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PROGRAMA CIENTÍFICO

Jueves 5 de septiembre 2024

8:30 Recogida documentación y acreditación

9:00 Inauguración

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Moderador: Dr Oussama Ahrazem (UCLM)

- **09:30 "The HMG-family protein HmbC is involved in the regulation of carotenoid genes in Fusarium fujikuroi".** Mª Carmen Limón. Department of Genetics, Faculty of Biology, University of Sevilla
- **09:50 "Identificación de carotenoides E/Z naturales con espectros UV/VIS atípicos".** Enrique Murillo Departamento de Bioquímica, Facultad de Ciencias Naturales Exactas y Tecnología, Universidad de Panamá, Panamá.
- **10:10 "Awakening of the native PHYTOENE SYNTHASE 1 promoter by correcting near-miss cis-acting elements activates carotenoid biosynthetic pathway in embryogenic rice callus".** Guillermo Sobrino-Mengual. University of Lleida-Agrotecnio CERCA Center, Lleida, Spain.
- **10:30 "Strategies and tools offering new opportunities for carotenoid biofortification in triticeae species".** María Dolores Requena Ramírez. IG-CSIC.
- **10:45 "Identification of proteins that bind Arabidopsis phytoene synthase to modulate its function".** Juan Navarro-Carcelen. Instituto de Biología Molecular y Celular Vegetal (IBMCP), CSIC-Universitat Politècnica de València.
- **11:00 "Unraveling Carotenoid Biosynthesis in Carrots: The Roles of DcHY5 and DcPIF3".** Claudia Stange. Universidad de Chile.

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Moderador: Dr Lorenzo zacarías (IATA-CSIC)

- **11:45 "Strigolactones: Essential Apocarotenoids for plant life".** Juan Antonio López Ráez. Estación Experimental del Zaidín (EEZ-CSIC), Granada, Spain.
- **12:05 "Viral vectors as a tool in the characterization of CCDs".** Lucía Inmaculada Morote Rodríguez. Instituto Botánico-UCLM. Albacete.
- **12:25 "Pearl millet genomes reveal a CARLACTONIC ACID METHYL TRANSFERASE as key determinant of strigolactone pattern and Striga susceptibility".** Derry Alvarez De Jesus. King Abdullah University of Science and Technology (KAUST), 23955-6900, Thuwal, Kingdom of Saudi Arabia.
- **12:45 "Strigolactones as a signal of "cry for help" in water-saving species: Conclusions drawn from a field experiment".** Lorena Ruiz de Larrinaga Vicente. Department of Plant Biology and Ecology, University of Basque Country (UPV/EHU), B/Sarriena s/n, 48940, Leioa, Bizkaia, Spain.
- **13:00 "Identification and characterization of putative zaxinone synthase enzymes in tomato".** Authors Eleonora Fabene. Agenzia Nazionale per le Nuove Tecnologie, l'Energia e lo Sviluppo Economico Sostenibile (ENEA), Roma, Italy.

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Sesión III. Carotenoides en la nutrición y en la salud

Moderadora: Dra Mª Luisa Bonet (UIB)

- **15:15 "Carotenoids with and without provitamin A activity in the prevention and reduction of risk in deficiency and chronic disease in humans".** Elena Rodríguez Rodríguez. Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC) y Facultad de Farmacia, Universidad Complutense de Madrid (UCM).
- **15:35 "Stereocontrolled synthesis of staphyloxanthin an biogenetic precursors".** Ángel Rodríguez de Lera. CINBIO, Departament of Organic Chemistry, University of Vigo.
- **15:55 "Phytoene and phytoene-rich microalgae extracts extend lifespan in C. elegans and protect against amyloid-β toxicity in an Alzheimer's disease model".** Morón-Ortiz, Ángeles. Food Colour and Quality Laboratory, Faculty of Pharmacy, Universidad de Sevilla, Spain.
- **16:15 "Differential Impact on Hepatic Metabolism of Retinoic Acid Isomers in Preclinical Models".** Sebastià Galmés. Laboratory of Molecular

Biology, Nutrition and Biotechnology, University of the Balearic Islands (UIB).

16:30 Café-Sesión pósters

- **17:10 "Carotenoid enrichment of fatty and dairy foods: consumer assessment and colorimetric stability study".** Ruperto Bermejo-Román, Mª Carmen Murillo, Jaen University.
- **17:30 "Early life programming of adipose tissue remodeling and browning capacity by Vitamin A as a potential anti-obesity strategy".** Joan Ribot, Laboratory of Molecular Biology, Nutrition and Biotechnology, University of the Balearic Islands (UIB).
- **17:50"Characteristics of red-fleshed sweet oranges and their impact on gut microbiota and health".** Jaime Zacarías-García. Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Valencia.
- **18:05 "Role of Saffron-derived crocetin into process of adipogenesis activated under diabetogenic conditions by subcutaneous adipose tissue".** Silvia Lloren Folgado. Department of Medical Sciences, Faculty of Medicine of Albacete, Instituto de Biomedicina de la UCLM.
- **18:20 "Halophilic archaea as a source of carotenoids with antioxidant and anti-inflammatory properties"** Patricia Gómez-Villegas. Universidad de Sevilla.

19:00 Visita Jardín Botánico Castilla-la Mancha

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Viernes 6 de septiembre 2024

Sesión IV. Biotecnología de carotenoides

Moderador: Dr. Javier Ávalos (US)

- **09:00 "Novel carotenoid biotechnology strategies".** Manuel Rodriguez Concepción. Institute for Plant Molecular and Cell Biology (IBMCP), CSIC-UPV. Valencia.
- **09:20 "From carotenoids to apocarotenoids: CCD engineering to produce crocins, picrocrocin, and safranal in tomato fruit".** Antonio Granell. Instituto de Biología Molecular y Celular de Plantas (IBMCP).

- **9:40 “Simultaneous accumulation of astaxanthin and lutein in continuous cultures of the microalga Chromochloris zofingiensis".** Ramos-González, Marcos. Institute of Plant Biochemistry and Photosynthesis (CSIC-US).
- **10:00 "Ecophysiology and in situ seasonal study of fucoxanthin content in the invasive exotic algae Rugulopteryx okamurae".** Nathalie Korbee. IBYDA-Universidad de Málaga.
- **10:20 "The flesh color of ripe melon (Cucumis melo) fruit from green and white cultivars is determined by a protein that prevents chloroplast differentiation".** Laura Valverde Carvajal. Instituto de Biología Molecular y Celular de Plantas (IBMCP), Valencia; Institute of Agrifood Research and Technology (IRTA), Bellaterra, Barcelona.
- **10:35 "GWAS and BSA-seq approaches reveal several genomic regions and candidate genes regulating carotenoid content in Cucurbita pepo fruit".** Alba López. Research Center CIAMBITAL, University of Almería.
- **10:50 Investigation of novel enzymes for Apocarotenoid Biosynthesis in “Azafran de bolita” (Ditaxis heterantha Zucc.). Matteo Nava. Department of Agricultural and Forestry Sciences (DAFNE), University of Tuscia, Italy.**

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Moderadora: Dra Nathalie Korbee (IBYDA-UMA)

- **11:35 "The role of BIOVEGEN as a public-private partnership and as an R&D&i services provider in plant production".** David Lapuente. BIOVEGEN.
- **11:55 "Innovation and technology: key elements for a sustainable agricultura".** Soledad de Juan Arechederra. Antama Foundation.
- **12:15 “Monitoring carotenoid content transitions by using RGB digital images as a transversal tool: from forest vulnerability to nutritional content".** Raquel Esteban. Department of Plant Biology and Ecology, University of Basque Country (UPV/EHU).
- **12:35 "Exosome-like nanoparticles from *Arbutus unedo L* mitigate LPS-induced inflammation via JAK [1]STAT inactivation".** Cristian Martínez Fajardo. Instituto Botánico-UCLM. Albacete.

- **12:50 "Nanotechnology behind scenes: New approaches of tomafran-nanodevices".** Enrique Niza. Naplatec.
- **13:05 "SDGs: the science perspective and its practical application in an academic context".** Carmen Belén Martínez Escobar. Instituto Botánico-UCLM.

13:20 Clausura

14:00 Pausa-comida

16:00-17:00 Sesión pósters y entrega Premios

19:00-21:00 Visita ciudad y recinto ferial. Encuentro en la Punta del Parque: Plaza de Gabriel Lodares

COMUNICACIONES ORALES

Pearl millet genomes reveal a CARLACTONIC ACID METHYL TRANSFERASE as key determinant of strigolactone pattern and *Striga* susceptibility

Hendrik NJ Kuijer[1,2,*], Jian You Wang[1,2,*], Salim Bougouffa[3,*], Michael Abrouk[2,4,5], Muhammad Jamil[1,2], Roberto Incitti[3], Intikhab Alam[3], Aparna Balakrishna[1,2], Derry Alvarez*[1,2], Cristina Votta[5,6], Guan-Ting Erica Chen[1,2,4], Claudio Martínez[6], Andrea Zuccolo[2,4,7], Lamis Berqdar[1,2], Salim Sioud[8,7] , Valentina Fiorilli[5], Angel R de Lera[6], Luisa Lanfranco[5], Takashi Gojobori[3], Rod A Wing[2,4,8], Simon G Krattinger[2,4], Xin Gao[3], and Salim Al-Babili[1,2,4]

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Carotenoids are vital isoprenoid photosynthetic pigments with diverse roles in photosynthesis and photoprotection. In plants, they play an important role as precursors of hormones and signaling molecules, such as Abscisic acid (ABA), strigolactones (SLs), zaxinone, anchorene and β-cyclocitral. Strigolactones (SLs) are a recently discovered hormone that inhibits shoot branching and is released by plant roots into the rhizosphere to attract symbiotic mycorrhizal fungi (AM), particularly under phosphate (Pi) starvation. SLs control secondary stem growth, plant height, and leaf shape. Additionally, SLs mediate physiological processes that include senescence, stomatal closure, and biotic/abiotic stress responses and determine root architecture. However, SLs also act as germination stimulants for root-parasitic weeds, such as Orobanche and Striga, posing severe agricultural problems worldwide.

