

I JORNADA DE BIENVENIDA DEL PROGRAMA DE DOCTORADO EN BIOTECNOLOGÍA AVANZADA



UNIVERSIDAD
DE MÁLAGA





I Jornada del Programa de Doctorado en Biotecnología Avanzada

5 de diciembre de 2019

- 9.40** **Carmen R. Beuzón López.** *Bienvenida a nuevos estudiantes e información sobre actualizaciones informativas*
- 10.00** **Francisco J. López Gordillo.** *Planificación e interpretación del trabajo científico*
- 10.20** **David Posé Padilla.** *Una trayectoria tras el Doctorado*
- 10.50 Sesión de charlas de alumnos 1**
- 10.50** **Lidia Blanco Sánchez.** *Regulatory mechanisms of tomato glandular trichomes against the aphid Macrosiphum euphorbiae.*
- 11.05** **Virginia Casas Arroyo.** *Estudio de polisacáridos de algas como posible fuente de compuestos nutraceuticos*
- 11.20** **Luz Divina María Gómez Pulido.** *A physico-chemical study of triterpenoids present in plant cuticles*
- 11.35 Pausa**
- 12.00 Sesión de charlas de alumnos 2**
- 12.00** **Lidia Jiménez Jiménez.** *Mejora de la calidad de fruto en especies modelo tomate y fresa: estudios metabólicos en transgénicas de tomate para el ciclo de Krebs y efectos del cambio climático en variedades comerciales de fresa europeas adaptadas a diferentes ambientes.*
- 12.15** **Javier Rueda Blanco.** *Suppression of plant defenses by bacterial effectors*
- 12.30** **Andrea Nieto Quero.** *Efectos del estrés en la neurogénesis hipocampal. Implicación del receptor LPA₁ para el ácido lisofosfatídico y la microglía*
- 12.45** **María de las Nieves López Pagán.** *Expresión heterogénea en genes relevantes para la virulencia de Pseudomonas syringae*
- 13.00 Sesión de pósters y almuerzo**
- 14.30 Fin de la jornada**

PRESENTACIONES EN PÓSTER

P.01. Lucía Cervantes Cárdenas. *Health-promoting potential of different strawberry varieties for breeding programs*

P.02. Ángel del Espino Pérez. *R gene regulation mediated by miRNA/phasiRNA during plant defense response against P. syringae*

P.03. Eva María Jiménez Enjuto. *Strategies for enhancing the osteogenic potential of BMP-9 in bone tissue engineering*

P.04. Liliya Kazantseva. *New uses for “old” drugs: Paclitaxel and its incorporation inside a thermo-sensitive polymer for osteosarcoma treatment*

P.05. Guillermo Moreno Ortega. *Mild water stress-induced priming enhance tolerance to Rosellinia necatrix in susceptible avocado rootstocks*

P.06. Precious Kwadzo Pomary. *Application of 1H NMR metabolomics for the diagnosis of neurological diseases*

P.07. Beatriz Romero Rodríguez. *Transcriptomic analysis of the interaction geminivirus-tomato*

P.08. Julia Vega Sánchez. *Cyanobacteria as potential sources of UV absorbing compounds: cosmeceutical applications*

P.09. Hafidh Almahrouqi. *The effect of fertilizer concentration on the productivity and biochemical composition of Spirulina (Arthrospira platensis) using urea as a nitrogen source*

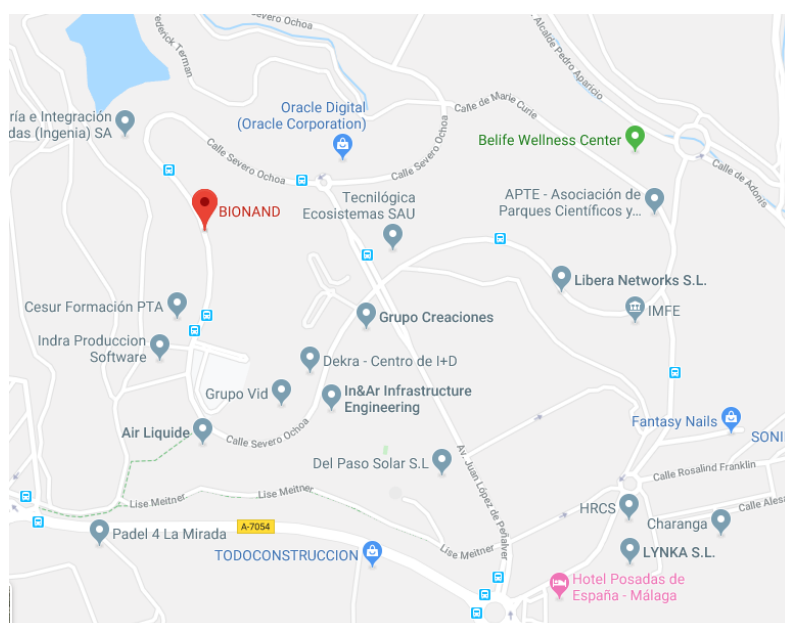
¿CÓMO LLEGAR?

