry Ltd. for it support with field assays.

# Understanding the role of nitric oxide (NO) during the hypoxic stress response through the N-degron pathway

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Nitric oxide (NO) is vital for regulating developmental processes and stress responses [1]. The PRT6 N-degron proteolytic pathway, along with ethylene (ET), NO and oxygen ($O_2$), controls the hypoxic stress response. In the absence of $O_2$, this pathway regulates the stability of ERFVII transcription factors, which induce the expression of hypoxia-responsive genes [2,3]. Although NO is crucial for the N-degron pathway, it is not clear whether it acts directly or indirectly through any of the components of this pathway.

Here, we aim to explore the role of the N-degron pathway in NO sensing and signaling during submergence and hypoxic stress, by using gain- and loss-of-function mutants and transgenic lines of *Arabidopsis thaliana*. Initially, we analyzed the molecular characteristics and subcellular localization of Methionine Aminopeptidases (MAP1A and MAP2 group), which constitute early components of the N-degron pathway, and their potential regulation by NO. Transient expression in *Nicotiana benthamiana* plants followed by confocal microscopy visualization showed that MAP1A and MAP2A fused with GFP in the N-terminus appeared to locate in the membrane and cytoplasm, and also in the nucleus in the case of MAP1A, with no change under hypoxic conditions. Additionally, we identified MAPs putative interactors related to hypoxia responses by performing a Co-Immunoprecipitation (Co-IP) assay using protein extracts from transgenic Arabidopsis lines under submergence and NO donor treatments. Results demonstrated that RAP2.3 interacts with MAP2A protein, even stronger when MAP2A and RAP2.3 are treated with the NO donor.

In conclusion, the localization of MAP1A and MAP2A in the plasma membrane, and in the case of MAP1A, in the nucleus, suggests their involvement in processing ERFVII factors in these compartments. Interestingly, RAP2.3 exhibits a stronger interaction *in planta* with MAP2A under submergence stress and NO treatments.

## References

[1] Manrique-Gil, I et al. Journal of Experimental Botany 72, 904–916 (2021).
[2] Licausi, F et al. Nature 479, 419–422 (2014).
[3] Gibbs, D et al. Molecular Cell 53, 369–379 (2014).

## Acknowledgments & Funding

We would like to thank the Spanish network RED2018-102397-T and RED2022-134072-T for the stimulating discussions. This work is funded by the projects PID2020-119731RB-I00 (Ministerio de Ciencia e Innovación), SA137P20 (Junta de Castilla y León) and Escalera de Excelencia CLU-2018-04 co-funded by the P.O. FEDER of Castilla y León 2014–2020 España (to O.L.). A. Fuentes-Terrón has a grant from JCyL orden EDU/1868/2022.

# Analysis of plant growth promotion and induction of stress resistance bacterial inoculants for olive crops

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Some plant-associated bacteria are able to promote plant growth (PGP) through different mechanisms (nutrient supply, synthesis of phytohormones and induction of resistance to biotic and abiotic stress). The development of bacterial bioinoculants can increase the efficiency and productivity of crops (Dias *et al.*, 2022), reducing the costs and the environmental impact compared to chemical fertilizers. These bioinoculants can also increase the resilience of crops to stress, being specially interesting for olive crops, due to its relevance in the countries of the Mediterranean basin and the hrydric stress they are exposed.

With this objective, a collection of 75 isolates was evaluated, including isolates of rhizosphere and endosphere of olive leaves, as well as endophytic strains from other plants with potential PGP available in our laboratory. There ability to mobilize nutrients and produce phytohormones, their resistance to abiotic stresses (temperature, salinity and water stress) and there ability to inhibit phytopathogenic fungi was analysed. Based on these data, it selected the best 24 bacterial strains were selected for further analyses, which were identified at the species level based on the sequence of its 16S RNA ribosomal gene.

In parallel, an analysis of the bacterial populations of 1104 rhizospheres of diferent crops, (155 belonging to olive trees) was performed. Taxa with an abundance significantly higher in this crop than in the rest were identified. Considering the taxa significantly associated with olive cultivation and the strains with the greatest potential to induce stress resistance, 10 strains were selected to be tested on olive seedlings under greenhouse conditions, showing that several strains were capable of significantly improve the development of this plant.

Future analyzes will include the development of microbial consortia and the analysis of their capacity to induce the resistance of the olive tree to biotic and abiotic stresses.

**References:** Dias, Mª.C., Araújo, M., Silva, S. & Santos, C. (2022). Sustainable Olive Culture under Climate Change: The Potencial of Biostimulants. *Horticulturae* 8, 1048. https://doi.org/10.3390/horticulturae8111048

**Acknowledgements:** this work has been funded by Grant PCI2022-132990 co-funded by PRIMA and the AEI

IAS2023
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# Garden waste compost tea: a sustainable approach to promote root growth in tomato plants

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In the coming years, the use of biostimulants will constitute an important approach to reduce chemical fertilization, increasing the efficiency of crops to cope adverse conditions. In this context, the application of garden waste compost teas (CT) can be used as an enviro-friendly alternative, since they provide soluble nutrients, beneficial compounds and microorganisms. In this work, we evaluated the physico-chemical properties of CT and its influence on tomato cv. Marglobe plants growth. The studied CT showed a relevant content in $K_2O$, N, and humic acids. Two applications of CT slightly increase root fresh and dry weights in tomato plants, whereas no differences were found in the aerial part development. Hence, to confirm the effect of CT in root development, an *in vitro* assay was carried out revealing that CT supply enhanced primary root length, lateral root number and root fresh weight, while decreasing shoot height and fresh weight in 10-days-old tomato seedlings compared to Hoagland solution. These results suggest that CT application promotes root growth at the first developmental stages. All together lead us to continue studying the metabolites contained in the CT, the pathways involved in the observed phenotype, the genotypes response and how does CT-treated plants face up to water deficit stress.

# "Immune responses in Czech poppy (*Papaver somniferum subsp. Somniferum*)"

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Breadseed poppy (*Papaver somniferum subsp. Somniferum*) is an important commercial crop in the Czech Republic. The Czech Republic is the world's biggest producer of breadseed poppy; recently, the Czech blue poppy got PGI (Protected Geographical Indication) mark from EU Commission. It underlines its importance in Czech agriculture. Surprisingly, no research is focused on unraveling how poppy immunity works on a molecular basis. However, such research has a high potential to gain knowledge to develop new strategies for improving poppy's yield and health.

In our work, we established poppy cultivation in controlled conditions. Additionally, our lab established two pathosystems: *P. somniferum* x *Xanthomonas papavericola* (bacterial pathogen) and *P. somniferum* x *Botrytis cinerea* (fungal pathogen). In parallel, we screen typical immune responses at eight poppy cultivars. Mainly, we analysed the burst of reactive oxygen species, seedlings, and plant growth inhibition, callose deposition, gene expression, and salicylic acid concentration after treatment with flg22, elf18, chitohexaose, and cellobiose, representing typical PAMPs (pathogen-associated molecular patterns) using in plant immunity research.

Our long-term goal involves obtaining novel knowledge about the poppy immune response, which is to use gene editing to create novel poppy genotypes with higher resistance against common poppy pathogens.

IAS2023
17th - 18th JULY
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# *Cannabis sativa* L. Varieties fingerprinting using different molecular markers

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*Cannabis sativa* L. is one of the oldest cultivated and domesticated plants and it is used as a source of fiber, in the agri-food industry and as a source of psychoactive substances (Clarke & Merlin, 2016). It is a crucial labor for the authorities to discriminate between drug-type" and "fiber-type" or hemp. They are morphologically identical but with different legal statuses. The necessity of clearly differentiating both types, along with the aim of fingerprinting different hemp strains available in the market, has led to the development of specific molecular markers (Onofri & Mandolino, 2017). Simple sequence repeats (SSRs) or microsatellites are highly polymorphic DNA sequences composed of a few bases (1-6 bp) that are repeated in tandem arrays. This type of marker is usually in a single locus with multiple alleles, and it is codominant, highly informative, and represents a reproducible technique that shows a high discrimination power. Single nucleotide polymorphisms (SNPs) are the most common form of variation in the genome and are extensively used to study genetic differences between individuals and populations (Dijk et al., 2014). These markers are characterized by a single base change in a DNA sequence (Semagn al., 2014) becoming a very useful approach due to their low assay cost, high genomic abundance, locus specificity and high-throughput analysis capacity. We have used SSRs and SNPs markers to classify and to fingerprint different populations of *Cannabis sativa* and to determine the photoperiod dependence of the strains. A set of 11 SSRs have been used in 3 different reactions using an ABI 3100 DNA sequencer to determine a specific fingerprint for the different varieties and the population structure has been determined using *Structure* Software. At the same time, several SNPs have been identified using Kompetitive Allele Specific PCR (KASP) genotyping to differentiate between varieties.

## References

Clarke, R., & Merlin, M. (2016). *Cannabis: evolution and ethnobotany*. Univ of California Press.

Onofri, C., & Mandolino, G. (2017). Genomics and Molecular Markers in Cannabis sativa L. *Cannabis sativa L.-botany and biotechnology*, 319-342.

Van Dijk, E. L., Auger, H., Jaszczyszyn, Y., & Thermes, C. (2014). Ten years of next-generation sequencing technology. *Trends in genetics*, *30*(9), 418-426.

Semagn, K., Babu, R., Hearne, S., & Olsen, M. (2014). Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. *Molecular breeding*, *33*, 1-14.

**Funding** *Centro Tecnológico Nacional Agroalimentario "Extremadura" (CTAEX)*

# IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF *FvAFS*, A GENE INVOLVED IN THE BIOSYNTHESIS OF α-FARNESENE IN *Fragaria vesca*

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Thanks to their unique flavour and aroma, strawberries are one of the most popular fruit among consumers. However, these properties have been lost in recent decades due to the prioritization of other agronomic characters in breeding programs, such as production, fruit size and resistance to pathogens.

Strawberry aroma is the result of a complex volatile composition (VOCs) of the fruit, being richer in the woodland strawberry, *Fragaria vesca*, than in the octoploid commercial species *Fragaria* × *ananassa*. A germplasm collection of European *F. vesca* accession has been re-sequenced and the volatile profiles of their fruit analyzed. Next, a Genome-Wide Association Analysis (GWAS) has allowed us to identify a series of polymorphisms linked to the accumulation of α-farnesene, a volatile terpenoid that has been shown to add sweet notes to the scent. These polymorphisms are located in the promoter region of a terpene synthase, named *FvAFS*, whose expression is induced during fruit ripening and correlated with α-farnesene accumulation, being therefore an excellent candidate for the biosynthesis of this volatile compound. In this study, we have performed a functional analysis and characterization of this gene. Thus, transient overexexpression of *FvAFS* in *Nicotiana benthamiana* and *in vitro* enzyme activity assays have confirmed the role of this gene in the accumulation of α-farnesene. Finally, assays based on scoring pest-induced damage in plants with different profiles for α-farnesene support a biological role of this volatile compound in strawberry plant defense.

**Funding & Acknowledgements**

Raquel Jiménez Muñoz is supported by Margarita Salas Grant from "Ministerio de Universidades" of Spain and Next Generation Fonds from the European Union.

María Urrutia and Carmen Martín-Pizarro are funded by the DOC_00278 and POSTDOC_21_00893 postdoctoral grants from the Junta de Andalucía, respectively.