Pearl millet is the sixth most important cereal crop after rice, wheat, maize, barley and sorghum. The yield of pearl millet, primarily cultivated in subtropical regions, including Sub-Saharan Africa and India, is significantly affected by Striga hermonthica, considered one of the seven major threats to global food security. The wild pearl millet line 29Aw (Aw) from Niger exhibits resistance to Striga through both pre- and post-attachment mechanisms. In contrast, SOSAT- C88 P10 (P10) is a susceptible millet line derived from the SOSAT variety, which originates from a cross between the landraces Sauna and Sanio and has high yields in West Africa. To investigate the underlying resistance mechanisms, we first verified the contrasting phenotypes of the two lines under greenhouse conditions. Root exudates from P10 induced higher Striga germination than those from Aw, suggesting that P10 susceptibility may be related to released SLs.

Here, we identified four SLs present in the Striga-susceptible P10, but absent in the resistant line Aw. The Aw genome lacks a 0.7Mbp section containing two putative CARLACTONIC ACID METHYLTRANFERASE1 (CLAMT1) genes. Upon transient expression, P10CLAMT1b formed methyl carlactonoate (MeCLA), an intermediate in SL biosynthesis. Feeding Aw with MeCLA resulted in the production of two P10-specific SLs. Screening a diverse pearl millet panel confirmed the pivotal role of the CLAMT1 section for SL diversity and Striga susceptibility. Our results uncover a new SL and the cause for Striga-susceptibility in pearl millet.

Keywords*: Carotenoids, Strigolactones, Pearl millet, Striga*.

Carotenoid enrichment of fatty and dairy foods: consumer assessment and colorimetric stability study

Mª Carmen Murillo[1P], Mª Carmen Hurtado[1], Antonio Estrella de Castro[2], Amparo Navarro[1], Mª Paz Fernández-Liencres[1], Tomás Peña[1] and Ruperto Bermejo-Román*[1]

[1] Department of Physical and Analytical Chemistry, Jaen University, 23700 Linares, Spain; [2] DSM Vitatene SAU-Nutritional Products, León, España.

Nowadays, interest in carotenoids has grown based on studies suggesting their protective antioxidant effect. Consequently, drugs which containing synthetic antioxidant compounds, have been used for treatment and prevention of diseases associated with oxidative stress and antioxidant deficiencies but unfortunately some of them have shown toxic properties in animal models [1]. Currently, the research is oriented towards the development and use of safer and more economical molecules of natural origin. On the other hand, the recommended doses of these antioxidants to prevent certain diseases are not easily covered by dietary modifications. Subsequently, an alternative way could be supplementing our diet using extracts enriched with them. In this context, there is a growing interest for developing new functional foods which are usually traditional foods enriched in one or several components that promote beneficial effects on human health [2].

In light of the above, our research group has developed a methodology to fortify different types of food with β-carotene from Blakeslea trispora fungi. The present work shows the carotene enrichment of fatty foods (butter and margarine) and dairy products (condensed milk and cheese spreads). These foods have been prepared and studied using colorimetry to evaluate their stability. In addition, food samples prepared with different levels of carotene fortification have been evaluated by a panel of tasters, who have shown their acceptability based on a visual assessment of the samples (Figure 1).

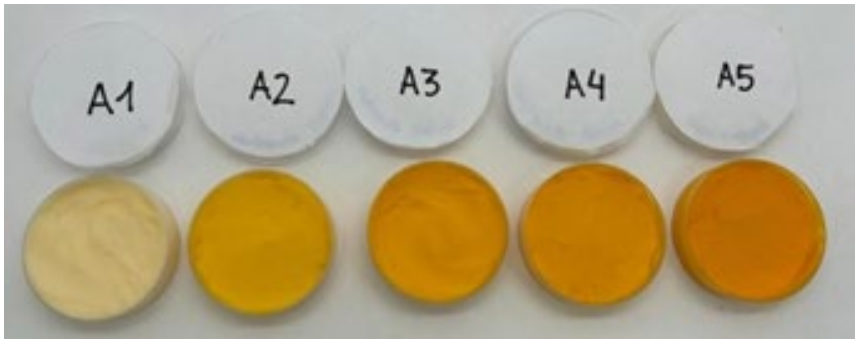

Figure 1.-Butter carotene fortification. A_1:0; A_2:24; A_3:48; A_4:72 and A_5:120 mg carotene / Kg of butter.

The outcomes obtained indicate that, in general terms, the prepared samples have a good color stability over the tested interval of 50 days. Thus, during the first 20 days the changes in color are practically imperceptible to the human eye. With regard to the consumer's perception, the best rated samples were those prepared with the lowest concentration of beta-carotene tested and which therefore had a color closer to the commercial samples. On balance, these enriched foods could represent an engaging way to incorporate carotenoids in the appropriate doses in human organism.

References

Meléndez-Martínez, A.J., Stinco, C.M. and Mapelli-Brahm, P., Skin carotenoids in public health and nutricosmetics: the emerging roles and applications. Nutrients, 2019, 11,1093, https://doi.org/10.3390/nu11051093..

Murillo, M., Rodrigues, N., Bermejo, R., Veloso, A.C., Pereira, J.A. and Peres, A., An electronic tongue as a tool for assessing the impact of carotenoids' fortification...., European Food Research and Technology, 2022, 248:1287-1298.

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Keywords: *Carotenoids, functional foods, stability*

Stereocontrolled synthesis of staphyloxanthin and biogenetic precursors

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CINBIO, Departament of Organic Chemistry, University of Vigo, Campus As Lagoas-Marcosende, 36310, Vigo, Spain

Human infections by Staphylococcus aureus are a concern in present times, since these bacteria have been included within the Priority 2 (HIGH) group in the WHO Bacterial Priority Pathogen List. More than 90% of all S. aureus clinical isolates are able to produce the golden pigment staphyloxanthin (1), an unphosphorylated saccharolipid connected to C30 apocarotenoid 4,4'-diaponeurosporenoic acid (3). The pigment, first reported in 1981,1 and more recently characterized,2 has been shown to condense in the functional membrane microdomain of S. aureus, providing the bacteria with membrane integrity, and with antioxidant activity, thus preventing its killing by neutrophiles. The ability of S. aureus to quench reactive oxygen species (ROS) acts as a virulence factor. We have developed the Pd-catalyzed Csp2-Csp2 cross-coupling reactions of bis- functionalized non-symmetrical 1,12-dodeca-1,3,5,7,9,11-hexaenes and the corresponding complementary alkenyl iodides for the synthesis of acyclic C30- apocarotenoids. Bidirectional Hiyama-Denmark cross-coupling reactions of the bis- functionalized hexaenes faced limitations related to the reactivity of the internal silanes. On the contrary, mixed hexaenylstannanes/boronates afforded the corresponding C30- apocarotenoids by stepwise Stille and Suzuki cross coupling reaction sequences. The protocol was extended to a series of non-symmetrical C30-apocarotenoids including staphyloxanthin (1) and related pigments (Figure 1), in efficient sequences that preserved the stereochemical information of the corresponding oligoene partners.

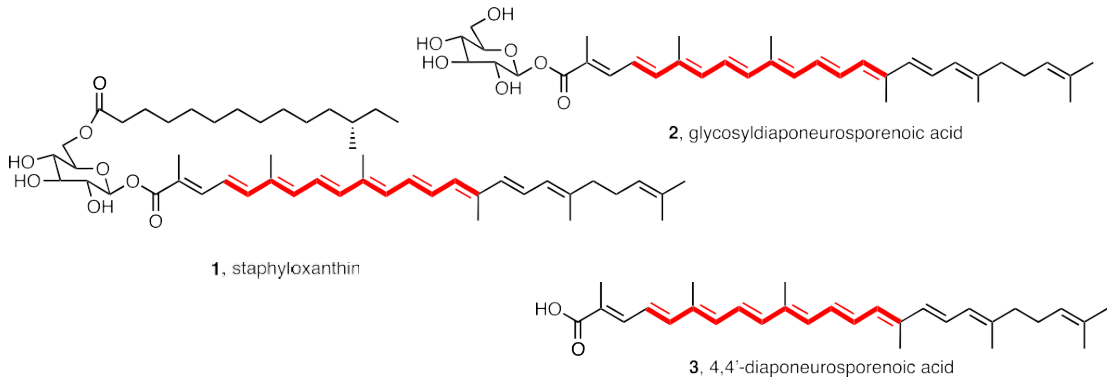

Figure 1. Structures of staphyloxanthin (1) and biogenetically-related pigments (2, 3).

References: [1] Marshall, J. H.; Wilmoth, G. J. J. Bacteriol. 1981, 2147, 900-913. [2] Götz, F. et al. J. Biol. Chem., 2005, 280, 32493-32498.