Centro Andaluz de Nanomedicina y Biotecnología (BIONAND)

C/ Severo Ochoa, 35. Parque Tecnológico de Andalucía

29590 Campanillas, Málaga

A la hora acordada saldrá un autobús desde la Facultad de Ciencias (Bulevar Louis Pasteur) hacia BIONAND



PROGRAMA DE DOCTORADO BIOTECNOLOGÍA AVANZADA
JORNADA DE BIENVENIDA

Resúmenes

1. Presentaciones Orales

BLANCO SÁNCHEZ, LIDIA

Mecanismos reguladores de los tricomas glandulares de tomate implicados en la defensa contra el pulgón *Macrosiphum euphorbiae*.

El tomate (*Solanum lycopersicum*) es uno de los cultivos hortícolas más importantes a nivel mundial, sin embargo, su producción se ve dañada por el ataque de numerosas plagas. Hoy en día, en distintas partes del mundo, el control de cultivos aún se basa principalmente en el uso de pesticidas, lo que acarrea serios problemas ya no sólo de salud pública sino además de resistencia en las plagas. Por ello, es imprescindible desarrollar estrategias de control de cultivo mucho más sostenibles, y para esto, es necesario tener un buen conocimiento del sistema de defensa natural. Los métodos de control de cultivos alternativos al uso de pesticidas requieren un buen conocimiento de la relación planta-plaga-enemigo natural. En tomate (*Solanum lycopersicum*), uno de los cultivos hortícolas más importantes a nivel mundial, la presencia de tricomas glandulares tipo IV y la producción de acilsacarosas está asociado con altos niveles de resistencia a diversas especies de artrópodos. Algunas especies de pulgones como es el caso de *Macrosiphum euphorbiae* resultan de especial interés ya que no sólo son las causantes de serios problemas en las plantas, sino que, además, actúan como vectores de numerosos virus. El objetivo principal de nuestro estudio es desentrañar los mecanismos que subyacen la defensa contra *Macrosiphum euphorbiae* desde una perspectiva tanto molecular como ecológica. En primer lugar, hemos analizado el comportamiento de *M. euphorbiae* en dos líneas isogénicas de tomate (ABL 10-4 y 'Moneymaker') que difieren en la presencia de tricomas glandulares tipo IV así como en la producción de acilazúcares. Al observar una reducción significativa en el crecimiento de los pulgones que se encontraban en la línea ABL 10-4, estudiamos el papel que podían desempeñar las acilsacarosas. Por otro lado, teniendo en cuenta que las fitohormonas pueden modular la densidad de los tricomas glandulares relacionados con esta defensa, se llevaron a cabo una serie de análisis transcriptómicos con el fin de analizar cambios en las principales rutas de señalización, las dependientes del ácido jasmónico (JA) y del salicílico (SA). Asimismo, advertimos que una herbivoría previa, tanto de insectos masticadores como succionadores, tenía un efecto antagónico en la actividad de los pulgones relacionada con la producción de tricomas glandulares tipo IV. Además, se llevaron a cabo una serie de experimentos que permitieron evaluar el comportamiento de los pulgones de líneas con las rutas de señalización del JA y SA dañadas. En conjunto, todos estos resultados, pusieron de manifiesto la relevancia de la ruta de señalización del JA en la defensa de las plantas de tomate frente a *Macrosiphum euphorbiae*.

CASAS ARROJO, VIRGINIA

Estudio de polisacáridos de algas como posible fuente de compuestos nutraceuticos