This work was supported by the ERC Starting Grant ERC-2014-StG 638134 and by the PAIDI P20_00385 (Junta de Andalucía).

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# Deciphering novel plant responses and plant-microbiome interactions under heat stress and nutrient scarcity

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According to predictions, the agricultural production of the 21st century will be facing radical changes in the climatic conditions and extensive scarcity of the current widely used fertilizing compounds. This combination of stresses will negatively affect the phytobiome as a whole and ultimately crop productivity. In nature, during extreme heat, the soil thermal geodynamics properties buffer the soil-root environment temperature, however, this phenomenon is not reflected in the experimental systems commonly used. To solve this limitation, we engineered a device called Temperature Gradient Root Zone (TG-RooZ) that mimics the soil temperature gradient observed in natural soils. Previous results from our lab indicate that high temperature reduces Pi assimilation and alters microbiota community, which can consequently limit plant productivity. This impact of temperature on plant development and Pi assimilation is in fact lost in the TG-RooZ conditions. In this collaborative project, we analyzed the developmental and physiological responses of tomato plants to different temperature regimes under low or high Pi availability. Additionally, preliminary results suggest that the fungal and bacterial composition of the rhizosphere is primarily driven by the phosphate level and secondly by the temperature conditions. Interestingly, the rhizosphere of plants in the TG-RooZ conditions is composed of similar communities as the plants in control temperature conditions. Moreover, we also built up a microbial collection, of approximately 400 bacterial and 100 fungal isolates, associated to tomato roots under heat stress that will be a critical resource to identify the molecular mechanism of microbe-mediated heat tolerance. To identify new genes and microbial functions implicated in the tolerance of plants to combined environmental stressors we will apply an integrative approach combining physiological and phenotypical analysis, transcriptomics, metabolomics and shotgun metagenomics.

**Funding** Research was supported by grants from the Spanish Government PID2020-113479RB-I00-/AEI/10.13039/501100011033 (MCIN/AEI) to JCdP and by the 'Severo Ochoa Program for Centres of Excellence in R&D' from the AEI of Spain (grant CEX2020-000999-S (2022–2025) to the CBGP

# Antimicrobial peptides: a potential tool to fight against plant pathogens

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Antimicrobial peptides (AMP) are short, usually amphipathic and cationic, gene-encoded peptides of different origins, which have been shown to be active against a broad spectrum of common pathogens, including bacteria, viruses, fungi, and protozoa (Léon *et al*., 2020). The mechanism of action of these peptides begins with an electrostatic interaction with the negatively charged cell membranes of the target cells, resulting in their permeabilization, which causes cell lysis. Eventually, AMPs can pass through the membrane and interact with different intracellular targets. The fact that AMPs have multiple final targets and the apparent non-specificity of their interaction with the membrane seem to be the basis for the difficulties that bacteria find in developing resistance against them. Although the antimicrobial activity of many AMPs has been demonstrated, their low stability has withdrawn their practical application (López-Sanmartín *et al*., 2022). In the present study we have demonstrated that D-Caerin, a synthetic peptide composed of the dextrorotatory enantiomeric isomers of the corresponding L–aa, chemically linked by peptidic bonds as in the natural L-Caerin. is much more stable than its natural corresponding isomer. Furthermore, the antimicrobial activity of this synthetic peptide was tested "*in vitro*" over a series of usual plant pathogenic bacteria, including *Agrobacterium tumefaciens*. *Xanthomonas vesicatoria*, *Xanthomonas campestris*, *Xanthomonas campestris*, *Erwinia tasmaniensis* and *Erwinia tasmaniensis* and the yeasts *Sacharomyces cerevesiae* and *Rhodoturala* sp. The determination of the lysis halos revealed that D-Caerin is more stable and has higher activity than natural L-Caerin. D-Caerin was especially effective against *Xanthomonas vesicatoria* and *Erwinia tasmaniensis*. Preliminary results show that lipidic extracts obtained from different microalgal species also show an important antimicrobial activity against these species, representing an important potential alternative tool for biocontrol and fight against pathogens in agriculture.

## References

- León R, Ruiz M, Valero Y, Cárdenas C, Guzman F, Vila M, Cuesta A. Exploring small cationic peptides of different origin as potential antimicrobial agents in aquaculture. *Fish Shellfish Immunol*. 2020, 98: 720-727.
- López-Sanmartín M, Rocío Rengel R, López-López M, Antonio J. Lebrón A.J., Molina-Márquez A, de la Rosa I, López-Cornejo P, Cuesta A, Vigara J, León R. D-Amino acid peptides as antimicrobial agents against vibrio-associated diseases in aquaculture. Aquaculture. 569, 2023, 739362.

**Funding.** This research was funded by the Andalusian government (I+D+i-JA-PAIDI-Retos projects 2020-PY20) and the Program Proyectos precompetitive de Transferencia del Conocimiento e Innovación de "FERTINAGRO-BIOTECH-UHU".2022.

**Acknowledgements.** We thank Fertinagro S.A.for kindly providing some bacterial species.

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# Comparing Soil Microbiomes in Citrus Orchards: Insights into the Impact of Organic Management Practices

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The soil microbiome comprises a diverse community of microorganisms, including bacteria, fungi, archaea, and viruses, which interact with each other and with plants in complex ways. These microorganisms are involved in key ecological processes such as nutrient cycling, organic matter decomposition, carbon sequestration, and plant-microbe interactions. Understanding the composition, diversity, and functioning of the soil microbiome is essential for optimizing agricultural practices, mitigating soil degradation, enhancing crop productivity, and promoting sustainable land management. Organic management practices, which exclude the use of synthetic fertilizers and pesticides, are known to promote soil biodiversity and enhance microbial activity. However, the specific effects of organic management on the composition and function of the soil microbiome in citrus orchards remain unclear.

This study aimed to compare the soil microbiome diversity between citrus orchards managed under regular and organic practices. Soil samples were collected from multiple orchards representing both management systems, and high-throughput sequencing techniques were employed to analyze the microbial communities.

Preliminary findings indicate that the different management highly affect the composition of soil microbiome modifying both, diversity and composition. Interestingly, the regular management has deeper impact in the diversity of fungi compared with organic management, whereas the composition and diversity of bacteria in the soil is not highly different between both treatments.

Understanding the differences in the soil microbiome between regular and organic management systems can provide valuable insights into the long-term effects of agricultural practices on soil health and productivity. This knowledge can aid in the development of sustainable management strategies that maximize crop production while minimizing environmental impact. Furthermore, it highlights the potential of organic management as a means to enhance soil microbial communities, contributing to the promotion of ecologically friendly citrus cultivation practices.

**Funding** This research was financially supported by Universitat Jaume I (UJI-A2019-19, UJI-B2022-30), 'Generalitat Valenciana' (GV/2019/028), and diputacion de Castellon

IAS2023
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# The beneficial fungi H2 enhances root development in Arabidopsis under phosphate- starvation conditions.

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Intensive agriculture is driven by the growing human population and contributes to climate change and various stresses that affect plant growth and yield. Soil nutrient deficiencies are major challenges for agricultural research due to nutrient scarcity and fertilizer reduction. To address this issue, we screened for beneficial interactions between A. thaliana and endophytic fungi (isolated from the same plant species in natural fields). We found the fungal isolate H2, which enhanced the primary root length and lateral root (LR) length and number under optimal and stress conditions, as phosphate starvation. GFP-expressing H2 transformed lines confirmed that the fungus is an endophyte that penetrates the Arabidopsis root, probably through the apoplast. *DR5:LUC* marker line showed that the interaction produces an increment in the number of prebranch sites, probably through the alteration of the  root clock. pHB53:mCherry was used to observe the stages of lateral root development in mock/treated plants. In addition, mutants with impaired LR formation and auxin signaling (arf7arf19, slr, gnom) were inoculated with H2 to assess its effect. We obtained transcriptome and miRNAome from roots and shoots from mock/treated plants after 3 and 6 days of co-incubation. We found a higher response to the fungi at starvation conditions, as expected by our screening results. Moreover, those genes induced under phosphate starvation in the presence of H2 are enriched in development and nutrient starvation gene ontology-related terms, indicating a boost in the response mediated by H2 presence.  We found 39 miRNAs (21 of them consider as novel miRNAs) induced in those plants incubated with H2 and grown under low phosphate concentrations, including the phosphate starvation-responsive miRNA399. Computationally predicted target genes for these 39 miRNAs are enriched in the same phosphate starvation and development gene ontology-related terms than those from our transcriptome. Our data suggests that the presence of H2 alleviate nutrient starvation in Arabidopsis by boosting the starvation response and enhancing the development of the root system.

Funding: Research was supported by grants from the Spanish Government PID2020-113479RB-I00-/AEI/ 10.13039/501100011033  and BIO2017-82209-R (MCIN/AEI) to JCdP and by the 'Severo Ochoa Program for Centres of Excellence in R&D' from the AEI of Spain (grant SEV-2016-0672 (2017–2021) to the CBGP. Ayuda  "PRE2021-098261financiada por MCIN/AEI /10.13039/501100011033 y por  FSE invierte en tu futuro" to  AL. Ayuda  "RYC2021-030913-I financiada por MCIN/AEI /10.13039/501100011033 y por la Unión Europea Next GenerationEU/PRTR" to JC.

# Changes in *Chenopodium quinoa* rhizosphere and endophytic bacteria with the genotype and water environmental conditions

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Drought episodes are expected to increase in intensity and frequency due to climate change limiting worldwide crop productivity. To minimize these impacts, different strategies have been followed including the incorporation of more water-efficient crop species and varieties or the use of plant growth-promoting bacteria (PGPBs). This last approach has been little explored in emergent crops such as *Chenopodium quinoa* Willd. (quinoa), an underutilized plant species with the potential to contribute to global food security due to the nutritional properties of its seeds and its tolerance to a diversity of abiotic stressors[1], including water stress[2]. This tolerance seems to be dependent on several factors, including the genotype and the intensity and type of stress[3] but could also be potentially determined by the microorganisms associated with the plant. Considering these aspects, the main goal of this work was to characterize the bacterial communities associated with the root and seeds of quinoa, identifying the taxa with the potential to alleviate drought stress. We, therefore, analyzed the influence of the genotype (using two different quinoa cultivars, F15 and F16), water treatment (applying long-term water stress and optimal irrigation conditions), and different bacterial infusions inoculated into the substrate in which quinoa plants grew (infusions obtained from different soils). We were able to associate some observed changes in the bacterial communities with differences in agronomical, physiological, and seed nutritional quality-related parameters measured during the experiment, highlighting the increase in the relative abundance of the family Nocardioidaceae in the rhizosphere of F15 (a water stress-sensitive cultivar) under water stress and the Pseudomonadaceae in the cultivar F16 (a water stress-tolerant cultivar). Overall, the results demonstrate that the genotype and the water conditions are determinant factors shaping quinoa associated-bacterial communities and highlight the potential of quinoa as a source of PGPBs to cope with water stress.