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Keywords: *apocarotenoids, staphyloxanthin, total synthesis, cross-coupling*

Monitoring carotenoid content transitions by using RGB digital images as a transversal tool: from forest vulnerability to nutritional content

Raquel Esteban*[1], Lorena Ruiz de Larrinaga[1], José María Becerril[1], Francisco San Miguel Oti[2], Unai Artetxe[1], María Teresa Gomez-Sagasti[1], Bruna Alberton[3,4], William W. Adams III[5], Barbara Demmig Adams[5], Jorge Curiel Yuste[2,6]

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The work addresses the intertwined challenges of climate change-induced forest vulnerability and declining crop nutritional quality. Although seemingly disparate, these threats are interconnected through the biochemical composition of chloroplasts within leaves. The enhancement of carotenoid content triggered by stress is widely employed as an early stress marker to evaluate and predict plant physiological status and as an early warning signal. At the same time, the higher carotenoid content translates into more nutritious food. Therefore, quantifying these compounds serves as a critical early detection tool not only for assessing tree health, essential for forest monitoring but also for plant nutritional quality. The nutritional quality of food is vital for human health by combating the many chronic diseases and disorders that are impacted by dietary carotenoids and other plant-based micronutrients. Approaches to scaling up leaf responses and assessing carotenoids from the leaf/tree level into pixels remain challenging. Using red-green-blue (RGB) channels of images assessed via digital cameras, and an additive colour model, we predict carotenoid content in two plant species and scenarios:

(i) Lemna minor (L.) Griff. (water lens or duckweed) colonies as a future food crop with an exceptional high content of high-quality protein and essential human micronutrient contents as desirable features for cultivation on Earth and in spaceflight environments, and (ii) Quercus faginea Lam. trees subjected to a drought experiment in the Basque Country (Spain) and monitored during spring and summer of 2023 for their physiological, including

carotenoid composition and functioning of the leaves' photosynthetic apparatus via concomitant imaging and functional/biochemical analysis. Use of repeated digital imaging allows for scalable, cost-effective, and non-destructive monitoring of foliar phytochemicals (such as carotenoids), enabling informed decision-making in both forest and agricultural systems. Overall, this work demonstrates the utility of integrating advanced imaging techniques into ecological and agricultural research as a multifaceted tool for monitoring plant health and nutritional quality. The findings underscore the importance of interdisciplinary approaches in addressing complex environmental and public health issues. This research is part of the strategic initiatives of the BEZ-EKOFISKO consolidated group, which has received the highest grade from the Basque Government classification for Scientific Groups (IT1648-22) and is funded by the national ATLANTIS project (PID2020-113244GA-C22; funded by MCIN/AEI/10.13039/501100011033).

Keywords*: carotenoids, real-time monitoring, repeated imagery*

Identification and characterization of putative zaxinone synthase enzymes in tomato

Eleonora Fabene*[1,2], Dorotea Ricci[1,3], Matteo Nava[1,2], Carla Sandri[1,2], Alessia Cuccurullo[4], Maria Lobato- Gomez[5], Jian You Wang6, Alessandro Nicolia[4], Antonio Granell[5], Salim Al-Babili[6], Luca Santi[2], Gianfranco Diretto[1], Olivia Demurtas[1]

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The cleavage of carotenoids, mediated by CCDs (Carotenoid Cleavage Dioxygenases) enzymes, provides the biosynthesis of apocarotenoids, a wide class of metabolites that includes phytohormones, signal molecules, chromophores and aroma constituents. In addition to the five CCD subfamilies identified and characterized so far (NCED, CCD1, CCD4, CCD7, CCD8), a sixth subfamily, named ZAS (zaxinone synthase), has recently been identified in Oryza sativa. The rice genome encodes four homologs, called OsZAS, OsZAS1b, OsZAS1c and OsZAS2. Among these, only the OsZAS and OsZAS2 functions were investigated: both enzymes cleave the apocarotenoid β-apo-10'-zeaxanthinal (C27) derived from zeaxanthin (C40), at the C13-C14 double bond, yielding zaxinone (C18). Zaxinone is an important growth regulating apocarotenoid metabolite in rice; it is involved in arbuscular mycorrhizal symbiosis and in the interaction with parasitic weeds, also influencing strigolactones levels in roots and exudates. Zaxinone has also been detected in Solanum lycopersicum, prompting our interest in exploring its function and biosynthetic pathway in this species.

We have identified three orthologs of the rice ZAS enzyme in tomato genome, named SlZAS, SlZAS-like 1 and SlZAS-like 2 through a series of bioinformatical analysis. We

decided to evaluate their enzymatical functions trough different experimental strategies: both heterologous expression in bacteria followed by in vitro assays and reverse genetic approaches, generating knock-out tomato plants in our genes of interest using CRISPR/Cas9 technique. In vitro assays were conducted to evaluate the enzymatic activities of tomato ZAS enzymes, focusing on their ability to cleave apo-10'-zeaxanthinal to produce zaxinone. Preliminary results indicate that only SlZAS enzyme catalyzes this reaction.

Using CRISPR/Cas9 technique, tomato plants (var. MoneyMaker) edited in SlZAS gene have been obtained. Phenotypic, biochemical, and molecular characterization of zas loss-of-function mutants (T4 generation) revealed reduced shoot growth and a less developed root system compared to wild-type plants. In addition, we have obtained T0 generation plants edited in SlZAS-like 1 and SlZAS-like 2, which are currently undergoing molecular screening.

Further investigations and analyses will elucidate the enzymatic functions of these genes and clarify the zaxinone biosynthetic pathway also in tomatoes, a globally important agricultural species. Understanding these physiological processes is crucial for advancing tomato cultivation in terms of food safety.

Keywords: *apocarotenoids, genome editing, tomato, zaxinone synthase, zaxinone*.

Differential impact on hepatic metabolism of retinoic acid isomers IN preclinical models

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Introduction and objective: Retinoic acid is the most active form of vitamin A. Its 13-cis isomer is widely used for the treatment of certain skin conditions, such as acne, although its use has been associated with adverse metabolic alterations. However, another of its isomers, all-trans retinoic acid (ATRA), is associated with metabolic benefits in various preclinical studies. The aim of this study was to characterize the differential effects of ATRA and 13-cis retinoic acid at the hepatic level by combining in vivo and in vitro models.

Methodology: The in vivo study involved 54 C57BL/6J mice, divided into two cohorts based on diet: normal diet (NF) and obesogenic diet (WD). The mice were treated orally with ATRA, 13-cis (both at 12 mg/kg/day), or olive oil (vehicle) for 15 days. Body adiposity, hepatic triglyceride and cholesterol content, and a transcriptomic study (RNA-seq) in the liver were evaluated. For the in vitro study, HepG2 cells were cultured and treated with different concentrations of ATRA or 13-cis. After 24 hours of treatment, RNA extraction and gene expression analysis were performed using qPCR.

Results: In male mice fed with WD, ATRA treatment was significantly associated with lower adiposity compared to the 13-cis and control groups. Similarly, the WD diet caused an increase in hepatic triglyceride and cholesterol content in the control and 13-cis groups but not in the ATRA group, which showed lipid levels similar to animals fed with NF. Gene expression analysis revealed a differential effect on the transcription levels of key lipid metabolism genes such as Cpt1a and Fabp5, and the regulation of retinoids in the liver by ATRA. In the in vitro model, ATRA treatment induced lower expression of lipid metabolism genes compared to 13-cis, which could be related to the lower accumulation of hepatic lipids observed in the in vivo study.

Conclusion: This study demonstrates the impact of ATRA on improving the metabolic profile, particularly in reducing adiposity and normalizing hepatic lipid parameters under obesogenic conditions. In contrast, these benefits are not observed with 13-cis. These findings suggest that ATRA could be a promising treatment option in clinical settings, especially for individuals with metabolic disorders.

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Keywords: *13cis isomer of retinoic acid, ATRA, Western diet, triglycerides, cholesterol*

Halophilic archaea as a source of carotenoids with antioxidant and anti-inflammatory properties

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The production of reactive oxygen species (ROS) has an important role in the progression of many inflammatory diseases. Impairment of the antioxidant defense systems causes the over-accumulation of these oxidative species, which produces oxidative damage in lipids, proteins, and DNA, inducing cell death. The search for antioxidants that can scavenge free radicals from the body cells and reduce oxidative damage is essential to prevent and treat these pathologies. Carotenoids constitute a group of isoprenoid pigments that are efficient physical quenchers of singlet oxygen and scavengers of other ROS. Most carotenoids consist of eight isoprene units with a 40-carbon skeleton, while some carotenoids named as higher carotenoids have a 45- or 50-carbon skeleton. About 40 kinds of higher carotenoids are present in some species of archaea. Haloarchaea are extremely halophilic microorganisms that inhabit hypersaline environments, such as saltworks or salt lakes, where they proliferate under high salinity, and elevated ultraviolet and infrared radiations. To cope with these extreme conditions, haloarchaea have developed singular mechanisms to maintain an osmotic balance with the medium, and are endowed with unique compounds, including carotenoids, not found in other species. This study evaluated the potential of haloarchaea as a new source of natural agents with antioxidant and anti-inflammatory properties.

Carotenoid-producing haloarchaea were isolated from Odiel Saltworks (SW, Spain) and identified on the basis of its 16S rRNA coding gene sequence as new strains belonging to the genera Haloarcula and Halobacterium. Carotenoids from these microorganisms were extracted in acetone and characterized by HPLC-DAD. The main carotenoid found was bacterioruberin, a 50-carbon carotenoid with 13 conjugated double bonds and 4 terminal

hydroxyl groups. The extracts containing bacterioruberin showed potent antioxidant capacity using in vitro DPPH and ABTS assays, and COX-2 inhibitory activity. In addition, lipopolysaccharide (LPS)-stimulated macrophages pretreated with these extracts showed a reduction in ROS production, a decrease in the pro-inflammatory cytokines TNF-α and IL-6 levels, and up-regulation of the factor Nrf2 and its target gene heme oxygenase-1 (HO-1), supporting the potential of the bacterioruberin extract analyzed as a therapeutic agent in the treatment of oxidative stress-related inflammatory diseases.