Las algas han sido usadas desde la antigüedad debido, a que son fuentes importantes de sustancias bioactivas naturales, atrayendo la atención de investigadores y compañías farmacéuticas por todo el mundo. Uno de los compuestos más estudiados son los polisacáridos. En el presente estudio se investiga los polisacáridos de diferentes algas (*Durvillaea antarctica*, *Euglena gracilis* y *Porphyridium cruentum*) como posible fuente de compuestos nutraceuticos. Se estudia su capacidad antioxidantes, antitumoral, citotóxica e inmunomoduladora de los polisacáridos caracterizándose previamente por FT-IR y CG-MS, se determina el porcentaje de lípidos, carbohidratos, ceniza, humedad y proteínas de la biomasa. De los polisacáridos sulfatados del estudio, se presentará tan sólo los de *D. antarctica* donde se observó una buena capacidad antitumoral principalmente en la línea celular HTC-116 (cáncer de colon humano) con una $IC_{50}=19.99 \mu\text{g mL}^{-1}$. Las líneas celulares ensayadas para ver la actividad antitumoral, mediante el ensayos colorimétrico MTT, fueron leucemia humana (U-937 y HL-60), cáncer de colon (HCT-116), cáncer de mama (MCF-7) y cáncer de pulmón (NCI-H460) y por citometría de flujo con las HL-60. El método usado para determinar la capacidad antioxidante de los polisacáridos fue el método ABTS. En cuanto a la capacidad inmunomoduladora se determina mediante la determinación de IL-6 y TNF- α usando la línea celular de macrófago de ratón (RAW 264.7).

GÓMEZ-PULIDO, LUZ DM

A Physico-Chemical study of triterpenoids present in plant cuticles

The plant cuticle is a lipid membrane that covers the surface of aerial parts of higher plants. Its main functions are to regulate water and gas exchange with the environment, attenuate UV radiation and provide mechanical support. The chief cuticle components are of lipid nature, the cutin matrix and waxes. Cutin is composed by esterified polyhydroxy fatty acids whereas waxes are represented by a complex mixture of very long chain alkanes, alcohols, fatty acids and triterpenoids. In this thesis, we present a new methodology for the structural characterization of amyrins, a class of triterpenoids found inside the tomato fruit cuticle. Two amyrin isomers (α and β), the most abundant in tomato cuticle, have been studied taking into consideration a hydrophobic molecular scenario that mimics the cuticle matrix. DFT calculations have been employed in combination with experimental data (Raman vibrational spectroscopy and X-Ray diffraction). Results have allowed a detailed description of the complex arrangement of these constructions in the cuticle environment.

JIMÉNEZ JIMÉNEZ, LIDIA

Mejora calidad de fruto en especies modelos tomate y fresa: Estudios metabólicos en transgénicas de tomate para el ciclo de Krebs y efectos del cambio climático en variedades comerciales de fresa europeas adaptadas a diferentes ambientes

Durante esta tesis se han llevado a cabo diferentes estudios referentes a la mejora de la calidad de fruto y usando dos especies modelo para fruto, tomate (*Solanum lycopersicum*), y fresa (*Fragaria x ananassa*). La investigación llevada a cabo con tomate estudia la regulación metabólica y funciones de los ácidos orgánicos en la planta de tomate y sus frutos durante la maduración y vida post- cosecha. La modificación usada para el estudio afecta al ciclo de Krebs y produce aumento en los niveles de Fumarato usando una enzima bacteria Maleato Isomerasa. Las plantas resultantes de la transformación presentan fenotipo enano, cambios metabólicos en sus hojas y frutos y diferencias en la vida post-cosecha de los frutos con lo que se infiere que los metabolitos afectados principalmente ácidos orgánicos desempeñan un papel regulatorio en el crecimiento de la planta así como en la degradación de la pared de fruto maduro. Por otro lado, en la línea de investigación con fresa se usan cinco variedades comerciales europeas de este fruto, establecidas y adaptadas a diferentes puntos geográficos de Europa, por lo que se trata de investigación más aplicada. Las variedades se han cultivado durante dos años o cosechas consecutivas en puntos del sur, centro y norte de Europa, sometiendo, por ello, a diferentes condiciones ambientales todas las variedades estudiadas. El análisis de diferentes aspectos del metabolismo de los frutos y de su expresión genética, sumado a que cada uno de los genotipos ha sufrido simultáneamente diversas condiciones climáticas y de cultivo revelará los efectos genotipo/ambiente más influyentes la composición organoléptica y sensorial de la fresa, ya que en este estudio se estudia tanto metabolismo primario como secundario (volátiles) de la fresa y la expresión genética implicada en estos cambios.