## References

1 Graf, B.L., Rojas-Silva, P., Rojo, L.E., Delatorre-Herrera, J., Baldeón, M.E., Raskin, I. (2015). Innovations in health value and functional food development of Quinoa (*Chenopodium quinoa* Willd.). Compr. Rev. Food Sci. Food Saf. 14 (4), 431–445

2 Hinojosa, L., González, J.A., Barrios-Masias, F.H., Fuentes, F.F., Murphy, K.M., 2018. Quinoa abiotic stress responses: a review. Plants 7 (4)

3 Maestro-Gaitán, I., Granado-Rodríguez, S., Orús, M.I., Matías, J., Cruz, V., Bolaños, L., Reguera, M. (2022) Genotype-dependent responses to long-term water stress reveal different water-saving strategies in C*henopodium quinoa* Willd. Environmental and Experimental Botany, 201, 104976.

4 Marasco R, Rolli E, Ettoumi B, Vigani G, Mapelli F, Borin S., Abou-Hadid, A., El-Behay, U.A., Sorlini, C., Cherif, A., Zocchi, G., Daffonchio, D. (2012) A drought resistance-promoting microbiome is selected by root system under desert farming. PLoS One 7: e48479

# Bacterial protection against fungal pathogens in strawberries

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## ABSTRACT

Strawberries are one of the most important horticultural crops in the world; however, agricultural practices associated with this crop that involve use of chemicals are the cause of soil and water contamination (García-Fraile *et al.,* 2017). This work has focused on the study of the endophytic bacterial microbiome, which information is scarce, to find potential beneficial strains which inoculation could allow the reduction in the use of chemicals for this crop. To do this, we studied the general taxonomic composition through massive parallel sequencing of amplicons from 9 plants collected in three Spanish locations. To carry out a more in depth and pioneering study, we selected the core microbiome (Shade & Handelsman ,2011), stable andpermanent members associated with a habitat with specific environmental conditions, where we found that the most abundant genera were *Bradyrhizobium, Steroidobacter, Bradyrhizobium, Moraxellaceae, Lactobacillus, Kineosporia,* and *Streptomyces*.

With the objective of finding PGPR bacteria capable of promoting the growth of strawberry plants while inhibit the development of phytopathogenic organisms, 44 strains were isolated from roots of this plant, of which 34 belonged to the *core* bacterime. Of the total of the isolates, 34.09% strains are capable of solubilizing $CaHPO_4$ or $Ca_3(PO4)_2$, 81.8% produce some type of siderophore and 72.72% fix atmospheric nitrogen. In addition, due to the great problems existing with phytopathogenic fungi in strawberry cultivation, we made dual cultures where we put each isolated strains in contact with two species of *Colletotrichum* sp. strawberry pathogens.

Due to its good results inhibiting the growth of these fungi and its potential as a biocontrol agent, we selected the LB9 strain, belonging to the species *Streptomyces anulatus*, a species included in the core bacteriome. We obtained it genome sequence for a deeper analysis of its potential as a strawberry inoculant.

## References

García-Fraile, P., Menéndez, E., Celador-Lera, L., Díez-Méndez, A., Jiménez-Gómez, A., Marcos-García, M., Cruz-González, X. A., Martínez-Hidalgo, P., Mateos, P. F., & Rivas, R. (2017).Bacterial Probiotics: A Truly Green Revolution. *Probiotics and Plant Health*, 131–162. https://doi.org/10.1007/978-981-10-3473-2_6.

Shade, A., & Handelsman, J. (2011). Beyond the Venn diagram: the hunt for a core microbiome. *Environmental Microbiology*, *14*(1), 4–12. https://doi.org/10.1111/j.1462- 2920.2011.02585.x

## Funding

We acknowledge the funding by the Regional Government of Castile and Leon (Escalera de Excelencia CLU- 2018-04), co-funded by the Operational Program of the European Regional Development Fund for Castile and Leon 2014–2020. ZSS acknowledge a Grant co-financed by the European NextGenerationEU, Spanish "Plan de Recuperación, Transformación y Resiliencia", Spanish Ministry of Universities, and University of Salamanca ("Ayudas para la recualificación del sistema universitario español 2021-2022").

# Evaluation of the synergic effect between plant probiotics and edible coating against fungal diseases to improve food security.

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Berries are an increasingly popular fruit with great nutritional content and a high economic value. However, red fruits are highly perishable and susceptible to a variety of illnesses, such as fungal contamination. Currently, pesticides are the most widely used treatment for dealing with this problem. Nevertheless, they constitute a risk to the environment, human health and food safety. Due to this, methods for controlling phytopathogens, such as biological control, have been developed. Plants probiotics are being used in this method to lessen or maintain the severity of fungal diseases. The innovative use of live microorganisms is an alternative way of improving food safety which also provides health benefits. Furthermore, there are materials in which protection against pathogens has been seen, such as edible coatings. The present work describes the isolation and identification of bacterial probiotics present in the microbiota of berries as potential biocontrol agents in synergy with edible coatings, to increase the shelf life of the product. Research on the antagonistic interactions between the isolates and the fungi *B. cinerea, Colletotrichum acotatum,* and *Alternaria* showed a significant inhibition ratio in which 26% of the strains had an antagonistic effect on the fungus. Consequently, the most effective isolates were monitored for at least three days to determine how strains respond to an edible alginate coating. Finally, an *in vivo* study on strawberries and grapes was done after confirming their good behaviour in alginate. Grapes were also chosen because of the problems they had with *B. cinerea* as well as their economic relevance. Thus, it can be concluded that probiotics can be potentially useful biological control agents when used in combination with edible coating as a pesticide substitute to control postharvest illnesses in berries.

## References:

Carmona-Hernandez, S., Reyes-Pérez, J. J., G. Chiquito-Contreras, R., Rincon-Enriquez, G., Cerdan-Cabrera, C. R., & Hernandez-Montiel, L. G. (2019). Biocontrol of Postharvest Fruit Fungal Diseases by Bacterial Antagonists: A Review. *Agronomy*, *9 (3)*, 121.

Massoud, R., Khodaeii, D., Hamidi-Esfahani, Z., & Khosravi-Darani, K. (2021). The effect of edible probiotic coating on quality of fresh fruits and vegetables: fresh strawberries as a case study. *Biomass Conversion and Biorefinery 13,* 2517–2526.

Petrach, S., Blanco-Ulate, B., J. Knapp, S., & A.L. Van Kan, J. (2019). Grey mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen Botrytis cinerea. *Molecular Plant Pathology,* 877-892.

Romero, J., Albertos, I., Díez-Méndez, A., & Poveda, J. (2022). Control of postharvest diseases in berries through edible oatings and bacterial probiotics. *Scientia Horticulturae.*

Wang, F., Xiao, J., Zhang, Y., Li, R., Liu, L., & Deng, J. (2021). Biocontrol ability and action mechanism of *Bacillus halotolerans* against Botrytis cinerea causing grey mould in postharvest strawberry fruit. P*ostharvest Biology and Technology.*

**Funding**: No funding support for this proyect.

**Acknowledgements**: The authors thanks Viveros Campiñas SCA (Segovia, Spain) for providing plants of the research.

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# Host-range tests and molecular studies of *Alternaria* sp., a potential biocontrol agent for *Ipomoea hederifolia* L.

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*Ipomoea hederifolia* L., (Convolvulaceae) is an invasive plant that infests various tropical and subtropical crops around the world. Its control has become difficult in recent years, due to its tolerance to some of the most common herbicides. In Brazil, we have previously detected the presence of fungal pathogens that could function as biocontrol agents. In particular, an isolate of *Alternaria* sp. stood out for causing foliar lesions with a significant incidence and severity. The objective of this study was to determine the specificity of our isolation of *Alternaria* sp., on some cultivated plants and confirm its identity through molecular studies. In the laboratory, through pathogenicity tests on the detached leaf, we evaluated the effect of the isolation of *Alternaria* sp, on corn, soybean, eucalyptus, and orange, using the agar disc with mycelium methodology. The inoculated leaves were incubated in a humid chamber for 10 days (±24°C). The response variable was the presence or absence of symptoms. Koch's postulates were realized when necessary. For each species evaluated, we performed four repetitions, taking each leaf as an experimental unit. The entire experiment was repeated twice in time. Molecular studies included DNA sequencing of the fungus, using the ITS (Internal Transcribed Spacer) region and the glyceraldehyde-3-phosphate dehydrogenase (GPDH) gene, as well as phylogenetic analyses. The results showed that the fungus did not infect any of the evaluated species, since symptoms were absent in all cases. The phylogenetic analysis for ITS confirmed that the isolate falls within the genus *Alternaria* sp.; however, the sequence of the GPDH gene needs to be improved to accurately establish the taxonomy of the species. We infer that our isolation of *Alternaria* sp. could represent an important resource for the development of new control methods for *I. hederifolia*. Additional studies are required.

**Acknowledgements** (We thank the Microbiology and Forest Pathology Laboratory of the São Paulo State University (UNESP) of Botucatu and the CAPES development agency.

# Extract of pistachio leaves as bioherbicide

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Based on the content of phenolic compounds, the bioherbicidal effect of pistachio leaf extracts is studied. Five extracts in water were prepared with summer and autumn pistachio leaves, with which germination bioassays were performed to analyse the possible inhibition of 4 weeds: *Lolium rigidum, Solanum nigrum, Scabiosa triandra and Lactuca serriola*. Germination bioassays were also performed on 4 crops: corn, barley, wheat, and lentil. The difference between the efficacy of autumn and summer leaves was analyzed. the 3% and 6% extracts produced a significant effect on the radicle length of the 4 species. The 12% extract produces a total inhibition of the germination of *Lactuca serriola, Scabiosa triandra* and *Solanun nigrum* and a reduction of the germination of *Lolium rigidum* of 21.9% with respect to the control. Essays were also carried out on lentil cultivation in pots in two different substrates to analyse the effect of two doses of extract as a preemergence herbicide. In each pot, 10 seeds of each of the 4 weed species were sown. 6 replicates per treatment were performed and the corresponding controls were included. The biomass of the crop and of each adventitious species was valued. The extract of pistachio leaves, applied in high doses, produced a reduction in the growth of broadleaf weeds in the pots with a low organic matter content. In addition, an analysis of the phenolic compounds of the extracts of both summer leaves and autumn leaves was carried out, observing statistically significant differences in the total phenolic content depending on the time the leaves were collected. We have identified 1 flavanol, 1 phenolic acid, 1 flavanone and 3 flavonols, also finding higher concentrations in autumn leaves. The results seem to indicate that autumn leaves are more suitable for the manufacture of a possible bio-herbicide.

**References**

Alma, M. H., Nitz, S., Kollmannsberger, H., Digrak, M., Efe, F. T., Yilmaz, N. (2004). Chemical composition and antimicrobial activity of the essential oils from the gum of Turkish pistachio (*Pistacia vera* L.). Journal of agricultural and food chemistry, 52(12), 3911-3914.

Pardo-Muras, M., Puig, C. G., Souto, X. C., Pedrol, N. (2020). Water-soluble phenolic acids and flavonoids involved in the bioherbicidal potential of Ulex europaeus and *Cytisus scoparius*. South African Journal of Botany, 133, 201-211. Puig, C. G., Revilla, P., Barreal, M. E., Reigosa, M. J., Pedrol, N. (2019). On the suitability of *Eucalyptus globulus* green manure for field weed control. Crop Protection, 121, 57-65.

Saludes-Zanfaño, M. I., Vivar-Quintana, A. M., Morales-Corts, M. R. (2022). Pistacia Root and Leaf Extracts as Potential Bioherbicides. Plants, 11(7), 916.