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Keywords*: bacterioruberin, carotenoids, haloarchaea, inflammation, oxidative stress*

From carotenoids to apocarotenoids: CCD engineering to produce crocins, picrocrocin, and safranal in tomato fruit

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Tomato ripe fruits are well-known for their high content of liposoluble antioxidants, especially lycopene, and to a lesser extent, β-carotene. Both carotenoids and vitamin C contribute significantly to the antioxidant activity of tomatoes, offering various health benefits. However, the water-soluble fraction of tomatoes generally lacks antioxidant compounds. In our laboratory, we developed a transgenic tomato line named Tomaffron (TF), which expresses Crocus sativus CCD2, leading to the accumulation of saffron apocarotenoids, such as crocins and picrocrocin. These compounds are water-soluble and possess strong antioxidant properties, resulting in TF tomatoes exhibiting greater overall antioxidant activity compared to the wild-type MoneyMaker (MM) cultivar.

The biosynthesis of saffron apocarotenoids in TF is derived from lutein and zeaxanthin, where zeaxanthin is absent, and lutein is present at low levels in MM ripe fruits. The expression of CsCCD2 depletes lutein levels in TF fruits. Saffron apocarotenoids are also found in Gardenia jasminoides, where GjCCD4a utilizes not only zeaxanthin and lutein but also β-carotene and lycopene to produce these valuable apocarotenoids.

To enhance the saffron apocarotenoid content in TF, we crossed it with Xantomato, a quadruple mutant that accumulates high levels of zeaxanthin and β-carotene in its fruit. Additionally, we introduced the GjCCD4a enzyme into MM and hp3/BSh, an intermediate mutant of Xantomato that also accumulates high β-carotene levels, to enable the production of saffron apocarotenoids from various carotenoid substrates. The progeny from crossing TF with Xantomato exhibited higher levels of saffron apocarotenoids compared to TF alone and showed diverse carotenoid accumulation profiles. In the T0 generation of GjCCD4a transformants, saffron apocarotenoid levels were significantly higher in hp3/BSh compared to MM transgenic plants.

Our research has resulted in the creation of various tomato lines that accumulate high levels of saffron apocarotenoids with distinct carotenoid compositions in their ripe fruits. These new tomato varieties also accumulate safranal, which is absent in wild-type fruits, along with α and β-cyclocitral, thereby significantly changing their volatile profile.

Keywords: *saffron apocarotenoids, tomato, CCD*

Innovation and technology: key elements for a sustainable agriculture

Soledad de Juan Arechederra

Fundación Antama, C/ Ferraz 28, Madrid (España)

The mission of the Antama Foundation is to promote new technologies applied to agri-food and the environment. A commitment to communication based exclusively on scientific evidence that aims to bring new tools closer to the media, politicians, legislators and public administrations, farmers, consumers, researchers, educators and anyone interested in the development of new technologies.

The Antama Foundation collaborates with other entities involved in this field, as well as with the national and international scientific community that works on plant and animal genetic improvement. A work focused on offering the most complete and up-to-date information on the latest agri-food technologies.

Research, innovation and technology are part of our agriculture and food production. All of these tools will allow us to practice an increasingly sustainable agriculture and to face the important food challenges we face today and in the near future.

We need to bring agriculture closer to society, as well as the importance of knowledge and science for our sector. And in our daily work, the Antama Foundation is committed to supporting all those entities that share its interest in providing proven scientific knowledge in the agri-food sector.

Keywords: *INNOVATION / TECHNOLOGY / SUSTAINABILITY / AGRICULTURE*

Ecophysiology and in situ seasonal study of fucoxanthin content in the invasive exotic algae *Rugulopteryx okamurae*

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Rugulopteryx okamurae is a brown macroalgae native from southeast Asia, invading the Mediterranean coasts since 2015. It has been causing drastic changes into socio-economic and environmental conditions of beaches everywhere around the southern Spain among other areas. The large amount of biomass produced can be valorized, thereby compensating the high economic expenses of the municipalities affected by the seaweed arrivals. Moreover, collecting the algae before they reach the coast would reduce the amount of algae that reaches the shore. In the BLUEMARO project, the fucoxanthin content, a carotenoid with multiple biotechnological applications, has been monitored over two years in three Andalusian locations (Tarifa, Estepona, and Maro- Cerro Gordo). Fucoxanthin is the majority carotenoid in the species. A seasonal variation in its internal content was observed, with Tarifa showing the highest average content. This makes Tarifa, which also coincides with the largest seaweed off-shore, the most suitable location for the extraction and potential biotechnological use of this carotenoid.

On the other hand, understanding the physiological behavior of *R. okamurae* is essential for preventing its spread to new areas. The Mediterranean Sea is known as an ultraoligotrophic area, where the availability of nutrients is low. This study focused on the effects of different N:P availability ratios at two temperatures in the growth and the accumulation of bioactive compounds (chlorophylls and carotenoids). Nutrient (nitrates and phosphates) uptake efficiency (NUE) and rates (NUR), photosynthetic responses, growth rates and biomass composition (chlorophylls and carotenoids) were evaluated at

the end of the experimental period. The species was able to remove entirely the nitrates available in the seawater to all N:P ratios after one week. NUR NO3- were higher with increasing of N:P ratio, and NUR PO43- was very similar to all treatments. Photosynthesis parameters increased with the highest N:P ratio utilized. The species showed positive and similar growth rates and constantly unchanged contents of chlorophylls and carotenoids. Our study presents relevant information to explain the huge ecophysiological plasticity of the species regarding nutrient availability and temperature, causing its spreading. The species is able to survive, grow and remove a quantity of nutrients much higher than those already available in the environment, indicating potential capacity of spreading from oligotrophic to eutrophic areas.

Keywords: *Algal valorization, Fucoxanthin, Invasive algae, Rugulopteryx okamurae*.

The HMG-family protein HmbC is involved in the regulation of carotenoid genes in *Fusarium fujikuroi*

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In the filamentous fungus Fusarium fujikuroi, the desaturase CarB, the phytoene synthase/cyclase CarRA, the oxygenase CarT and the aldehyde dehydrogenase CarD synthesise the xanthophyll neurosporaxanthin. The expression of the corresponding genes is regulated by light through the action of photoreceptors, among which the flavoprotein WcoA plays an important role. On the other hand, the ubiquitin ligase CarS maintains the expression of the genes of the pathway at moderate levels, since mutants lacking this protein show a carotenoid-overproducing phenotype due to the derepression of these genes. However, this protein does not exert its function directly on DNA, so one of our goals is to identify direct regulators of carotenoid synthesis genes.

For this purpose, we carried out pulldown experiments on proteins capable of binding to the promoters of the carB, carX and carRA genes in the wild type and in an overproducer mutant. In the list of proteins identified by mass spectrophotometry we found two particularly abundant in all conditions belonging to High Mobility Group family (HMG), which play regulatory roles in other organisms. To find out their function, we investigated the consequences of deleting the gene of one of them, which we call HmbC. The ΔhmbC mutants obtained produce more carotenoids than the wild type in both light and dark, although their levels were lower than those of the carS mutant. Analysis of car gene mRNA levels by qRT-PCR showed increased transcription of the structural genes carB and carRA in the ΔhmbC mutants, as well as decreased transcription of the carS gene. These results suggest that HmbC participates in the control of carotenogenesis by modulating the levels of the CarS repressor protein.

The hmbC mutants also showed other phenotypic alterations. Incubation of mycelia of these mutants with hydrolytic enzyme cocktails produced a lower number of protoplasts than the incubation of the wild type, suggesting differences in cell wall structure or composition in the mutants. Morphological differences were also observed in the hyphae

of the mutants compared to the wild type under osmotic stress conditions, such as 1.2 M sorbitol. In conclusion, this protein is not only involved in the regulation of carotenoid biosynthesis but also controls other biological processes such as cell wall formation.

Keywords: *Fusarium, regulation, neurosporaxanthin, HMG protein*

Role of saffron-derived crocetin in the process of adipogenesis activated under diabetogenic conditions by subcutaneous adipose tissue

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Type 2 diabetes mellitus (DM2) is a metabolic disorder strongly associated with obesity. DM2 is considered a public health problem and is among the 10 leading causes of death. Obesity is known to lead to cardiometabolic pathology through adiposopathy or hypertrophic adipose tissue (AT) growth related to altered adipogenesis by obesogenic signals. Most DM2 patients are obese or develop obesity over the years, however, not all obese individuals develop DM2. This difference indicates that the signals that regulate the obesity that develops in DM2 (diabesity) must be different from the obesogenic signals, which are called diabetogenic signals. The application of a healthy diet, such as the Mediterranean diet, prevents diabesity. Saffron (Crocus sativus L.) is often used in this diet. Crocetin (CCT) is a bioactive apocarotenoid in saffron that has several health-promoting properties. Our aims were to 1) evaluate the effect of diabesogenic secretome (SdDM) obtained after differentiation of human preadipocytes (PA) (DM2) derived from subcutaneous AT (SAT) and 2) the role of CCT in the process of adipogenesis in a metabolically detrimental diabesogenic microenvironment. The study focused on the effect on viability and intracellular fat accumulation of differentiated healthy visceral and subcutaneous adipocytes, in the presence or absence of SdDM and/or CCT (10 µM). The adipokine profile of SdDM was determined by chemiluminescence. Intracellular fat was quantified by Oil Red O staining and viability by MTT assay. Our results showed that diabesogenic conditions induced cytotoxicity and provided a proadipogenic environment

only for visceral PA. CCT acted as an anti-adipogenic and cytoprotective compound. This study shows the hidden face of SAT, a tissue that under physiological conditions is beneficial, but under diabetogenic conditions may be detrimental, which may lead to a change in therapeutic strategies to combat diabesity. Our results point to a role for CCT as a possible candidate for inclusion in pharmacological therapies aimed at reversing AT accumulation in DM2.