RUEDA BLANCO, JAVIER

Suppression of plant defenses by bacterial effectors

During the plant-pathogen interaction, disease or resistance are determined in the plant by a series of molecular events. The plant detects Pathogen-Associated Molecular Patterns (PAMPs), such as flagellin, triggering a defence response called PTI (PAMP-Triggered Immunity). Bacterial pathogens can in turn suppress such defence response through the translocation into the plant cell cytosol of virulence proteins, called effectors, via a Type Three Secretion System (T3SS). In resistant plants, intracellular receptors known as R proteins recognize these effectors, triggering a second line of defence, more specific and intense, called ETI (Effector-Triggered Immunity), which usually leads to programmed cell death known as HR (Hypersensitive Response). Bacterial infection can also trigger a systemic plant defense response that protects the plant against additional pathogen attacks known as SAR (Systemic Acquired Resistance). *Pseudomonas syringae* is a phytopathogenic bacterium whose virulence depends on a T3SS and its effector repertoire. We are interested in the molecular and cellular mechanisms involved in effector-mediated defense evasion by *P. syringae*, in particular 3 effectors and their putative targets to suppress such defenses. i HopZ1a, an unusual

effector which is able to suppress in *Arabidopsis* both local (PTI and ETI), and systemic (SAR) defences. ii HopAF1, a widely distributed effector in *P. syringae* which suppress local defences (PTI and ETI). iii HopZ3, an effector that suppress the ETI triggered by several effectors from the same effector repertoire.

NIETO QUERO, ANDREA

Efectos del estrés en la neurogénesis hipocampal. Implicación del receptor LPA₁ para el ácido lisofosfatídico y la microglía

El estrés puede tener un impacto en la salud mental, induciendo numerosas psicopatologías, particularmente depresión, un trastorno cuya incidencia está creciendo de forma alarmante y que conlleva numerosos procesos patológicos asociados. Sin embargo, los mecanismos responsables de los trastornos del estado de ánimo resultan pobremente conocidos. A nivel neurobiológico, es un hecho constatado que el estrés reduce la neurogénesis hipocampal lo que aumenta la vulnerabilidad a los efectos negativos del estrés. Numerosos procesos pueden estar implicados en la reducción neurogénica inducida por estrés, entre ellos la neuroinflamación. La microglía puede jugar un papel clave en este proceso. Una respuesta microglial alterada se ha vinculado a la etiopatogénesis de la depresión. Por otro lado, otra de las vías que recientemente están recibiendo interés por su capacidad para modular la neurogénesis y por su posible relación con la depresión es la vía LPA-LPA₁ (LPA, ácido lisofosfatídico). En este sentido, se ha observado que su bloqueo aumenta la vulnerabilidad a los efectos negativos del estrés, exacerba la patología hipocampal, induce una mayor reducción de la tasa neurogénica producida por estrés y se ha relacionado recientemente con la etiología de la depresión. Además, se ha comprobado que los receptores LPA₁ se expresan 'in vivo' en microglía, por lo que podrían modular su respuesta y así, constituir un mecanismo a través del cual la vía LPA-LPA₁ modula la neurogénesis hipocampal. Sin embargo, en la actualidad no se conoce la posible relación entre microglía, neurogénesis y LPA en la depresión inducida por estrés. Es por ello que concretamos como hipótesis que, el mediador lipídico, LPA, estaría asociado a los cambios en la estructura y función de la microglía inducidos por estrés, lo que a su vez se relaciona con cambios neurogénicos y con aparición de sintomatología depresiva. Por todo ello, para abordar este proyecto de tesis doctoral se han planteado dos objetivos principales que están siendo desarrollados en modelos animales: 1) estudiar la posible relación entre LPA, respuesta microglial y reducción neurogénica en la depresión inducida por estrés. 2) Estudiar el bloqueo del sistema LPA endógeno en los efectos del estrés en la respuesta de la microglía y la neurogénesis hipocampal y su relación con la inducción de sintomatología depresiva. Estos objetivos están siendo abordados en dos grandes bloques experimentales: 1^{er}) bloque en el que se emplea como tratamiento ambiental el uso de diferentes estresores y se está estudiando la respuesta de la microglía, la neurogénesis y el sistema LPA endógeno y la relación con la aparición de sintomatología depresiva; 2º) bloque que ha sido diseñado para estudiar el efecto del bloqueo del sistema LPA endógeno en impacto del estrés sobre el estado de la microglía, la neurogénesis hipocampal y su relación con la aparición de sintomatología depresiva.