Xuan, T. D., Shinkichi, T., Khanh, T. D., Chung, I. M. (2005). Biological control of weeds and plant pathogens in paddy rice by exploiting plant allelopathy: an overview. Crop protection, 24(3), 197-206.

**Funding**: PC_TCUE21-23_019

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# Microbiome and resistome composition in feces and manure of dairy cows

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Livestock production effluent has been implicated in the transmission of antimicrobial resistance (AMR) bacteria into aquatic, terrestrial and atmospheric ecosystems (Sura *et al.,* 2015; McNab *et al*., 2007 and McEachran *et al.,* 2015). According to the World Health Organisation, by 2050, drug-resistant diseases will cause roughly 10 million death per annum; that is why, AMR is concidered an urgent global public health concern (WHO, 2014). In the dairy cattle sector, the massive use of antibiotics has led to the presence of antibiotics in the environment through manure and slurry (Liu et al., 2019). And even if they have benefits on the soil when they are used as a fertilizer, they constitute at the same time a potential source of contamination, both environmental and biological. The aim of this work is to prove that cattle effluents could carry a significant load of bacteria and antibiotic resistance genes (ARB and ARG), which could be significantly different between production systems. For this purpose, we selected 18 dairy cattle farms from three different production systems (intensive, conventional grazing and organic grazing; six per production system) and we defined sample size as follows: 300 feces samples from first-calving Holstein cows (100 samples per production system) and 100 manure samples (30 samples per production system). Using metagenomis and nanopore sequencing (Oxford Nanopore Technology; MinIon platform), we studied the composition of the microbiome and resistome in both sample types and here are some preliminar results: metagenomes studied are mostly composed of bacteria (84%), followed by archaea (4%) and viruses (<1%). The most abundant metagenomes in feces are *Methanobrevibacter* sp. YE315, *Bifidobacterium pseudolongum* and *Bifidobacterium angulatum.* In manure, the most abundant metagenomes are *Nostoc* sp. C057, *Escherichia coli* and *Pseudomonas nitroreducens.* In feces, we found 30 gen groups of AMR and in manure samples 67.

## References

Liu, J., Taft, D.H., Maldonado-Gomez, M.X. et al. 2019. The fecal resistome of dairy cattle is associated with diet during nursing. Nat Commun 10:1–15

McEachran, A. D. et al. 2015. Research. Antibiotics, Bacteria, and Antibiotic Resistance Genes: Aerial Transport from Cattle Feed Yards via Particulate Matter. Environ. Health Perspect. 123, 337–343

McNab, Walt W., Singleton, M. J., Moran, J. E. & Esser, B. K. 2007. Assessing the Impact of Animal Waste Lagoon Seepage on the Geochemistry of an Underlying Shallow Aquifer. Environ. Sci. Technol. 41, 753–758

Sura, S. et al. Transport of three veterinary antimicrobials from feedlot pens via simulated rainfall runoff. 2015. *Sci. Total Environ.* 521, 191–199

WHO. 2014. Antimicrobial Resistance: global report on surveillance. World Health Organization

## Funding

Government of Cantabria: funding of regional research project

## Acknowledgements

Government of Cantabria; Regional Ministry for Rural Development, Livestock, Fisheries and Food
State Investigation Agency; FPI2019 aid for pre-doctoral researchers

# New insights into the *Trichoderma atroviride*-tomato interaction microRNAs

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The agricultural system must move towards more sustainable and context-sensitive solutions. In this situation, *Trichoderma* spp. could be a model fungus to sustain crop productivity needed under climate change exposure and devastating disease outbreaks. *Trichoderma* (*Ascomycota*, teleomorph *Hypocrea*) is a plant-beneficial fungus of high interest in agriculture as it is used as a biological control agent against plant pathogenic microorganisms and as a biostimulant, favoring seed germination, plant growth and adaptation to abiotic stresses (Woo et al., 2022). In plant-*Trichoderma* systems, after root colonization, the fungus affords the plant with long-lasting resistance against biotic and abiotic stresses by balancing different phytohormone-dependent pathways, a phenomenon known as priming, which provides the plant with a faster and stronger induction of plant basal resistance mechanisms upon the perception of a future triggering stimulus (Morán-Diez et al., 2021). Although progress has been made in understanding the mechanisms of action of *Trichoderma*, changes in plant small non-coding RNAs (sncRNA) produced by the fungus have not yet been fully addressed (Morán-Diez et al., 2021). Hence, we analyzed by high-throughput Illumina sequencing the microRNA (miRNA) population in four-week-old tomato plants whose seeds were treated with *Trichoderma atroviride* T11 compared to those from untreated plants. Differential regulation of different miRNA populations was observed in T11-treated plants. Moreover, we have identified *Trichoderma*-derived miRNAs and explored how these events can have a long-lasting impact on gene expression and plant immunity. Several miRNAs were differentially expressed. Among the miRNAs significantly repressed compared to those in untreated plants, miR166 showed a high read number, it is highly conserved with functions in various developmental processes, as well as regulatory roles against biotic and abiotic stresses in major crop plants. Furthermore, we are investigating other significant differentially expressed miRNAs such as miR6024 and miRNA6027.

**References**

Woo et al. (2022). Nature Reviews Microbiology 21: 312–326.
Moran-Diez et al. (2021). Journal of Fungi 7: 318.

**Funding**

This work has been supported by grants PID-2021-126575OB-I00 and TED2021-130934B-I00 funded by Spanish MCIN/AEI/ 10.13039/501100011033 and by “European Regional Development Fund A way of making Europe” by the European Union/Next Generation EU/PRTR, and by Castile and Leon grants SA094P20, Escalera de Excelencia CLU-2018-04 and IR2020-1-USAL05, and grant FJC2021-047100-I funded by MCIN/AEI/10.13039/501100011033 and by “European Union NextGenerationEU”/PRTR».

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# Nitric Oxide Modulation of Molecular Targets in Pollen and Trichome Development

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Climate change is a global concern that affects landscapes across all continents, causing disruptions to ecosystems and the living conditions of plants and animals. While plants have a natural ability to adapt to changing environments, they are struggling to keep up with the rapid pace of climate change and its consequences.

In this context, the economic significance of crop plants for food production and security is heightened in the context of a rapidly growing global population. The quality of pollen plays a crucial role in crop productivity, but it is influenced by various internal and external factors. Additionally, trichomes are structures involved in mechanical defense and play a significant role during abiotic and biotic stresses. Furthermore, trichomes act as light reflectors, impacting leaf heat balance and the interception of photons, which, in turn, affect gas exchange traits [1,2].

In this scenario, nitric oxide (NO) is an important molecule involved in multiple stages of plant development. It is produced through various pathways and influences molecular processes such as seed dormancy, germination, hypoxia, and interactions with microorganisms [3,4]. Research has shown that NO also affects pollen development and efficiency, with external NO negatively impacting tube growth [5]. Moreover, NO has been found to influence trichome branching patterns in the model plant species *Arabidopsis thaliana* [6].

In light of this, the principal objective of our study is to uncover the specific mechanisms through which NO influences pollen (development, quality, and efficiency) and trichome branching patterns. With this aim we are identifying molecular targets in both processes, in particular the transcription factors TGACG MOTIF-BINDING PROTEIN 9 (TGA9) and TGA10 for pollen analysis and a selection of trichome branching related genes, including TONEAU 2 and ANGUSTIFOLIA.

## References

[1] https://doi.org/10.1071/FP16095
[2] https://doi.org/10.1007/s11676-019-01034-4
[3] https://doi.org/10.1038/ncomms9669
[4] https://doi.org/10.1093/jxb/eraa442
[5] https://doi.org/10.1242/dev.01153
[6] https://doi.org/10.3389/fpls.2021.630792

## Funding

We would like to thank the Spanish network RED2018-102397-T and RED2022-134072-T for the stimulating discussions. This work is funded by the projects PID2020-119731RB-I00 (Ministerio de Ciencia e Innovación), SA137P20 (Junta de Castilla y León) and Escalera de Excelencia CLU-2018-04 co-funded by the P.O. FEDER of Castilla y León 2014–2020 España (to O.L.).

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# Antimicrobial activity of grape pomace extracts based on natural deep eutectic solvents (NADES)

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Grapes (*Vitis spp*) are esculent berries whose crops are over the world, being Italy, France and Spain the main producers. About 80% of the harvest is used in the winemaking sector producing a great amount of a solid residue: the grape pomace [1]. This by-product is rich in phenolic compounds, specifically anthocyanins that could be used as colourants and preservatives in the food industry. The recovery of this waste enables the implementation of the circular economy. Moreover, the development of green extraction methods is a further step towards sustainable development. Natural Deep Eutectic Solvents (NADES) present an opportunity to carry out green and sustainable extraction of polyphenols [2].

Initially, the anthocyanin and flavonol extraction capacity of 16 different NADES based on choline chloride (ChCl) and sucrose, in combination with different organic acids and propylene glycol, was evaluated. 2 mg of lyophilised grape pomace peel was extracted with 40 mL of each of the NADES of interest in a shaker for 1h at 40 ºC and 210 rpm. The recovery yields were compared with the standard extraction using methanol acidified with HCl 0.5 N. The extraction yields obtained with some NADES were higher than those obtained in the methanolic extraction, standing out the combinations of ChCl with lactic acid and propylene glycol, in the extraction of both anthocyanins and flavonols. Finally, the antimicrobial capacity of the extracts with the highest amount of anthocyanins and flavonols was evaluated, showing good activity against foodborne bacteria such as *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*; and also clinical bacteria such as *Proteus mirabilis* or *Pseudomonas aeruginosa*.

NADES are effective to obtain polyphenol-rich extract with antimicrobial activity, increasing in this way their suitability as preservatives. However, their toxicity and other bioactive properties need to be evaluated to determine their potential application in the industry.

## References

[1] Sirohi et al. Green processing and biotechnological potential of grape pomace: Current trends and opportunities for sustainable biorefinery, Bioresour. Technol. 314 (2020) 123771. [2] A. Palos-Hernández et al. Obtaining green extracts rich in phenolic compounds from underexploited food by-products using natural deep eutectic solvents. Opportunities and challenges, Sustain. Chem. Pharm. 29 (2022).

## Acknowledgements

This research was funded by the European Union through FEDER-Interreg España-Portugal project TRANSCoLAB (Grant 0612_TRANS_CO_LAB_2_P) and by Project PID2019-106167RB-I00 financiado por MCIN/ AEI /10.13039/501100011033.

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# A case of university-industry collaboration: Developing a collaborative environment to promote sustainable agriculture

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This work constitutes a real collaboration between the business world and the university to promote sustainable agriculture in one of the most vulnerable regions of Europe, where extensive agriculture occupies most of its territory. A collaboration has been established between the company Jamones Blázquez and the Universities of Salamanca and Valladolid to evaluate the agronomic use of the digestate obtained as a by-product in the generation of biogas from slurry in the Biogas Plant owned by the company located in Juzbado (Salamanca, Spain). The use of digestate in agricultural production may be considered a viable approach because of its high organic matter and nutrient (mainly nitrogen and phosphorus) contents. Thus, the revaluation of a by-product that is currently considered waste, which must be treated in accordance with the current legislation, is revalued in a circular economy context. Hence, the main goal is the use of this organic product to reduce the use of chemical fertilizers totally or partially. To accomplish this objective, we will determine the physicochemical properties of the digestate and carry out the field tests. Field tests will be conducted by applying different digestate doses to corn, barley, rapeseed, and sunflower crops.