Keywords: *Type 2 diabetes mellitus, adipogenesis; crocetin; diabesogenic microenvironment*.

GWAS and BSA-seq approaches reveal several genomic regions and candidate genes regulating carotenoid content in *Cucurbita pepo* fruit.

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Carotenoids are a group of fat-soluble pigments of plant origin that are required in the human diet as provitamin-A, but also act as functional foods to reduce the incidence of certain diseases. For this reason, increasing carotenoid content in fruits and vegetables is a current challenge in plant breeding. In the present work, we have used two approaches to study the genetic control of color and carotenoid accumulation in *C. pepo* fruit. A screening for lutein, zeaxanthin, α-carotene, and β-carotene content in 257 accessions of *C. pepo* showed a high variation in the content of these pigments. Phenotype and genotype data, the latter obtained by GBS in the CucCAP project, were combined in a genome-wide association study (GWAS) that revealed some genomic regions of interest. For lutein content, a polymorphism was identified on chromosome 11 (S11_3084639) linked to the Cp4.1LG11g05170 gene, which codes for a carotenoid cleavage dioxygenase (CCD) and apocarotenoid formation. The study identified 9 regions associated with α-carotene and β-carotene content. Among them, a region on chromosome 13 includes allelic variants of the Hsp70 protein (Cp4.1LG13g00040), a chaperone that regulates carotenoid accumulation in tomato fruit. On the other hand, orange fruit accessions with high carotenoid contents have been used in biparental crosses with white fruit accessions and low carotenoid contents. F2 segregated 1:2:1 for white:yellow:orange fruit with low, intermediate, and high β-carotene and lutein contents in two consecutive years. Whole genome re-sequencing of several bulks from white, cream or orange fruiting plants in the F2 generation was used in a BSA-seq analysis that identified a major QTL on chromosome 14 and a minor QTL on chromosome 5 regulating lutein and β-carotene content. Candidate genes within these QTLs that could control the carotenoid content of zucchini and squash are discussed.

Keywords: *fruit colour, Cucurbita pepo, GWAS, QTLseq*

Strigolactones: essential apocarotenoids for plant life

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Strigolactones (SLs) are carotenoid-derived compounds, belonging to the apocarotenoid family, that modulate plant responses under environmental stresses. They are paramount under nutritional stress conditions, especially Pi deficiency, acting as 'cry for help' signals. SLs are unique molecules, having dual functions as phytohormones regulating plant growth and development, and as rhizosphere cues promoting the association with beneficial soil microorganisms such as arbuscular mycorrhizal (AM) fungi and rhizobacteria. Recently, it has been proposed that SLs are evolutionary 'old' compounds that originated as AM symbiosis-promoting signals in the rhizosphere, and later recruited as plant hormones by flowering plants. This association dates back more than 400 million years ago, and it is considered a key component of the plant microbiome and crucial in plant evolution. Indeed, more than 80% of land plants, including most agricultural and horticultural crops, form AM symbiosis. Interestingly, in addition to a better nutrition, AM symbiosis offers many other benefits to plants, including an improved defense response to pathogens and pests, having a great potential as biostimulants, biofertilizers and bioprotection agents in agriculture.

We are interested in understanding how nutrient fertilization regulates SL biosynthesis and how this impacts AM symbiosis establishment, plant development and defense responses. The goal is to find the optimal balance between chemical and biological fertilization by favoring plant-AM fungus communication, in order to reduce the use of agrochemicals (fertilizers and pesticides) in a modern and sustainable agriculture. To close the cycle, we are also assessing how AM symbiosis affects carotenoid biosynthesis in tomato plants to produce healthier fruits.

Keywords: *Strigolactones, Chemical communication in plans, Rhizosphere, Environmental stress*

SDGs: the science perspective and its practical application in an academic context

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The United Nations' 2030 Agenda (A2030) is increasingly prominent in European political priorities, emphasizing integrated perspectives (Borchardt et al., 2022). This focus extends to funding programs for scientific research (Horizon Europe), which also aim to achieve the UN's Sustainable Development Goals (SDGs). Alongside these programs, there has been significant development of accessible and concise research resources and tools, providing scientific evidence for analysing the implementation scope of the SDGs in scientific advancements. These resources facilitate better result transfer and communication, making the SDGs more understandable for the public. From a scientific evidence perspective, evaluating the definition of the SDGs and their associated targets involves assessing whether they are supported by scientific evidence and whether they comprehensively address economic, social, and environmental dimensions of sustainability. Approximately 30% of the SDGs are well-defined, 54% could be more specific, and 17% require significant work for better definition (ICSU-ISSC, 2015).

Building on this foundation, the alignment of research actions using the SDG Mapper tool from the European Commission's Joint Research Centre should be reevaluated based on expert knowledge. SDG Mapper employs text mining and an AI-based approach to transparently and robustly identify semantic links between the SDGs and analysed documents (Borchardt et al., 2022). It enhances understanding of the sustainability framework and facilitates SDG integration into decision-making processes. However, it's essential to recognize that the tool does not capture context or the level of commitment to the SDGs. Expert review is crucial to ensure proper contextualization of mapping results and prevent misuse for "greenwashing" purposes (Borchardt et al., 2022).

In summary, SDG Mapper represents a powerful tool for creating supplementary reports in research projects.

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Keywords: *Agenda 2030, sustainability, scientific evidence, SDG mapping*.

Phytoene and phytoene-rich microalgae extracts extend lifespan in *C. elegans* and protect against amyloid-β toxicity in an Alzheimer's disease model

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Carotenoids are versatile isoprenoids in diverse biological processes, ranging from vitamin A synthesis to anti-inflammatory responses. More than 800 carotenoids have been identified in nature, of which just some are colorless. One of them is phytoene, a dietary carotenoid that has been poorly studied until recently and has been associated with various biological actions that promote health. *Caenorhabditis elegans* is a valuable model for biomedical research as it shares many biochemical pathways with humans, is genetically tractable, and offers a broad set of research tools including models of human disease and aging. The objective of the main study was to investigate the role of phytoene-rich microalgal extracts and pure phytoene in aging and proteotoxicity models of C. elegans.

Extracts of *Chlorella sorokiniana* and *Dunaliella bardawil* rich in phytoene, and a phytoene standard, all dissolved in DMSO, were tested. For lifetime assays, 120 synchronized wild-type L4s per condition were transferred to fluorodeoxyuridine-treated plates and maintained at 20 °C. Live/dead nematodes were counted every other day. The assay was performed in triplicate. For the proteotoxicity model, strain GMC101 was used, which expresses human Aß42 in the body wall muscle, causing progressive paralysis. Paralysis and survival of 90 synchronized L4s per condition were analyzed for four days, and performed in triplicate. DMSO was used as a control in both assays.

Supplementation with the three samples significantly increased the survival rates of the nematodes, from a half-life of 16.1 days in those treated with DMSO to 19.1, 18.6, and 17.7 days in those treated with extracts of *D. bardawil*, phytoene, and *C. sorokiniana*, respectively. All nematodes were dead on day 27. Additionally, from day 2 to 4, supplements significantly reduced paralysis in nematode strain GMC101 compared to

DMSO on day 2, from 62% to 40, 37, and 43% in *Dunaliella*, *Chlorella*, and phytoene treatments, respectively.

In conclusion, supplementation with phytoene-rich microalgal extracts or a phytoene standard increases lifespan and resistance to proteotoxicity in C. elegans compared to the control.

Keywords: *phytoene, oxidative stress, amyloid-β42 proteotoxicity, Chlorella sorokiniana, Dunaliella bardawil*

Viral vectors as a tool in the characterization of CCDs

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Carotenoids constitute an important group of natural products widely used as food colorants, nutraceuticals, cosmetic additives and health supplements. Due to the high presence of conjugated double bonds in their carbon chain, these molecules are susceptible to non-enzymatic and enzymatic cleavage reactions generating a series of biologically important derivatives, known as apocarotenoids. Enzymatic cleavage is catalyzed by a series of enzymes belonging to the Carotenoid Cleavage Dioxygenases (CCD) family. Among the best studied and most valuable apocarotenoids for the pharmaceutical and food industry are pigments such as crocins, mainly present in saffron, or bixin, characteristic of the plant Bixa Orellana. Due to the importance of these molecules, the search for enzymes capable of generating these apocarotenoids and their characterization becomes a task of high biotechnological value.

This characterization has been carried out by means of "in vitro" or "in vivo" activity assays, the latter with bacteria previously genetically modified for the accumulation of the different carotenoid substrates.

These techniques have several limitations such as the lack of appropriate substrates, and the metabolization by *E. coli* of the apocarotenoids generated, which leads to an added difficulty in the identification of metabolites and therefore in the characterization of these enzymes. Plants are therefore the ideal platform for the analysis of these activities, but an efficient system for such characterization is required. Expression systems based on viral

vectors are a fast and efficient option for the generation of biosynthetic enzymes that will interact with the natural metabolism of the host plant.