LÓPEZ PAGÁN, NIEVES

Expresión heterogénea en genes relevantes para la virulencia de *Pseudomonas syringae*

La heterogeneidad o variación fenotípica ha sido descrita en poblaciones clonales microbianas desde hace décadas. Bajo el control de ciertos circuitos regulatorios, la heterogeneidad en la expresión génica puede dar lugar a un perfil de expresión bimodal en ambientes homogéneos, proceso conocido como biestabilidad. La relevancia de este proceso se ha demostrado en *Salmonella entérica* y en otros patógenos humanos durante el establecimiento de la resistencia a antibióticos, y se ha observado la implicación de este proceso en la expresión de genes de virulencia. *Pseudomonas syringae* es una bacteria fitopatógena de gran importancia económica que requiere del Sistema de Secreción Tipo III (T3SS) para suprimir las respuestas de defensa de la planta. Nuestro equipo ha descrito que genes de diferentes elementos del T3SS muestran biestabilidad en su expresión en condiciones de inducción en el laboratorio y que dicha expresión es marcadamente heterogénea durante la colonización de la planta. Por otro lado, el flagelo es otro elemento importante tanto en el estilo de vida de *P. syringae* como en su interacción con el huésped, donde dispara inmunidad basal. Asimismo, el flagelo presenta expresión heterogénea, tanto en cultivos, como durante la proliferación en los espacios intercelulares de la hoja huésped. De esta manera, esta tesis pretende demostrar cómo la expresión heterogénea de ciertos genes en *Pseudomonas syringae* es beneficiosa tanto para la interacción con la planta como para su crecimiento a nivel poblacional.

2. Posters

ALMAHROUQUI, HAFID

The effect of fertilizer concentration on the productivity and biochemical composition of *Spirulina (Arthrospira platensis)* using urea as a nitrogen source

A cost effective fertilizer based on urea as nitrogen source and low concentration of fertilizer was formulated for the mass production of *Spirulina*. Growth and properties of two different *Spirulina* strains were compared using different percentage (100, 50 and 25%) of the standard Zarrouk medium and the modified medium with urea. The Malaysian strain (MS) showed the highest growth biomass in 50% standard medium ($1.39 \text{ g L}^{-1} \pm 0.07$) and the lowest one was with the strain from Spain (SS) in 25% medium with urea ($0.95 \text{ g L}^{-1} \pm 0.01$). The highest protein content ($60.11\% \pm 0.85$) was found in SS in 100% medium with urea, while the lowest one ($33.82\% \pm 0.32$) was in 25% medium with urea with MS. MS had the highest carbohydrate content ($19.37\% \pm 0.93$) in 25% medium with urea but SS had the lowest content ($4.71\% \pm 0.56$) in 100% medium with urea. The highest lipids content ($13.5\% \pm 0.50$) was observed for SS in 100% medium with urea and the lowest content ($7.83\% \pm 0.29$) was found for MS in 25% medium with

urea. The chlorophyll a content ($13.60 \text{ mg g}^{-1} \pm 0.08$) in SS was the highest in 50% standard medium, while the lowest content ($5.05 \text{ mg g}^{-1} \pm 1.01$) was found in MS in 25% fertilizer with urea. Both strains were able to grow with low fertilizer concentrations without losing their properties, which suggested that the cost of fertilizer can be significantly up to 75%.

CERVANTES CÁRDENAS, LUCÍA

Health-promoting potential of different strawberry varieties for breeding programs

Strawberry fruits are characterized for their high content in antioxidant compounds with health properties, which are involved in the prevention of some diseases such as cancer and cardiovascular diseases. Among these health-promoting compounds, with diverse chemical nature, the polyphenol group stands out. In Spain, strawberries are mainly cropped in the Huelva region, where their cultivation has evolved from a monovarietal or few varieties scenario to a wide range of varieties in recent years. This multivarietal spectrum has increased the diversity of fruit quality attributes since cultivars differ in their fruit characteristics; such as, the amount and type of antioxidants. These differences among varieties will probably involve differences in their bioactive potential. Besides, it is known that the digestion process interferes in the release and/or transformation of these compounds determining their potential absorption (*i.e.* bioavailability) and bioactivity. Thus, in this work we assessed fruit antioxidant capacity of five strawberry varieties before and after *in vitro* digestion. We found no correlation between the antioxidant capacity in the non-digested and bioavailable extracts (*i.e.* absorbed for the organism). This result suggests that antioxidants quantification on non-digested extracts may not reflect properly the health benefits attributed to strawberries, highlighting the relevance of the digestion process for determining the health potential of strawberry and for a proper varietal selection in breeding programs aiming to obtain varieties with enhanced wholesome characteristics.