# Nitric oxide regulation of seed dormancy and germination traits through DELAY OF GERMINATION 1 (DOG1)

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The interplay between different internal cues (i.e. hormones) and environmental conditions (i.e. temperature) is key for understanding numerous plant growth and development processes. Seed dormancy is usually described as the incapacity of a mature seed to complete its germination even when external conditions are favorable [1]. The balance between seed dormancy and germination is highly regulated by the interaction between abscisic acid (ABA), gibberelins (GAs) and the gasotransmitter nitric oxide (NO). In previous works, *DELAY OF GERMINATION 1* (*DOG1*) has been identifed as a master regulator for seed dormancy [2], whose transcript levels are controlled by the temperature at which seed maturation takes place, promoting higher dormancy levels when it is lower [3].

With this background, our study focuses on the interplay between DOG1, different seed maturation temperatures and the role of NO in the accumulation of DOG1 protein. Our results show that changes in temperature have an important effect on the accumulation pattern of DOG1. Under lower maturation temperature seeds present a higher accumulation of the DOG1 monomeric form, related with an increase in dormancy levels. Corresponding with the breakage of dormancy and therefore the promotion of germination, the accumulation pattern is altered towards the formation of the dimeric form of DOG1. We observed that lines with overaccumulation of NO have an increase in the levels of dimeric DOG1. In this context, we propose a hypothetical working model to explain the dormant state of seeds and the promotion of germination through the balance between DOG1 monomer and dimer and the highly specific role of NO during the regulation of these processes, where *S*-nitrosylation is the key modification regarding this molecular switch.

## References

1. Née et al., 2017. *Nature Communications*, 8, 72.
2. Bentsink et al., 2006. *PNAS*, 103, 17042-17047.
3. Murphey et al., 2015. *Environmental and Experimental Botany*, 112, 33-43.
4. Albertos et al., 2015. *Nature Communications*, 6, 1-10.

## Funding and acknowledgments

This work is funded by the projects PID2020-119731RB-I00 (Ministerio de Ciencia e Innovación), SA137P20 (Junta de Castilla y León) and Escalera de Excelencia CLU-2018-04 co-funded by the P.O. FEDER of Castilla y León 2014–2020 España (to O.L.). F. Pollo-Rodríguez has a grant from Junta de Castilla y León (Orden EDU/875/2021).

# From models to crops: Epigenetic regulation of flowering time in *Brassicas*

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In plants, the trimethylation of histone H3 lysine 27 (H3K27me3) is a silencing epigenetic mark relevant for developmental transitions like flowering. H3K27me3 is deposited at target genes by the Polycomb repressive complex 2 (PRC2), and removed by the histone demethylase activity of specific Jumonji C (JmjC) proteins (Crevillén 2020). The floral transition is a key agronomic trait because untimely flowering impairs fruit production and yield. However, the epigenetic mechanisms regulating flowering time in crops remain poorly understood. During the last years, we have studied the epigenetic regulation of flowering time by H3K27me3 in the model plant *Arabidopsis thaliana* and *Brassica rapa*, a worldwide agrieconomically important crop that include root and leafy vegetables, and oilseed cultivars (e.g. turnip, packchoi and oilseed sarson, respectively) (Payá-Milans *et al.*, 2019). Regarding the role of the main H3K27me3 demethylases in *B. rapa*, we found that *braA.ref6* mutants were late flowering without altering the expression of the *B. rapa* homologs of the floral repressor *FLC*. Strikingly, BraA.REF6 regulated a number of gibberellic acid (GA) biosynthetic genes and, furthermore, GA-treatment complemented the late flowering phenotype of the *braA.ref6* mutant. On the other hand, BraA.ELF6 modulated the expression of *B. rapa FLC*-like genes (Poza-Viejo *et al.*, 2022). This study shed light to understand the epigenetic regulation of flowering time in *B. rapa*, highlighting differential regulatory mechanisms between model and closely-related crop species in adjusting agronomical traits such as flowering time.

Crevillén P (2020) Histone demethylases as counterbalance to H3K27me3 silencing in plants, iScience 23, 11:101715.

Payá-Milans M, Poza-Viejo L, San Martín-Uriz P, Lara-Astiaso D, Wilkinson M and Crevillén P (2019) Genome-wide analysis of the H3K27me3 epigenome and transcriptome in Brassica rapa, GigaScience 8, 12:1-13.

Poza-Viejo L, Payá-Milans M, San Martín-Uriz P, et al., Crevillén P (2022) Conserved and distinct roles of H3K27me3 demethylases regulating flowering time in Brassica rapa, Plant, Cell & Environment 45(5):1428-1441.

This work was supported by grants PID2021-1222410B-I00 and BIO-2015-68031-R to PC from the Agencia Estatal de Investigación of Spain.

# EXPLORING THE SYNERGISTIC POTENTIAL OF CO-CULTURED ENDOPHYTIC FUNGI FROM PERSEA INDICA: AMPLIFYING SECONDARY METABOLITE PRODUCTION FOR ENHANCED BIOPESTICIDAL EFFECTS

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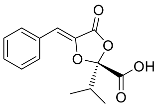
Endophytes, a fascinating group of microorganisms that can be found in asymptomatic plant tissue, hold great potential in the field of agri-environmental research. Among these endophytic fungi, the majority belong to the phyla Ascomycota and Deuteromycota and are known to produce secondary metabolites that can serve as plant defensive compounds. One intriguing example is *Guignardia manguiferae*, an endophytic fungus isolated from the endemic Canarian tree *Persea indica* **[1]**. This fungus has been found to produce several noteworthy secondary metabolites, including guignardic acid **(1)**, metguignardic acid **(2)**, guignardionone C **(3)**, guignardionone D **(4)**, and phenguignardic acid methyl ester **(5)**. Similarly, another endophytic fungus, *Penicillium glabrum*, which was also isolated from the same plant species, has demonstrated the production of 1,3-dihydroxy-2,4-dimethoxy-5-methylbenzene **(6)**, asterric acid **(7)**, and methyl asterrate **(8)** (data not published). These secondary metabolites hold potential for their biopesticidal properties.

To further explore and optimize the production of these metabolites, we conducted co-cultivation experiments using *Guignardia manguiferae* and *Penicillium glabrum* on a modified Czapek-Dox culture medium **[2]**. Our goal was to assess the production levels and variability of secondary metabolites in the liquid fermentation extracts and evaluate their biopesticidal potential. Remarkably, the co-cultivation approach led to significant variations in extract yields compared to individual fungal cultures, accompanied by noticeable insecticidal and nematicidal effects. Chromatographic analyses, specifically GC-MS, revealed notable differences between fermentation batches. Our findings emphasize the significance of exploring microbial interactions and co-cultivation strategies for harnessing the full potential of agri-environmental resources.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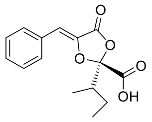
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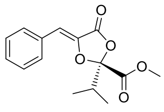
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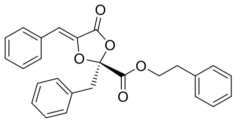
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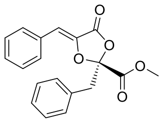
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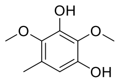
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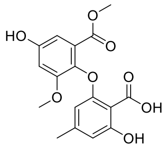
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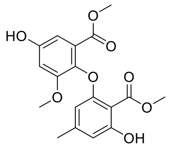
(8)

## References

**[1]** Gonzalez-Coloma, A.; Andrés, M.F.; Contreras, R.; Zúñiga, G.E.; Díaz, C.E. Sustainable Production of Insecticidal Compounds from Persea indica. Plants **2022**, 11, 418.

**[2]** Xu, S.; Li, M.; Hu, Z.; Shao, Y.; Ying, J.; Zhang, H. The Potential Use of Fungal Co-Culture Strategy for Discovery of New Secondary Metabolites. *Microorganisms* **2023**, *11*, 464.

## Funding

Research Project PID2019-106222RB-C31 funded by MCIN/ AEI /10.13039/501100011033/ ERDF "A way of doing Europe”

# METABOLIC POTENTIAL OF ENDOPHYTIC BACTERIAL COMUNITIES OF RUBUS ULMIFOLIUS SCHOTT AGAISNT BIOTIC AND ABIOTIC STRESSES

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Plants have to deal with biotic and abiotic stresses, such as pathogen infections and drought, which interfere in their development. Endophytic bacteria are components of the plant microbiota which colonize internal tissues and establish non-harmful relationships. Many of the them have been described to produce bioactive compounds, taking an active role in plant growth, but also helping them to tolerate biotic and abiotic stresses.

Thus, the objective is to study bacteria communities which inhabit blackberry and link their metabolic potential with biotic and abiotic stress tolerances.

Blackberry from three different niches were sampled, a standard, a river-sided and a human-impacted one. Total DNA was extracted and seven hypervariable regions of the 16S rRNA gene were amplified. QIIME2 was used for quality filtering, bacterial composition and diversity analysis and the metabolic pathways were inferred with Picrust2 and MicrobiomeAnalyst.

There were slight differences among niches. Some remarkable genera related with plant tolerance to stresses were identified. Sphingomonas has been the main component of all samples, accounting for 19.6% in standard; 22.5% in riverside and 22.2% in human-impacted samples. This genus has shown to be beneficial against drought stress [1]. Similarly, Streptomyces and Rhizobium are related to biotic stresses tolerance. This genera has been also identified in blackberry samples, accounting for 1.6% and 1.2% in standard; 1.7% and 2.3% in riverside; and 3.2% and 1.0% in human-impacted samples. The metabolic analysis predicted statistically significant metabolic pathways associated with the synthesis of tryptophan and the synthesis of proline, both metabolite accumulation in the plant increases tolerance against drought stress; and the synthesis of antibiotic molecules such as vancomycin or tetracycline [2, 3].

Endophytic bacteria have been considered as a novel approach to cope with these stresses. Thus, studying endophytes communities may be a source of new mechanisms to fight against biotic and abiotic stresses.

## References:

1. Ullah, A., Nisar, M., Ali, H., Hazrat, A., Hayat, K., Ayaz, &, Keerio, A., Ihsan, M., Laiq, M., Ullah, S., Fahad, S., Khan, A., Aamir, &, Khan, H., Akbar, A., & Yang, X. (2019). Drought tolerance improvement in plants: an endophytic bacterial approach. *Applied Microbiology and Biotechnology.* 103:7385–7397 https://doi.org/10.1007/s00253-019-10045-4
2. Tahir, M., Sajjad Mirza, M., Zaheer, A., Dimitrov, M. R., Smidt, H., & Hameed, S. (2013). Isolation and identification of phosphate solubilizer Azospirillum, Bacillus and Enterobacter strains by 16SrRNA sequence analysis and their effect on growth of wheat (Triticum aestivum L.). In *AJCS* (Vol. 7, Issue 9). https://doi.org/10.1016/j.bjm.2015.11.035
3. Singh, S., Singh, S., Singh, A., & Kalra, A. (2022). Endophytic microbes mitigate biotic-abiotic stresses and modulate secondary metabolite pathway in plants. *Microbial Syntrophy-Mediated Eco-Enterprising*, 87–124. https://doi.org/10.1016/B978-0-323-99900-7.00001-8

## Funding:

This work has been funded by MCIN/AEI/ 10.13039/501100011033 and by “ERDF A way of making Europe”.