Among them, a system derived from tobacco etch virus (TEV, potyvirus genus) is chosen as a tool for cloning different CCDs, obtaining recombinant clones that are agroinoculated in *N. benthamiana* plants. This transient expression This transient expression system constitutes a rapid alternative for the characterization of the activity of the different CCDs under study, making it possible to obtain significant quantities of crocins and other apocarotenoids in adult N. benthamiana plants in only two weeks. It is thus a fast and simple alternative for the high-throughput analysis of the enzymes under study, allowing to solve the problems associated with the characterization in other platforms.

Key words: *Apocarotenoids, CCDs, crocins, viral vectors*

Identificación de carotenoides e/z naturales con espectros UV/vis atípicos

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Los carotenoides poseen muchos dobles enlaces conjugados y cada doble enlace tiene la posibilidad de encontrarse en configuración E o Z (cis o trans), pero en la mayoría de los carotenoides naturales todos los dobles enlaces son de configuración E. Cuando en la cromatografía HPLC-DAD encontramos picos con espectros UV/VIS que no corresponden a carotenoides conocidos, es posible que se trate de isómeros E/Z. Tradicionalmente, los isómeros E/Z se han identificado por el "Pico cis" del espectro UV/VIS, comparado con el todo E. Sin embargo, en algunos casos nos encontramos con isómeros E/Z que poseen espectros UV/VIS atípicos, en donde el "Pico cis" no está bien definido o la estructura fina del espectro no se parece a la de un carotenoide. Nosotros identificamos los isómeros E/Z, combinando el uso del "Far-UV Peak" y la micro fotoisomerización con Iodo. En el fruto del Pibá (Bactris gasipaes) demostramos la presencia de un E/Z-γ-caroteno y un E/Z-licopeno, que otros investigadores los identificaron como δ-caroteno y E/Z-γ-caroteno respectivamente. En el sapote amarillo (*Quararibea cordata*) identificamos E/Z-anteraxantina y en la sandía amarilla (*Citrullus lanatus*) varios isómeros E/Z de licopeno, con espectros atípicos. En las flores del tulipán africano (Spathodea campanulata) y el framboyán (*Delonix regia*) identificamos una E/Z-rubixantina, su respectivo precursor metabólico E/Z-γ-caroteno y diversos isómeros E/Z de licopeno con espectros atípicos. Lo encontrado nos permite plantear alternativas metabólicas que explican la presencia de carotenos y xantofilas E/Z naturales.

Palabras clave: *Isómeros E/Z, espectros UV/VIS, fotoisomerización*

Investigation of novel enzymes for Apocarotenoid Biosynthesis in "Azafran de bolita" (*Ditaxis heterantha* Zucc.)

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Ditaxis heterantha, a plant belonging to the *Euphorbiaceae* family, thrives in the semi-arid regions of Mexico. It is commonly known as "azafran de bolita" or "azafrancillo" due to its seeds, which feature a vibrant orange endosperm traditionally used by local populations as a dye and food additive, similar to the use of Crocus sativus (saffron).

Previous studies have demonstrated that *Ditaxis heterantha* seeds are rich in fatty acids, tocopherols, and phytosterols, all of which contribute to their significant antioxidant activity. In addition to these known compounds, in 2005 two novel apocarotenoids were found in the endosperm: Ditaxin (C31) and Heteranthin (C27). These compounds add a new dimension to the already impressive phytochemical profile of the plant.

To further explore the potential of *Ditaxis heterantha*, we evaluated the bioactivity of its endosperm extracts on various cancer cell lines. Our results revealed a significant reduction in cell viability even at low extract concentrations, indicating potent anti-cancer properties. This finding underscores the potential of *Ditaxis heterantha* as a source of new therapeutic agents.

In order to fully understand the plant's metabolic profile, both targeted and untargeted metabolomic analyses were conducted on the endosperm, peel, leaves, and roots.

Additionally, analyses on DNA content and karyotype through flow cytometry and fluorescence microscopy were conducted to enhance the understanding of this species.

This comprehensive profiling provides a detailed understanding of *Ditaxis heterantha*'s phytochemical landscape and emphasizes its potential as a source of bioactive compounds with applications in pharmaceuticals and nutraceuticals.

Moreover, our analyses are focused on elucidating the biosynthetic pathway of the two aforementioned apocarotenoids: Ditaxin and Heteranthin. We are integrating both metabolomics and transcriptomics approaches in order to pinpoint potential carotenoid precursor/s and identifying the specific genes involved in the biosynthesis of these apocarotenoids; in a first stage, we decided to focus on the carotenoid cleavage dioxygenases (CCDs) responsible for the biosynthesis of these new apocarotenoids, and in silico studies are still in progress. Future research will also consider other enzymes involved in the biosynthesis of carotenoids and apocarotenoids of *D. heterantha*. Once the list of candidate genes will be compiled, their roles will be verified using enzymatic activity assays in bacteria, yeast, and plants.

Keywords: *Ditaxis heterantha, apocarotenoids, endosperm, transcriptomics, metabolic profiling*

Identification of proteins that bind *Arabidopsis* phytoene synthase to modulate its function

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Carotenoids are powerful antioxidants and the main source of vitamin A that we can obtain through diet. The first enzyme in the carotenoid biosynthesis pathway is phytoene synthase (PSY), which catalyzes the production of phytoene from geranylgeranyl diphosphate (1). PSY activity is crucial, not only for carotenoid production, but also for the differentiation of chromoplasts, which are plastids specialized in accumulating carotenoids and other plastidic isoprenoids such as tocopherols (2, 3). In fact, chloroplasts from leaves can be transformed into artificial chromoplasts by transient expression of the crtB gene, which encodes a bacterial PSY. However, overexpression of plant PSY genes is unable to trigger this process because they do not reach sufficient phytoene production. Our hypothesis is that endogenous regulatory mechanisms limit the activity of plant PSY enzymes in chloroplasts, but not that of bacterial enzymes (2, 3).

Since a very important part of the regulation of PSY activity in plants occurs at the post-translational level and is mediated by binding to other proteins (1), our goal is to find proteins that interact with PSY (PIPs) that can improve its enzymatic activity and/or the production, transport and storage of phytoene, in order to increase carotenoid production and even trigger the artificial chromoplastogenesis phenotype for the generation of biofortified crops. One of the identified PIPs is FIBRILLIN6, a fibrillin able to interact with PSY and to improve phytoene production directly promoting the enzyme's activity (4). These and other results with other PIPs will be presented at the meeting.

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Keywords*: Phytoene synthase, Chromoplast, PSY-interacting protein*

Nanotechnology behind scenes: New approaches of tomafran-nanodevices

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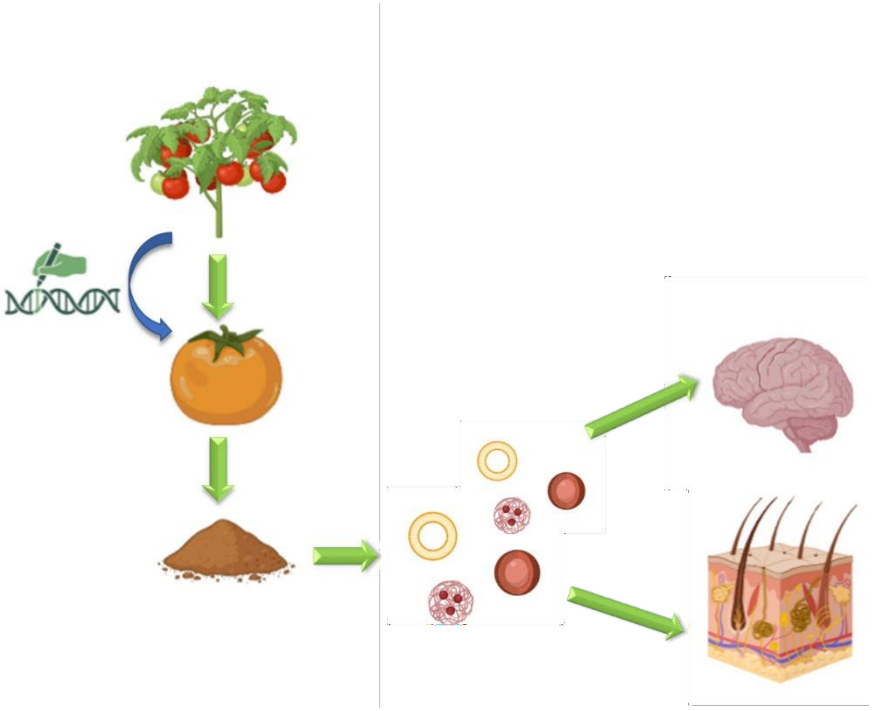
We are currently in a new scientific era, "the age of nanotechnology". The latest scientific, biomedical and technological advances, such as the recent SarSCOV2 vaccines, new aerospace materials and even everyday household appliances such as televisions are incorporating nanotechnology to improve their efficiency and, above all, to improve our quality of life. One of the most widespread uses of nanotechnology focuses on the encapsulation, stabilization and vectorization of compounds in order to increase the efficacy of many active molecules such as terpenes, alkaloids and apocaroteoids among others. Tomafran is a new genetically modified tomato variety that produces a high level of saffron-like apocaroteoids such as crocin, picocrocin and crocetin. Despite the broad utilities and functions of its extract, as a nutraceutical and cosmeceutical ingredient, its use may be limited by numerous biological barriers, such as the blood-brain barrier or the skin barrier. The Bioforce research group and the spin-off Naplatec have developed different nanotechnological approaches to improve the efficacy of tomafran extract activity. In this presentation we will take a long tour through the different applications of tomafran with the enhancement of its activity through novel nanodevices.