DEL ESPINO PÉREZ, ÁNGEL

R gene regulation mediated by miRNA/phasiRNA during plant defense response against *P. syringae*

In plants, two main types of noncoding small RNA molecules have been found: microRNAs (miRNAs) and small interfering RNAs (siRNAs), differing these in their biogenesis and mode of action, but sharing similar sizes (20-24 nt). In plants, their mature forms are products of the activity of DCL proteins and can act as negative regulators of gene expression. In recent years, the role of miRNAs in regulation of gene expression in plant responses against bacterial pathogens is becoming clearer. Comparisons carried out in our lab between expression profiles of different *Arabidopsis thaliana* mutants affected in gene silencing, and plants challenged with *Pseudomonas syringae* pathovar tomato DC3000, led us to identify a set of uncharacterized R genes,

25% medium with urea. The chlorophyll a content ($13.60 \text{ mg g}^{-1} \pm 0.08$) in SS was the highest in 50% standard medium, while the lowest content ($5.05 \text{ mg g}^{-1} \pm 1.01$) was found in MS in 25% fertilizer with urea. Both strains were able to grow with low fertilizer concentrations without losing their properties, which suggested that the cost of fertilizer can be significantly up to 75%.

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belonging to the TIR-NBS-LRR gene family, as differentially expressed in these conditions. By bioinformatics tools, we found a miRNA* of 22 nt putatively responsible for down-regulating expression of these R genes. We have also found that the corresponding pri-miRNA is down-regulated after PAMP-perception. We demonstrate that plants with altered levels of this miRNA* (knockdown lines or overexpression lines) exhibit altered PTI-associated phenotypes, suggesting a role for this miRNA* in this defence response against bacteria. We have characterized the expression pattern of both pri-miRNA and its best target R genes. Finally, we identify phasiRNAs that arise from the transcript of this R gen in a miRNA*-RDR6-DCL4-dependent manner.

JIMÉNEZ ENJUTO, EVA M

Strategies for enhancing the osteogenic potential of BMP-9 in bone tissue engineering

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor beta (TGF- β) superfamily and are mostly known for being highly pleiotropic growth factors with a great osteoinductive potential. Two of these cytokines (BMP-2 and BMP7) have approval from the FDA and the EMA to be used in reparative orthopaedic surgery, although the clinical doses that are applied are far above the physiological levels, what can lead to important adverse side-effects. BMP-9 is a less characterized member of this group, but has been shown to possess a higher osteogenic activity than the above mentioned BMPs. The main objective of this project is the search for molecular strategies based on the use of BMP-9 to increase or accelerate osteogenesis in bone tissue engineering. Therefore, BMP-9 will be combined with molecules that have demonstrated to have a synergistic or additive effect with other BMPs (EGF, PDGF, FGF-2 and IGF- 1) to enhance bone formation. These combinations will be tested in vitro on osteoprogenitor cells to determine their ability to induce cell differentiation and/or proliferation and, in a later stage, the most promising combinations will be tested in both ectopic and orthotopic in vivo osteogenesis models in rats, performing histological and histomorphometrical studies, as well as qRT-PCR and biochemical characterization. A second strategy that will be explored is that of combining the use of BMP-2 and -9. The results obtained so far corroborate the high potential of BMP-9 for inducing osteoprogenitor cell differentiation in vitro, although its ability to induce ectopic osteogenesis is somewhat limited. In vitro, the combinations of BMP-9 with other cytokines show different degrees of interaction, which will have to be further explored in vivo.

KAZANTSEVA, LILIYA

New uses for “old” drugs: Paclitaxel and its incorporation inside a thermo-sensitive polymer for osteosarcoma treatment

Osteosarcoma is one of the most common malignant tumours of bone, primarily affecting children and adolescents. The implementation of chemotherapy in 1970, combined with the advancements in surgery and radiotherapy, has substantially improved cancer therapy in patients with localized disease. However, it is almost inefficient for patients with metastasis, and the survival rate has remained unchanged over the last decades. Drug repositioning, which is based on “recycling” an already known drug from one disease to another, is a rapid and cost-efficient approach to the development of new compounds. Paclitaxel (PTX), a microtubule stabilizing agent responsible of M-phase cell cycle arrest, is commonly used for the treatment of breast and lung cancer, but there are few studies on its efficiency against osteosarcoma. On other side, nanotechnology is nowadays getting attention in cancer treatment, as nanoparticles are biocompatible and susceptible of different modifications that make them specific for targeting tumours, and for releasing its cargo under specific stimuli, like changes in temperature or pH. Apart from nanoparticles, there is a growing interest in mesenchymal stem cells (MSC) as cellular vehicles, because they are hypoimmunogenic and have the ability of tumour homing. For this reason, combining nanotechnology and cell therapy gives chance to generating a “nano-engineered” MSC that would be able to both actively target the tumour site and protect the PTX loaded nanoparticle against macrophage clearance. Thus, the aim of our work is to evaluate the efficiency of PTX as a chemotherapeutic drug alone and incorporated inside a thermo-sensitive polymer, Poly(N-isopropylacrilamide) (PNIPAM) for osteosarcoma treatment. To test different concentrations of the drug, traditional 2D and 3D spheroids cell culture system were evaluated. The results showed PNIPAM to be biocompatible, as no cytotoxic effect was detected in either 2D or 3D systems. PTX, alone and loaded inside PNIPAM nanoparticles, was able to reduce the viability of different osteosarcoma cell lines in 2D, with each cell line presenting different sensitivity to the treatment. In a microtissue model, PTX loaded nanoparticles presented better outcome as compared to PTX alone. However, less sensitivity to both types of PTX was detected when cells were grown in 3D within a supportive collagen matrix. According to our findings, PTX loaded PNIPAM might be a good candidate for osteosarcoma treatment and its incorporation inside MSC is a promising system, although additional studies are still required to implement the technology.