## Acknowledgements:

RRC acknowledges the grant PID2019-109960RB-I00 and the FPI grant PRE2020-094762 funded by MCIN/AEI/ 10.13039/501100011033 and by “ERDF A way of making Europe”. Also acknowledges the Agribiotechnology Research (CIALE) Regional Government of Castile and Leon (Escalera de Excelencia CLU-2018-04) and co-funded by the Operational Program of the European Regional Development Fund for Castile and Leon 2014–2020.

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# Changes in plant growth promotion abilities of the root-associated bacteria along *P. vulgaris* L. phenological stages

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Due to the growing global population, there will be significant pressure on food production. The unstable climate, growing urbanization, industrialization, and pesticide contamination are threats to current agricultural practices. Sustainable practices must be undertaken in order to attain food security. One of the most widely grown legumes in the world and a dependable source of high-quality protein, the common bean (*Phaseolus vulgaris* L.) can reduce the environmental impact of meat production, while helping human population to intake the recommended daily amount of protein. High crop yields, however, require intensive and expensive fertilization. Rhizobacteria that promote plant growth (PGPR) are emerging as a viable option for boosting production of crops, although this interaction is not fully understood, particularly the temporal fluctuations in the microbiome. Thus, a better understanding of the dynamics and interactions between microbes and plants may enhance the positive impacts of microorganisms on plants. To do this, the cultivable bacteria of the bean root (rhizoplane, endosphere, and nodules) were isolated and characterized at different phases of plant development (early vegetative growth, late vegetative growth, flowering, and pod). Indole-3-acetic acid production, siderophore synthesis, and antifungal activity of isolated bacteria were identified and related to plant phenology, suggesting that several strains seem to respond to the plant needs at each developmental stage. Several strains were singled out for their capacity to exhibit one or more PGP abilities, making them great candidates for use as stage-specific biostimulants in precision agriculture.

## References

Chaparro, J. M., Badri, D. V., & Vivanco, J. M. (2014). Rhizosphere microbiome assemblage is affected by plant development. ISME Journal, 8(4), 790–803. https://doi.org/10.1038/ismej.2013.196

Eid, A. M., Fouda, A., Abdel-rahman, M. A., Salem, S. S., Elsaied, A., Oelmüller, R., Hijri, M., Bhowmik, A., Elkelish, A., & El-Din Hassan, S. (2021). Harnessing bacterial endophytes for promotion of plant growth and biotechnological applications: An overview. Plants, 10(5), 1–33.

Ganugi, P., Martinelli, E., & Lucini, L. (2021). Microbial biostimulants as a sustainable approach to improve the functional quality in plant-based foods: a review. Current Opinion in Food Science, 41, 217–223. https://doi.org/10.1016/j.cofs.2021.05.001

## Funding

This research was funded by FCT through the financial support to the H2O value project (PTDC/NAN-MAT/30513/2017) also supported by FCT/MEC through national funds, and the co-funding by the FEDER, within the PT2020 Partnership agreement and Compete 2020 (CENTRO-01-0145-FEDER-030513). We also acknowledge financial support to CESAM by FCT/MCTES (UIDP/50017/2020, UIDB/50017/2020, LA/P/0094/2020) through national funds.

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# Molecular interaction between BRs and NO in Arabidopsis early development

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Plant development is promoted by plant hormones and growth regulators. Among the plant hormones, brassinosteroids (BRs) are steroids hormones involved in multiple physiological processes, such as germination, cell elongation, plant growth, and defense against biotic and abiotic stresses. Nevertheless, the molecular interaction of BRs with other growth regulators such as the reactive nitrogen species (RNS) nitric oxide (NO), remains elusive. It has been described that BRs increase the expression of *NR* and *NOA*, two genes that codify enzymes involved in NO biosynthesis in plants (1). Both, BRs and NO participate in the architecture of the primary root (1) and NO is necessary in the stomatal closure induced by BRs (2). Moreover, NO signal is perceived by different postranslational modifications (PTMs) inlcuding cysteine *S*-nitrosylation, tyrosine nitration, nitrosylation of metals and lipid nitration (3). Interestingly, it has been described that *S*-nitrosylation of the recombinant kinase BIN2, the main negative regulator of BRs signaling pathway, inhibits its kinase activity *in vitro* (4). However, the physiological effect of this PTM *in planta* remains unclear. In order to furhter investigate the molecular interaction between BRs and NO, different phenotypical, bioinformatic, molecular and trasncriptional approaches have been performed. The phenotypical assays showed differences to BR sensitivity on several mutants with impair endogenous levels of NO. Moreover, changes in the phosphorylation status of BES1 and BZR1, two transcription factors (FTs) that are master regulators of BR responses, have been detected in the presence of NO related compunds. Finally, differences in gene expression studies of BR marker genes using mutant backgrounds with altered NO levels have been uncovered. These results will help to expand the current knowledge of the molecular crosstalk between NO and BRs and its effect on plant gowth and development.

## References

1. Tossi *et al* (2013). *Plant Signaling & Behavior*, *8*(7), e24712.
2. Shi *et al* (2015). *The Plant Journal*, *82*(2), 280–301.
3. Sun *et al* (2021). *Horticulture Research*, *8*(1), 71.
4. Wang *et al* (2014). *Proceedings of the National Academy of Sciences USA*, *112*(2), 613–618.

## Funding

J. R-H. Scholarship Programa Clave, JCyL.
L. H-M. PhD Fellowship University of Salamanca.
P. A. María Zambrano Grant, NextGenerationEU.
O. L. research: Spanish Ministry of Science and Innovation (PID2020-119731RB-I00), JCyL (SA137P20), Escalera de Excelencia (CLU-2018-04) co-funded by P.O. FEDER CyL 2014-2020

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# Interaction of volatiles with *Arabidopsis thaliana* and *Rhizobium* in non-stressed and Cd-stressed conditions

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Plants interact with soil microorganisms, such as rhizobacteria, which can result in benefits for the plants[1,2]. Studies show that rhizobacteria volatile organic compounds (VOCs) can stimulate plant growth, improve nutrition, and increase stress tolerance, but effects on bacteria are less known[3,4]. The aim of this study is to unveil if volatiles able to promote plant growth influence soil bacteria in the same way and whether these effects are maintained or altered in the presence of Cd stress. Divided culture plates were used in the experiments. On one side bacteria (*Rhizobium* sp. strain E20-8) or *Arabidopsis thaliana* were placed and on the other side a disk soaked with a concentration (0 nM, 1 nM, 100 nM, 10 µM, 1 mM, and 100 mM) of of the tested volatiles (2,3-butanediol, 3-methyl-1-butanol, and 2-butanedione). Three independent replicates were performed for each volatile and concentration. Growth and biochemical analysis (protein content, oxidative damage, and antioxidant response) were performed to compare volatile effects in plants and bacterial[5]. Volatile compounds influenced plants and bacteria differently and this influence was also changed by Cd stress. The three volatiles increased plant tolerance to Cd since their antioxidant activity protected plant cells from Cd toxicity. However, this effect was less marked on bacteria, with two compounds (3-methyl-1-butanol and 2,3-butanediol) increasing Cd toxicity, and only one (2-butanone) being able to protect *Rhizobium* from Cd stress.

The use of volatiles to promote plant growth or increase stress tolerance must be considered carefully, as the positive effects of some compounds observed for plants are not replicated in bacteria and might change soil microbial communities. Taking this precaution, the application of volatiles can be a safe, sustainable alternative to increase crop yields and reduce crop environmental susceptibility. They can also be used in bioremediation to boost land revegetation and restore ecosystems.

## References

1. Harman, G.; Khadka, R.; Doni, F.; Uphoff, N. Benefits to Plant Health and Productivity from Enhancing Plant Microbial Symbionts. Front. Plant Sci. 2021, 11, 65.
2. Roman-Reyna, V.; Pinili, D.; Borja, F.N.; Quibod, I.L.; Groen, S.C.; Mulyaningsih, E.S.; Rachmat, A.; Slamet-Loedin, I.H.; Alexandrov, N.; Mauleon, R.; et al. The Rice Leaf Microbiome Has a Conserved Community Structure Controlled by Complex Host-Microbe Interactions. bioRxiv 2019.
3. Ryu, C.M.; Farag, M.A.; Hu, C.H.; Reddy, M.S.; Wei, H.X.; Pare, P.W.; Kloepper, J.W. Bacterial Volatiles Promote Growth in *Arabidopsis*. Proc. Natl. Acad. Sci. USA 2003, 100, 4927–4932.
4. Fincheira, P.; Quiroz, A.; Tortella, G.; Diez, M.C.; Rubilar, O. Current Advances in Plant-Microbe Communication via Volatile Organic Compounds as an Innovative Strategy to Improve Plant Growth. Microbiol. Res. 2021, 247, 126726.
5. Sá, C.; Matos, D.; Cardoso, P.; Figueira, E. Do Volatiles Affect Bacteria and Plants in the Same Way? Growth and Biochemical Response of Non-Stressed and Cd-Stressed Arabidopsis thaliana and Rhizobium E20-8. Antioxidants, 2022, 11.

## Funding

We acknowledge funding by Fundação para a Ciência e Tecnologia (FCT) through PhD scholarships of Carina Sá (2020.05872.BD) and Diana Matos (2020.07142.BD). We also acknowledge financial support to CESAM by FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020), through national funds.

**Poster 43**

# Factors shaping the microbiome composition of two different plant species co-located in Iberian forests

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Plant-associated microbial communities play important roles in host nutrition, development and defence. In particular, those living within internal plant tissues can affect plant metabolism in a more intimate way. Understanding the factors that shape plant microbial composition and discovering enriched microbes within endophytic compartments would thus be valuable to gain knowledge on potential plant–microbial coevolutions. However, these interactions are usually studied through reductionist approaches (*in vitro* models or crop controlled systems). Here, we investigate these ecological factors in wild forest niches using proximally located plants from two distant taxa (blueberry and blackberry) as a model.

Although the microbial communities were quite similar in both plants, we found that sampling site had a high influence on them; specifically, its impact on the rhizosphere communities was higher than that on the roots. Plant species and sample type (root–rhizosphere) affected the bacterial communities more than the fungal communities. For instance, Xanthobacteraceae and Helotiales taxa were more enriched in roots, while the abundance of Gemmatimonadetes was higher in rhizospheres. Acidobacteria abundance within the endosphere of blueberry was similar to that in soil. Several taxa were significantly associated with either blackberry or blueberry samples regardless of the sampling site. For instance, we found a significant endospheric enrichment of *Nevskia* in blueberry and of *Sphingobium*, *Novosphingobium* and *Steroidobacter* in blackberry.

There are selective enrichment and exclusion processes in the roots of plants that shapes a differential composition between plant species and sample types (root–rhizosphere). The special enrichment of some taxa in each plant species might suggest the presence of ancient speciation processes and might imply specific symbiosis. The selection of fungi by the host is more pronounced when considering the fungal trait rather than the taxonomy. This work helps to understand plant–microbial interactions in natural ecosystems and the microbiome features of plants.