Simultaneous accumulation of astaxanthin and lutein in continuous cultures of the microalga *Chromochloris zofingiensis*

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Microalgae are a natural source of high-value compounds, most notably carotenoids. The combination of inherent characteristics of terrestrial plants, such as oxygenic photosynthesis and simple nutritional requirements, together with the biotechnological attributes of microbial cells, make these organisms a perfect model for the generation of these compounds. The microalga *Chromochloris zofingiensis* has been widely recognized as a source of astaxanthin.

The aim of the present study was to investigate the accumulation of carotenoids in the microalga *C. zofingiensis* in response to nitrogen and light availability, factors that modulate the synthesis of these pigments. The cultures were maintained under photoautotrophic conditions, in a continuous regime, modifying the dilution rate, the nitrate supplied in the medium and the incident irradiance. The obtained results made it possible to determine the conditions that favored the accumulation of the different carotenoids. Thus, when the nitrogen present in the biomass was less than 4% of the dry weight, astaxanthin accumulated, while 9% nitrogen in the biomass favored the synthesis of lutein. As for light, average irradiance positively affected lutein accumulation under both high and low nitrate conditions, however, astaxanthin content only increased with irradiance under nitrogen-limiting conditions.

By jointly analyzing carotenoid accumulation profiles and expression levels of genes involved in the biosynthesis pathway of these pigments, it was determined that the factor triggering the most significant response in astaxanthin and lutein accumulation was nitrogen availability, and that a synergy between this parameter and available irradiance existed, which modulated individual responses to both stresses. Regulation appears to be orchestrated at several levels: the expression pattern of cyclases suggests a redirection of

lycopene to β-carotene under nitrogen-limiting conditions, which is transformed into astaxanthin by overexpression of BKT1. In turn, under these conditions, slightly higher lutein values are reached by alteration of the α-carotene/lutein ratio, due to up-regulation of CYP97A1 and CYP97C, especially when coupled with high irradiance conditions.

Adequate nitrogen availability modulated by light availability would allow adjusting the simultaneous accumulation of lutein and astaxanthin, enhancing the potential of C. zofingiensis as a natural source of both pigments.

Keywords*:* *lutein, astaxanthin, microalgae, continuous cultures*

Strategies and tools offering new opportunities for carotenoid biofortification in Triticeae species

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Carotenoids are responsible for the yellow pigment content (YPC) in cereal grains, which is an important quality trait in both durum wheat and common wheat breeding programmes. Tritordeum, the amphiploid derived from the cross between the wild barley *Hordeum chilense* and durum wheat, has a remarkably high carotenoid content, giving its derivatives products a characteristic golden colour. The significance of YPC has promoted genetic studies which identified the main QTL (Quantitative trait loci) for YPC on the homoeologous group 7 in Triticeae species. Later studies showed that this QTL was associated with variations in the Phytoene synthase 1 gene.

Lutein is the most abundant carotenoid in the endosperm of Triticeae species and it can be found in its free form or esterified with fatty acids forming monoesters or diesters. Esterification with fatty acids is a common mechanism for carotenoid sequestration and accumulation in plants. Esterified carotenoids are more stable than non-esterified carotenoids, thereby preserving their antioxidant activity. The catabolic loss of carotenoids in cereals is largely due to their inherent industrial processing and storage. Consequently, carotenoid esterification could be incorporated as a trait in breeding programmes with the objective of enhancing the carotenoid content of cereal grains and improving carotenoid retention throughout the food chain. Until recently, the genetic and biochemical mechanisms underlying the esterification of carotenoids with fatty acids in plants were poorly understood. Our work is contributing to a more detailed understanding of this process, particularly in cereals.

In this work, we summarise the recent advances in our research line on carotenoid content and esterification in cereals, including: (1) The first report of durum wheat accessions with carotenoid esterification ability; (2) The advances in determining the genetic basis of carotenoid esterification with the identification of the XAT-7A1 and XAT-7Hch genes from durum wheat and *H. chilense*, respectively, and their potential for durum wheat breeding programmes; (3) The identification of MTAs (marker-trait associations) for specific carotenoids and total grain carotenoid content by genome wide association scan (GWAS); (4) The development of enriched bread wheat lines for grain carotenoid content by inter- specific breeding. These results offer new opportunities for the biofortification of wheat and related species with carotenoids.

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Keywords: *Carotenoids, esters, lutein, tritordeum, wheat.*

Early life programming of adipose tissue remodeling and browning capacity by Vitamin A as a potential anti-obesity strategy

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The early stages of life, especially the period from conception to two years, are crucial for shaping metabolic health and the risk of obesity in adulthood. Adipose tissue (AT) plays a crucial role in regulating energy homeostasis and metabolism, and brown AT and the browning of white AT are promising targets for combating weight gain. Evidence in animal models indicates that Vitamin A (VA) during prenatal and early postnatal stages can influence the development and function of AT, affecting the likelihood of obesity later on.

Overall, these studies show that VA supplementation in early postnatal life can favor adipocyte hyperplasia, perhaps as a consequence of inhibiting the differentiation of fully mature adipose cells by epigenetic mechanisms. This effect is interesting since adipocyte hyperplasia has a protective impact on metabolic dysfunction induced by excessive caloric intake. An insufficient number of adipocytes favors adipocyte hypertrophy, hypoxia, and inflammation, a key cause of metabolic dysfunction. Moreover, recent studies indicate that VA supplementation in early life may ameliorate malprogramming caused by an inadequate maternal diet.

In addition, the timing of VA supplementation during development may critically condition the consequences on offspring adiposity. VA supplementation promotes AT hyperplasia in the offspring when administered to pregnant and lactating dams, during pregnancy only, or directly to suckling or recently weaned animals, albeit not necessarily by the same mechanisms, while the stimulation of beige/brown adipogenesis apparently requires VA supplementation during fetal life; it is worth noting that in mice, many cells acquire the commitment to brown/beige adipocytes during fetal life.

Provitamin A carotenoids, such as β-carotene, are generally considered to have lower toxicity and teratogenicity than preformed VA and may, therefore, represent a safer form of VA provision. Little is known about the eventual metabolic programming effects of β-carotene. What seems clear from different studies, is that supplementing preformed VA or β-carotene in early life may entail different outcomes regarding adiposity programming; β-carotene is not only a precursor to VA, it has VA-independent effects and can be metabolized to other apocarotenoids besides the VA retinoids.

Insights into the molecular mechanisms by which micronutrients and bioactive compounds influence AT remodeling and browning capacity at early life stages open up novel avenues towards feasible and effective strategies for combating obesity and associated metabolic disorders.

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Keywords: *Metabolic programming, Vitamin A, Carotenoids, White Adipose tissue, Brown adipose tissue*

Novel carotenoid biotechnology strategies

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The nutritional and economic relevance of carotenoids has spurred a large number of biotechnological strategies to enrich plant tissues with these phytonutrients. Most approaches to alter carotenoid contents in plants have been focused on manipulating their biosynthesis or degradation, whereas improving carotenoid sink capacity in plant tissues has received much less attention. Also, biofortification of green leafy vegetables with pro-vitamin A carotenoids such as β-carotene has remained challenging to date. In our lab we combined two strategies to overcome these challenges. One of them involves the conversion of chloroplasts into non-photosynthetic, carotenoid-overaccumulating plastids (named artificial chromoplasts) in leaves agroinfiltrated or infected with constructs encoding the bacterial phytoene synthase crtB, leaving other non-engineered leaves of the plant to sustain normal growth. The second approach involves producing β-carotene in the cytosol of leaf cells to avoid the negative impacts on photosynthesis derived from changing the balance of carotenoids and chlorophylls in chloroplasts. Combination of these two strategies together with light treatments promoting the proliferation of plastoglobules (a major site for β-carotene production and sequestration in artificial leaf chromoplasts) not only improved β-carotene accumulation but it also resulted in a much higher bioaccessibility. While these strategies were initially tested in Nicotiana benthamiana leaves, they also work to improve β-carotene contents and bioaccessibility in edible lettuce (Lactuca sativa) leaves.

Carotenoids with and without provitamin A activity in the prevention and reduction of risk in deficiency and chronic disease in humans.

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The study of carotenoids in relation to human health/disease has focused on those most abundant in the blood, three carotenoids (α-carotene, β-carotene, lycopene) and three xanthophylls (lutein, zeaxanthin, β- cryptoxanthin). Although all of them show diverse biological activities in the human organism, two stand out for their importance and specificity, they are those provitamin A (two carotenes and one xanthophyll) and lutein and zeaxanthin for their protective role in ocular tissue and visual function. As a provitamin, β-carotene is considered to be the most important, but recent studies have shown that β-cryptoxanthin may contribute much more to vitamin A intake than previously thought. Two studies will be presented, one on the calculation of apparent dietary bioavailability (ratio of blood concentration of each carotenoid to its concentration in the diet) in subjects with different dietary patterns, and another on an intervention with orange juice (analysis of juice and blood concentrations of each carotenoid). Further study of these aspects is essential for a better understanding of vitamin A requirements and also for the adequate management of proposals to prevent its deficiency.