MORENO ORTEGA, GUILLERMO

Mild water stress-induced priming enhance tolerance to *Rosellinia necatrix* in susceptible avocado rootstocks

White root rot (WRR) disease caused by *Rosellinia necatrix* is one of the most important threats affecting avocado orchards in temperate regions. The eradication of WRR is a difficult task and environmentally friendly control methods are needed to lessen its impact. Priming plants with a stressor (biotic or abiotic) can be a strategy to enhance plant defense/tolerance against future stress episodes but, despite the known underlying common mechanisms, few studies use abiotic-priming for improving tolerance to forthcoming biotic-stress and *vice versa* (*‘cross-factor priming’*). To assess whether *cross-factor priming* can be a potential method for enhancing avocado

tolerance to WRR disease, 'Dusa' avocado rootstocks, susceptible to *R. necatrix*, were subjected to two levels of water stress (mild-WS and severe-WS) and, after drought-recovery, inoculated with *R. necatrix*. Physiological response and expression of plant defense related genes after drought-priming as well as the disease progression were evaluated. Water-stressed avocado plants showed lower water potential and stomatal limitations of photosynthesis compared to control plants. In addition, NPQ and q_N values increased, indicating the activation of energy dissipating mechanisms closely related to the relief of oxidative stress. This response was proportional to the severity of the water stress and was accompanied by the deregulation of pathogen defense-related genes in the roots. After re-watering, leaf photosynthesis and plant water status recovered rapidly in both treatments, but roots of mild-WS primed plants showed a higher number of overexpressed genes related with plant defense than severe-WS primed plants. Disease progression after inoculating primed plants with *R. necatrix* was significantly delayed in mild-WS primed plants. These findings demonstrate that mild-WS can induce a primed state in the WRR susceptible avocado rootstock 'Dusa' and reveal that '*cross-factor priming*' with water stress (abiotic stressor) is effective for increasing avocado tolerance against *R. necatrix* (biotic stressor), underpinning that plant responses against biotic and abiotic stress rely on common mechanisms. Potential applications of these results may involve an enhancement of WRR tolerance of current avocado groves and optimization of water use via low frequency deficit irrigation strategies.

POMARY PRECIOUS, KWADZO

Application of 1H NMR Metabolomics for the diagnosis of Neurological diseases

The definitive causes of most neurological diseases are currently generally elusive to researchers. However, the pathological changes that occur in the nervous system begin early (in some instances, several years prior to the observation of clinical symptoms), leaving behind measurable pathological markers. These biomarkers can be detected in the cerebrospinal fluid which is the best proxy for central nervous system biopsy. Since there are several molecules in the CNS, each involved in several overlapping metabolic pathways, there is the need for a holistic approach rather than targeting a singular causative agent. In this work we present the coupling of nuclear magnetic resonance spectroscopy with computational modalities in the stratification of neurological diseases based on the metabolic fingerprint of patients. CSF samples were collected from patients with multiple sclerosis, clinically isolated syndrome, benign intracranial hypertension and migraine. The samples were stored at -80°C prior to acquisition of NMR spectra based on established protocols and standard operating procedures (SOPs). The NMR spectra were pre-processed by Fourier transformation, phase and baseline correction and then converted to a text file. Subsequently automated peak picking and peak grouping were performed with the R statistical programming language to generate an $n \times m$ data matrix. Taking advantage of the intrinsic properties of NMR spectrum, the data matrix was normalised with the internal standards in each sample to eliminate variability due to varying linewidth. Feature selection was performed using both multivariate and univariate statistical analysis. The important variables that contributed

to group separation were identified using statistical correlation and ratio analysis in conjunction with metabolite databases. These metabolites were then quantified, statistically compared and later used in the construction of correlation metabolic networks and also the identification of the metabolic pathways affected. It was possible to differentiate patient groups based on the metabolic fingerprint even in the absence of metabolite identification. Also, metabolites that differ among the groups represent different metabolic pathways that have been altered especially those associated with energy production such as citrate and pyruvate. The combination of SOPs with appropriate computational algorithms can improve early diagnosis and monitoring of neurological diseases using ^1H NMR with or without metabolite identification. Metabolic pathways involved in energy production are affected in neurologic diseases.