**Funding**

We acknowledge the grant PID2019-109960RB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and by “ERDF A way of making Europe” and funding by the Regional Government of Castile and Leon (Escalera de Excelencia CLU-2018-04), co-funded by the Operational Program of the European Regional Development Fund for Castile and Leon 2014–2020. ZSS acknowledge a Grant co-financed by the European NextGenerationEU, Spanish "Plan de Recuperación, Transformación y Resiliencia", Spanish Ministry of Universities, and University of Salamanca ("Ayudas para la recualificación del sistema universitario español 2021-2022").

# *Lentzea* sp. 6', an interesting strain in the development of biopesticides for crop protection

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Actinobacteria are microorganisms of great interest due to their ability to produce a wide range of secondary metabolites. Recent studies focusing on their genomes have unveiled their metabolic versatility, particularly in the production of new antimicrobials with potential applications against multidrug-resistant organisms, as well as their mechanisms of tolerance to different stresses (Carro et al., 2018; Jose et al., 2021). Particularly, some bacteria have been discovered in desert, arid or semi-arid regions, which represent a novel source of secondary metabolites. Among them, the genus *Lentzea*, belonging to the family *Pseudonocardiaceae*, stands out as a promising producer of bioactive compounds and other metabolites of interest (Maiti & Mandal, 2022). This finding highlights this genus's broad range of potential applications to counteract the detrimental effects associated with climate change.

The aim of this study was to taxonomically and functionally characterize a strain partially identified as *Lentzea sp*., isolated from a desert area in Western Sahara. Several experiments were conducted to assess its potential for plant growth promotion, inhibiting phytopathogenic microorganisms, and tolerating different abiotic stresses. We performed a functional annotation of the available genomes of *Lentzea* genus strains, which revealed the presence of genes encoding secondary metabolite producers, polymer-degrading enzymes, as well as both common and uncommon bioclusters within this genus. Additionally, some assays were conducted to evaluate its ability to produce secondary metabolites *in vitro* and *in planta*. Results demonstrated that this strain exhibited inhibitory activity against plant pathogens, such as *Acidovorax valerianellae* and the fungus *Leptosphaeria maculans*. These findings underscore the untapped potential of the genus *Lentzea* in the production of secondary metabolites and bioactive compounds, positioning it as a promising plant probiotic and antimicrobial agent for crop protection.

## References

Carro, L., Nouioui, I., Sangal, V., Meier-Kolthoff, J. P., Trujillo, M. E., Montero-Calasanz, M. D. C., Sahin, N., Smith, D. L., Kim, K. E., Peluso, P., Deshpande, S., Woyke, T., Shapiro, N., Kyrpides, N. C., Klenk, H.-P., Göker, M., & Goodfellow, M. (2018). Genome-based classification of micromonosporae with a focus on their biotechnological and ecological potential. Scientific Reports, 8(1). https://doi.org/10.1038/S41598-017-17392-0

Jose, P. A., Maharshi, A., & Jha, B. (2021). Actinobacteria in natural products research: Progress and prospects. Microbiological research, 246, 126708. https://doi.org/10.1016/j.micres.2021.126708.

Maiti, P.K., Mandal, S. Comprehensive genome analysis of Lentzea reveals repertoire of polymer-degrading enzymes and bioactive compounds with clinical relevance. Sci Rep 12, 8409 (2022). https://doi.org/10.1038/s41598-022-12427-7

Nouioui, I., Carro, L., García-López, M., Meier-Kolthoff, J. P., Woyke, T., Kyrpides, N. C., ... & Göker, M. (2018). Genome-based taxonomic classification of the phylum Actinobacteria. Frontiers in microbiology, 9, 2007.

## Funding:

This result is part of the I+D+i TED2021-129160B-I00, funded by MCIN/AEI/ 10.13039/501100011033 and by "Unión Europea NextGenerationEU/PRTR". EM expresses gratitude for the EU HORIZON 2020 Marie Sklodowska Curie Actions (Grant Agreement nº 897795)).

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# Hexanoic acid as an inductor of *Solanum lycopersicum* defenses against *Xanthomonas vesicatoria*

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Tomato plants are one of the most widespread, economically important crops in the world. However, biotic stresses caused by pathogenic microorganisms can be a limiting factor for plant production. These stresses can trigger different responses, from altered molecular expression to changes in the development of the plants. Several strategies have been used for disease control, mainly based on chemicals that may cause a problem for the environment. Plants can defence themselves from pathogens using pre-existing physical and chemical barriers. In addition to this basal resistance, plants can also develop a state of enhanced defensive capacity when they are properly stimulated, called induced resistance (IR). As IR can be triggered by natural or synthetic compounds, we propose the natural compound hexanoic acid (Hx) as an inductor of the tomato defenses.

Hx is a natural compound that has been demonstrated to act as resistance-inducing agent against a wide spectrum of pathogens (Vicedo et al 2009, Scalschi et al, 2013, Llorens et al, 2015a,b). *Xanthomonas vesicatoria* (Xv) is the causal agent of bacterial spot disease on pepper and tomato, which causes an important economic loss.

In this work, we studied the effect of the application of Hx to five-week old tomato plants, before the inoculation with Xv under controlled conditions. We observed that the treated plants were able to protect themselves more efficiently than the control ones, showing less injures on the leave surface. Also, the recovery of the bacteria indicated that Xv was not able to spread and grow as fast as in non-treated plants. This may indicate that hexanoic acid could improve the defense capacity before the attack. Analysis about callose and lignin depositions, and expression of marker genes involved in plant defence are also carried out. This could contribute to identify new defense strategies against Xv for improving crop yields.

## References

Llorens, E.; Vicedo, B.; López, M.M.; Lapeña, L.; Graham, J.H.; García-Agustín, P. Induced resistance in sweet orange against *Xanthomonas citri* subsp. citri by hexanoic acid. Crop Protection, 2015 a. DOI: 10.1016/j.cropro.2015.04.008

Llorens, E., Scalschi, L., Fernández-Crespo, E., Vicedo, B., Lapeña, L., García-Agustín, P. Hexanoic acid provides long-lasting protection in 'Fortune' mandarin against *Alternaria alternata*. Physiol. Mol. Plant Pathol. 2015b. Jul;91:38–45. doi: 10.1016/j.pmpp.2015.05.005.

Scalschi L, Vicedo B, Camañes G, Fernandez-Crespo E, Lapeña L, González-Bosch C, García-Agustín P. Hexanoic acid is a resistance inducer that protects tomato plants against *Pseudomonas syringae* by priming the jasmonic acid and salicylic acid pathways. Mol Plant Pathol. 2013 May;14(4):342-55. doi: 10.1111/mpp.12010. Epub 2012 Dec 23. PMID: 23279078; PMCID: PMC6638675.

Vicedo B, Flors V, de la O Leyva M, Finiti I, Kravchuk Z, Real MD, García-Agustín P, González-Bosch C. Hexanoic acid-induced resistance against *Botrytis cinerea* in tomato plants. Mol Plant Microbe Interact. 2009 Nov;22(11):1455-65. doi: 10.1094/MPMI-22-11-1455. PMID: 19810814.

**Funding**: Grant PID2021-123600OR-C4 funded by MCIN/AEI/ 10.13039/501100011033 and by FEDER A way of making Europe.

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# Micronutrient seed priming with iron, zinc and boron in wheat and barley: improvement of germination and early plant development.

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Some of the major goals pursued in current agriculture are to reduce chemical inputs, to increase the yield and the nutritional value of the crops and to improve stress resistance of seeds and seedlings. *Priming* and *biofortification* are environmental-friendly techniques that can be used to achieve these objectives. *Priming* consists in stimulating seeds or plants by different physico-chemical or biological methods to prepare them to face biotic or abiotic stress. Among the priming techniques is nutripriming, which consists in soaking initial seeds in solutions with specific nutrients to improve subsequent crop yield. *Biofortification* is instead based on an extra supply of nutrients to the plant during the growth season, in this case to increase the nutritional value of its edible part. Biofortified seeds can be used for seeding with the hypothesis that it may also result in a better germination and early plant development. For both nutripriming and seed biofortification, solutions of $FeSO_4$, $ZnSO_4$ and $Na_2B_4O_7$ at different concentrations were used (in ranges from 10 to 5000 mg/L, depending on the compound) setting individual and combined treatments (all four possible combinations). The only difference was that in nutripriming initial seeds were treated, while for biofortification the treatment was applied to mother plants. The vigour and performance were evaluated through *in vitro* germination and initial growth of seedlings. Preliminary results were found quite promising, as they showed a great homogenisation in germination times and a substantial improvement in the initial growth, especially noticeable in intermediate concentrations of $FeSO_4$ and $ZnSO_4$. Altought more experiments are needed to corroborate these results and verify the effect of the treatments under field conditions, these first results are an encouraging starting point towards the yield improvement of such important cereal crops in our region.

## Funding

This study was funded by Diputación de Palencia (Generando Valor Rural Provincia de Palencia, Proyectos I+D+i 2022; ref.: 00200200514), Fundación General de la Universidad de Valladolid (FUNGE) (Concept proofs call within the Plan TCUE 2021-2023, co-funding by the Regional Gobernment of Castilla y León and the European Regional Development Fund (ERDF) (Ref.: 067/229421) and the University of Valladolid - Banco Santander pre-doctoral contract to T. S-G. (Call 2022).

## Acknowledgements

We would like to thank Felicidad López and Evelio Alonso for their invaluable help with laboratory and field work.

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# Using *Kmer*-GWAS to Explore the Genetics of Powdery Mildew Resistance in a Swiss Wheat Landrace Collection

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Man-made wheat domestication and breeding led to genetic bottlenecks minimizing genetic diversity with which commercial varieties cannot cope with biotic and abiotic stresses imposed by climate change. Bread wheat landraces, a crop status between wild relative and elite cultivars display, compared to modern breeding material, higher genetic diversity likely to be important for adaptation. To exploit this untapped genetic diversity potential, we assembled a diverse collection of 500 Swiss bread wheat landraces (*Triticum aestivum* L.) within the H2020 Activated GEnebank NeTwork (AGENT) project to identify sources of resistance against the wheat yield-reducing fungal disease powdery mildew caused by *Blumeria graminis* f. sp. *tritici*.

To identify novel sources of resistance, a *Kmer*-based genome-wide association study (GWAS) was conducted on the landrace panel genotyped with DArTseq technology for seedling resistance using ten powdery mildew isolates with contrasting virulence spectra. We developed a pan-genome-wide association pipeline based on multiple wheat reference genomes, maximizing the chances of tagging responsible loci for mildew resistance, likely absent in a single reference genome. The association mapping detected multiple known *Pm* genes like *Pm1*, *Pm2* or *Pm4b* but also novel regions associated with powdery mildew resistance in chromosomes 3D, 5D and 6A. One prime candidate for further validation studies is a Werner syndrome-like exonuclease on chromosome 3D.

Of note, unlike standard GWAS, our method identifies associations with structural variations and sites not present in a single reference genome, highlighting the relevance of landraces stored at genebanks as a source of novel genetic variation important not only for mildew resistance but any other agronomic trait important for adaptation to a changing environment.

**Funding** This work is supported by the European Union’s Horizon 2020 research and innovation programme under the grant agreement No 862613 (Activated GEnebank NeTwork [AGENT])

# Antioxidant response of *Solanum lycopersicum L.* to stress due to the presence of metalloids

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Currently there is an increasing presence of heavy metals in soils and water due to anthropogenic activities. The biggest problem caused by this increase is the difficulty in recycling these compounds and their high permanence in soils, which can facilitate their entry into food chains. There are plants with a great capacity to assimilate heavy metals and metalloids or make them less accessible to other organisms present in the soil. In the present study, we have analyzed the behavior of *Solanum lycopersicum L.*, an important crop with great agronomic interest, under the stress caused by antimony (Sb). In our research we wanted to evaluate the antioxidant response, mainly the components of the Ascorbate-Glutathione (AsA-GSH) cycle, throughout different exposure times to the metalloid. Within the components of the AsA-GSH cycle, we have deepened our knowledge about Glutathione S-transferase (GST) and Glutathione Reductase (GR), since previous investigations show their involvement in the elimination of xenobiotic components. We reckon that a better understanding of how these enzymes work could be key to developing more tolerant varieties to this kind of abiotic stress. For that, we have decided to analyze whether there are key domains that also appear in other model species, which could explain a greater or lesser phytoremediation capacity. After analyzing the gene expression data, we report a particularly relevant influence of dehydroascorbate reductase (DHAR), GR and GST, especially in plants exposed to Sb at the final exposure time (14 days). This observation is especially striking as it shows a gradual attenuation of the expression as the exposure time progresses. Finally, the bioinformatics study showed a high conservation of the domains in the GRs analyzed between model species and other Solanaceae and considerable divergence in the GSTs, which could explain a different behavior of the species under this type of stress.

**Funding** Universidad de Extremadura

**Acknowledgements** Centro Tecnológico Nacional Agroalimentario "Extremadura" (CTAEX)

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# Phenotypic and molecular characterization of *Amaranthus*

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Amaranth is a pseudocereal originated in Mesoamerica belonging to the genus *Amaranthus*. It is currently cultivated in regions with temperate and warm climates for its nutritional properties, but also for the ornamental value. In this work we characterize different species of wild and cultivated amaranth, through phenotypical and molecular approaches to investigate how the domestication influenced in developmental cues and stress responses.

We studied three stages during the life cycle of the plants, such as dormancy, germination and post-germinative development. Related to seed biology, the cultivated species have a greater seed weight and size, but lower germination rates after seed harvesting caused by increased dormancy levels and no to the lack of seed viability. Interestingly, these cultivated species show higher response to several treatments that break seed dormancy as seed imbibition and temperature compared with its wild relatives. We studied the ABA sensitivity of Amaranth seed during germination, and we found out that this inhibition was related to the accumulation an ABI5-like protein, described as a master repressor of seed germination in Arabidopsis and other species (Albertos et al., 2015). During post-germinative development, we analysed the presence of lipid bodies as indicators of postgerminative correct development since oleosomes act as a primary energy source during seed germination, but no differences between wild and cultivated species were detected. Likewise, the sensitivity to the gasotransmitter nitric oxide (NO) on the hypocotyl elongation did not show differences in our experimental set up. Finally, we analyzed the response to drought stress through measuring of water loss on detached leaves, detecting that that some of the cultivated species have reduced water loss that may be an indication of having higher tolerance to abiotic stresses.

## References

Albertos P. et al. (2015). S-nitrosylation triggers ABI5 degradation to promote seed germination and seedling growth. *Nat. Commun*. 6, 8669.

## Acknowledgements & Funding

P. A. María Zambrano Grant, NextGenerationEU. This work is funded by the projects PID2020-119731RB-I00 (Ministerio de Ciencia e Innovación), SA137P20 (Junta de Castilla y León) and Escalera de Excelencia CLU-2018-04 co-funded by the P.O. FEDER of Castilla y León 2014–2020 España (to O.L.).

# Plant Growth-Promoting Bacteria from Cape Verde to Increase Maize Tolerance to Salinity

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Salinity constitutes a major abiotic factor that negatively affects crop productivity. Inoculation with plant growth-promoting bacteria (PGPB) is proven to increase plant tolerance to abiotic stresses and enhance plant growth, development and productivity. The present study aims to increase the resilience of crops to salinity using bacteria from the microbiome of plants growing in saline environments. For that, the halotolerance of bacteria present in the roots of natural plants growing on saline soils from Sal Island (Cape Verde), which is characterized by its arid environment and maritime influence, was determined, with some strains displaying extreme halotolerance (5.5% < $IC_{50}$ < 6%). Their ability to produce plant growth-promoting traits was evaluated, with most strains increasing indole acetic acid (26–418%), siderophore (>300%) and alginate (2–66%) production and phosphate solubilization (13–100%) under salt stress. The strains evidencing the best performance were inoculated in maize (*Zea mays* L.) plants and their influence on plant growth and biochemical status was evaluated. Results evidenced bacterial ability to especially increase proline (55–191%), whose osmotic, antioxidant and protein-protecting properties reduced protein damage in salt-stressed maize plants, evidencing the potential of PGPB to reduce the impact of salinity on crops. Enhanced nutrition, phytohormone production and osmolyte synthesis along with antioxidant response all contribute to increasing plant tolerance to salt stress.

## References

Cardoso, P.; Freitas, R. & Figueira, E. (2015). Salt tolerance of rhizobial populations from contrasting environmental conditions: understanding the implications of climate change. *Ecotoxicology*, *24*, 143 – 152. https://doi.org/10.1007/s10646-014-1366-8

Shilev, S. (2020). Plant.Growth-Promoting Bacteria Mitigating Soil Salinity Stress in Plants. *Applied Sciences*, *10* (20), 7326. https://doi.org/10.3390/app10207326

Zhao, C.; Zhang, H.; Song, C.; Zhu, J. & Shabala, S. (2020). Mechanisms of Plant Responses and Adaptation to Soil Salinity. *The Inovation*, *1* (1), 100017. https://doi.org/10.1016/j.xinn.2020.100017

## Funding

This research was also funded by FCT/MCTES through the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020), through national funds.

IAS2023
17th - 18th JULY
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# Unleashing the Power of Nitrogen-Fixing Microorganisms to Foster Sustainable Agriculture

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In modern agriculture, the problem of nitrogen deficiency in the soil is addressed by applying chemical fertilizers, which often lead to soil, water, and environmental contamination. Developing strategies to enhance nitrogen use efficiency is crucial for establishing sustainable agriculture and represents a significant challenge of this century. One promising approach is the utilization of nitrogen-fixing microorganisms as efficient biofertilizers, particularly endophytic microorganisms that reside within the plant and provide fixed nitrogen directly to it. Wild plant species, which harbor a greater diversity of endophytic microorganisms compared to cultivated species and can thrive without nitrogen fertilization, are excellent candidates for endophyte isolation.

The aim of this project was to identify microorganisms with the capacity for atmospheric nitrogen fixation, aiming to supply nutrients to plants and reduce reliance on chemical fertilizers and the associated problems. Endophytic microorganisms were isolated from wild plants belonging to agronomically important botanical families such as *Solanaceae* and *Rutaceae*. Isolation was performed from the roots, stems, and leaves using a nitrogen-free culture medium, resulting in the collection of seventy bacterial isolates. To confirm their nitrogen-fixing ability, a screening for the presence of the *nifH* gene, which is responsible for nitrogen fixation in the nitrogenase complex, was conducted. Subsequently, the strains were identified as belonging to the genera *Paenibacillus*, *Pseudomonas*, *Burkholdelia*, and *Rahnelia* through analysis of the 16S ribosomal RNA sequence.

Selected microorganisms were then inoculated into agronomically important plants such as citrus and tomato and various physiological parameters including height, total fresh and dry weight, root and shoot weight, total chlorophyll content, photosynthetic rate, and total nitrogen content were determined. The results demonstrated that certain bacterial isolates were capable of improving the measured parameters under nitrogen-deficient conditions. These bacteria hold potential for use in the field to enhance plant growth while reducing the need for excessive nitrogen fertilizer application, thereby lowering costs and mitigating environmental pollution.

**Funding:** Project funded by Diputación de Castellón, Convenio UJI: Fijación Nitrógeno Cítricos

**Acknowledgements:** We express our gratitude to Diputación de Castellón, specifically Santiago Pérez Peñarroya and Ramón Martinez Peiro, for their invaluable support in making this project possible.

# Identification of a Syntetic Bacterial Community that enhances plant growth under heat stress

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Climate change induces elevated temperatures that impair plant growth and fitness[1]. The root system plays a crucial role in plant adaptation to heat stress, adjusting its development according to temperature and mediating water and nutrient uptake and interaction with the microbiota[2].

To properly study the effect of extreme temperatures on plant growth, del Pozo´s group have engineered a new device called Temperature Gradient in the Root Zone (TGRooZ). The TGRooZ generates a controlled temperature gradient in the root growth zone while keeping the aerial tissues exposed to the environmental temperature, generating conditions similar to those in natural ecosystems[3]. Using this system, we have studied the microbiome of tomato plants grown in natural soil and exposed to three different temperature regimes: 22°C, 36°C, and 36TGRooZ. Comparing bacterial community profiles in the rhizosphere and root, we observed that across all temperatures used, soil and rhizosphere samples supported higher bacterial alpha diversity and richness indexes than roots samples. It was also shown that homogeneous high temperature changes the beta diversity of both rhizosphere and root-associated microbiome[3].

Using the TGRooZ system a bacterial community has been identified that could be important for the adaptation of plants to high temperature, so that a synthetic bacterial community (SynCom) has been designed in collaboration with Dr. Castrillo. We are using this SynCom to investigate the effects of heat stress on *Arabidopsis thaliana* and found that this SynCom clearly enhances the shoot growth and phosphorus accumulation of Arabidopsis under heat stress conditions, especially when plants are grown in the TGRooZ. Furthermore, this SynCom induces the auxin signaling in the root. We are now identifying the specific bacteria that could be responsible for these phenotypes. Taken together, our results indicate that the use of the TGRooZ will help to test new microbes to enhance plant tolerance to heat under closer-to-field conditions.

## **References**:

(1) Bhattacharya. (2019). Effect of High Temperature on Crop Productivity and Metabolism of Macro Molecules, 1–114.
(2) Koevoets et al. (2016). Frontiers in plant science, 7, 1335.
(3) González-García et al. (2023). Plant Communications, 4(3).

## **Funding**:

Research was supported by PID2020-113479RB-I00 grant by MCIN/AEI/10.13039/501100011033/ to J.C.P and by the "Severo Ochoa Program for Centres of Excellence in R&D" from the AEI of Spain (grant SEV-2016-0672 (2017-2021) to the CBGP. ITGM is supported by a predoctoral fellowship from Programa Propio I+D+I UPM-Santander Universidades 2022

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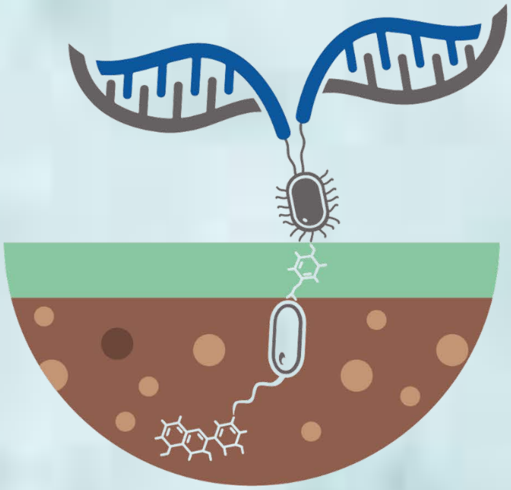

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