ROMERO-RODRÍGUEZ, BEATRIZ

Transcriptomic analysis of the interaction geminivirus-tomato

Geminiviridae family is one of the main families of plant pathogenic viruses with large relevance as they cause great losses worldwide in commercial crops and crops destined to food production. Geminiviruses present a little single-stranded DNA genome and a capsid composed of two twin icosahedral parts. *Tomato Yellow Leaf Curl Virus* (TYLCV) belongs to the *Begomovirus* genus and is transmitted by the whitefly *Bemisia tabaci*. With only 6 viral proteins, this geminivirus must create a proper environment for viral replication, transcription and propagation. Behind the apparent simplicity of geminiviruses lies a complex network of molecular interactions with their host and even their natural vector, which induces a wide variety of transcriptional, post-transcriptional and chromatinic changes in both the plant and the geminivirus. In order to study these changes and decipher the effects of the transmission vector on the infection, we carried out a global approximation of the TYLCV-tomato interaction to generate integrated single-base resolution maps by NGS (next-generation sequencing) of the transcriptome, smallRNAome and methylome of the pathogen and the host. Tomato plants (MoneyMaker) were infected with TYLCV under controlled conditions of light and temperature using *Agrobacterium tumefaciens* or its natural vector. Apical tissue from these plants was collected at different time points (2, 7, 14 and 21 days after inoculation), and three biological replicas were generated for each treatment and time. Total RNA and DNA was extracted and analysed by RNA-Seq, smallRNA-Seq and Bisulfite-Seq. The transcriptome of the tomato-TYLCV interaction will be presented and discussed.

VEGA, JULIA

Cyanobacteria as potential sources of UV absorbing compounds: Cosmeceutical applications

In the last decades, there is a great concern about the negative effects of the ultraviolet (UV) radiation on the human health. The intensive use of synthetic sunscreens to protect our skin from this type of radiation can provoke a negative impact on the aquatic environment (i.e. bleaching of coral reefs, hormone disorders in mammals). Finding ecological alternatives without negative environmental impacts is on demand. In this way, Cyanobacteria, have acquired a great biotechnological interest due to their capacity to synthesize different high-value photoprotective compounds, such as pigments, polyphenols or mycosporine like aminoacids (MAAs). This work is focused on the research of three molecules with UV-absorbing capacity in Cyanobacteria: mycosporine like aminoacids (MAAs), polyphenols and scytonemin. Extractions from algal thalli were conducted in five species (*Anabaena* sp., *Calothrix* sp., *Lyngbya* sp., *Nostoc* sp. and *Scytonema* sp.). Five different natural solvents with varying polarity were used. The content of the three above mentioned molecules in the extracts and the antioxidant activity were determined. Considering the MAAs, only *Lyngbya* sp. and *Scytonema* sp. presented a relevant content (approx. 2.5 and 0.8 mg g⁻¹ DW, respectively). The most predominant MAAs was asterina-330 (λ_{max} =330 nm) in *Lyngbya* sp. and mycosporine-glycine (λ_{max} =310 nm) in *Scytonema* sp., and the best solvent was ethanol 80% in all cases. The highest content of polyphenols was observed also in *Scytonema* sp. (18.2 mg g⁻¹ DW), followed by *Nostoc* sp. (10.3 mg g⁻¹ DW), being water the best solvent in most of the cases. The highest content of scytonemin (λ_{max} =384 nm) was also found in *Scytonema* sp. (2.2 mg g⁻¹ DW), followed by *Lyngbya* sp. and *Nostoc* sp. (1.2 and 0.3 mg g⁻¹ DW), using ethanol as solvent. The highest antioxidant activity was also found in *Scytonema* sp. (36.3 $\mu\text{mol TE g}^{-1}$ DW), followed by *Anabaena* sp. and *Nostoc* sp. (28.5 and 20.6 $\mu\text{mol TE g}^{-1}$ DW, respectively). In this case, a lot of variability among solvents was observed. We point out that *Scytonema* sp. and *Lyngbya* sp. can be potential sources of antioxidant and photoprotective compounds to be used in cosmetic products. Other species, like *Nostoc* sp. or *Anabaena* sp., are also good options due to the possibility to increasing their biomolecules content by growing under stress conditions i.e. increased UV radiation under high N supply as it has been reported with MAAs in red algae.

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