Lutein and zeaxanthin are the only carotenoids in the blood that are deposited in the retina and brain. They are associated with photoreceptors and form the macular pigment. Their increase in ocular tissue is associated with a lower risk of macular degeneration, and their supplementation in the diet also slows its progression. This presentation will show the results of intake studies in Spain, blood concentrations, bioavailability study of lutein (free and ester forms) and intervention with food and capsules to see the effect on markers of antioxidant status, visual function and macular pigment density. The status of lutein and zeaxanthin in the macula is currently being linked to their presence in the brain and to aspects of cognitive imbalance. Although much progress has been made over the last few

decades, there are still aspects that need to be studied in depth, such as those relating to doses and the characteristics of individuals who could benefit most from supplementation.

Keywords: *carotenes, xantophylls, vitamin A, deficiency disease, macular degeneration*.

Strigolactones as a signal of "cry for help" in water-saving species: conclusions drawn from a field experiment

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Strigolactones (SLs) are carotenoid-derived plant hormones that play a crucial role in plant adaptation to abiotic stresses. By promoting symbiosis with beneficial soil microorganisms, these hormones enhance nutrient and water uptake, as well as stress resilience. Therefore, the analysis of SLs under water deficit scenarios could be a promising strategy for anticipating drought-induced health transitions in trees, reducing tree mortality, and minimizing forest decline. In this study, the expression of several SL biosynthesis genes (D27, CCD8, and MAX 1) were analyzed. To simulate drought scenarios at the tree level throughout the growing season, rain exclusion umbrellas were installed near Vitoria-Gasteiz (Basque Country, Spain) from March to October 2023. This experiment involved a total of eight trees of a water-saving species (*Pinus pinea* L.), with four trees subjected to drought simulation and the other four serving as controls (no drought simulation). This study aimed first to establish a successful protocol for extracting high-quality RNA and second to analyze the effect of drought on the expression of SL biosynthesis genes using quantitative RT-PCR (qPCR). To analyze the impact of drought at the leaf level, the physiological status of each tree, including carotenoid composition and the functioning of the photosynthetic apparatus, was monitored during the spring and summer of 2023. At the root level, non- structural carbohydrates and root architecture were measured at the end of the growing season. Afterward, four different RNA extraction

methodologies were used for root samples: (i.) “RNeasy Plant Mini Kit” (Qiagen, Hilden, Germany), (ii.) Trizol method, (iii.) CTAB method and (iv.) a novel modified CTAB method. Our results showed that only the novel modified CTAB method allowed for obtaining high-quality RNA to conduct molecular subsequent analyses. Moreover, our observations highlighted relevant differences in the responsiveness of the physiological apparatus of *Pinus nigra* L. individuals. Besides, an inhibition trend for the expression of the three SL genes under drought stress was observed. Overall, although this work will provide a crucial understanding of plant physiology under intense drought periods, providing valuable insights into tree health loss, further research is required to understand why trees reduce SL production under drought stress and whether this is related to the recruitment of beneficial soil microorganisms.

Funded by Red Nacional de Carotenoides (CaRed)

Keywords*: Strigolactones, drought experiment, roots, RNA extractions*

Awakening of the native PHYTOENE SYNTHASE 1 promoter by correcting near-miss cis-acting elements activates carotenoid biosynthetic pathway in embryogenic rice callus

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Metabolic engineering in plants typically involves the introduction of transgenes and/or the mutation or silencing of endogenous genes. An alternative approach is promoter modification where small changes in the promoter sequence allow genes to be switch on or off in particular tissues. We screened promoters for near-miss cis-acting elements that can be converted into functional endosperm- specific regulatory motifs by changing 1–4 nucleotides. Such minimal interventions would awaken “silent latent endosperm-enabled promoters” (SLEEPERs). To test this hypothesis, we chose rice PHYTOENE SYNTHASE 1 (PSY1), encoding the enzyme responsible for the first committed step in the carotenoid biosynthesis pathway, because it is not expressed in rice endosperm. Sequence analysis of the promoter region identified six motifs within a 120-bp region, upstream of the transcriptional start site, which differed from endosperm-active elements by up to four nucleotides. We mutated four motifs to match functional elements in the endosperm-active BCH2 promoter, and this promoter was able to drive GFP expression in callus and in seeds of regenerated plants. The 4 M promoter was not sufficient to drive PSY1 expression, so we mutated the remaining two elements and used the resulting 6 M promoter to drive PSY1 expression in combination with a PDS transgene, which encodes phytoene

desaturase, the subsequent enzyme in the pathway. Callus transformed with the corrected PSY1 construct and PDS resulted in deep orange tissue indicating the accumulation of carotenoids, which was subsequently confirmed by targeted metabolomics analysis. PSY1 expression controlled by the uncorrected or 4 M variants of the promoter plus a PDS transgene produced callus that lacked carotenoids. These results confirm that the correction of promoter elements can facilitate the ectopic activation of endogenous plant promoters in rice callus and endosperm and most likely in other tissues and plant species.

Keywords: *Callus, Carotenoids, cis-acting element, Endosperm, Metabolic engineering, Promoter*

Unraveling carotenoid biosynthesis in carrots: the roles of DCHY5 and DCPIF3

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During photomorphogenesis in plants, light plays a crucial role in regulating gene expression and the biosynthesis of chlorophylls and carotenoids. In darkness, plants elongate their hypocotyls due to the action of transcription factors known as PIFs (Phytochrome Interacting Factors), which also repress the expression of PSY genes, leading to a reduction in carotenoid synthesis. But in shade, the photoreceptor PHYA is activated and translocated to the nucleus where, together with PAR1, it targets PIFs for degradation. This process allows the bZIP transcription factor HY5 (ELONGATED HYPOCOTYL5) to bind to PSY promoters, thereby inducing their expression and promoting carotenoid synthesis.

Daucus carota (carrot) accumulates high levels of carotenoids in its storage root when it grows underground, and PHYA is required for this process. Therefore, the aim of this study is to present evidence of DcHY5 and DcPIF3 functionality to elucidate the mechanism of carotenoid synthesis in carrot storage roots. We determined that DcHY5 and DcPIF3 have 75% and 48% sequence identity with AtHY5 and AtPIF3, respectively. Both proteins localize to the nucleus and interact with DcPHYA and DcPAR1. Their expression level is also higher in carrot roots grown underground. Using ChIP assays, we found that DcPIF3 binds to G-box elements, which was further confirmed by an in vivo transient expression system. Our results indicate that both DcHY5 and DcPIF3 modulate the expression of DcPSY1 and DcPSY2 and modulate carotenoid synthesis. Together, these findings suggest that DcPIF3 inhibits and DcHY5 promotes carotenoid biosynthesis in *D. carota*.

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***Keywords**: Daucus carota, DcHY5, DcPIF3, DcPSYs, carotenoids*

Volatile apocarotenoids might work as molecular signals in plant-to-plant communication

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Apocarotenoids, compounds product of carotenoids oxidative cleavage, are a very diverse group of metabolites with essential functions in plants. Some of these, such as strigolactones (SLs) or abscisic acid (ABA), are phytohormones whose functions as signaling molecules / developmental regulators and their mode of action are very well described. Conversely, the scientific knowledge generated over the role of other apocarotenoids as signaling molecules is still quite limited. (1).

Our project is focused on the study of the role as signaling molecules of two volatile apocarotenoids coming from β-carotene oxidative cleavage: β-ionone (β-ion) and β-cyclocitral (β-cc). Both of these compounds are volatile organic compounds (VOCs) whose production is enhanced when plants grow in proximity shade conditions and start degrading their carotenoids (2). Our working hypothesis claims that these two VOCs play a pivotal role in plant-to-plant communication as signaling molecules, modulating the growth and development of vegetation in proximity shade conditions. The results obtained support that β-ion, and also β-cc to a lesser extent, contribute to the repression of the growth observed after the exposure of Arabidopsis thaliana seedlings to the volatiles emitted by tomato plants in conditions of proximity shade. Additionally, β-cc is able to also repress A. thaliana seeds germination, delaying their development. In that sense, these results led us to think that plants exposed to proximity shade change their volatilome to suppress neighboring plants' development. The reviewed results abovementioned, as well as the latest progresses made towards the elucidation of the molecular mechanisms by which these compounds trigger their effects will be presented.

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(2) Martinez-Garcia & Rodriguez-Concepcion M (2023) Molecular mechanisms of shade tolerance in plants. New Phytol. 239:1190-1202.

Keywords: *Apocarotenoids, VOCs, β-ionone, β-cyclocitral, plant-to-plant communication, proximity shade*

The flesh color of ripe melon (*Cucumis melo*) fruit from green and white cultivars is determined by a protein that prevents chloroplast differentiation

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Fruit flesh color in melon (*Cucumis melo*) is a major attribute of fruit quality related to the accumulation of carotenoids but also chlorophylls. This character is determined by two loci, Green-flesh (Gf) and White flesh (Wf). Gf (responsible of the orange flesh color) is epistatic over Wf (the main determinant of white and green coloration). The Gf gene is MELO3C005449, also known as ORANGE (CmOr). The encoded protein has been shown to stimulate the production of carotenoids and prevent the degradation of β-carotene. The dominant allele CmOrHis leads to β-carotene accumulation and results in an orange flesh phenotype. In the absence of this allele, the flesh of ripe melon can be white or green depending on Wf, being white dominant over green. The identity of Wf has remained unclear despite several candidates have been proposed. We have obtained compelling evidence that Wf encodes a fruit-specific protein that prevents the differentiation of chloroplasts and hence the accumulation of carotenoids and chlorophylls during fruit ripening. Previous analysis of a recombinant inbred line (RIL) collection generated by crossing the white-fleshed melon variety Piel de Sapo (PS, gf gf / Wf Wf) and the orange- fleshed Vedrantais (VED, Gf Gf / wf wf) allowed to identify a QTL associated with white and green flesh color. Fine mapping of this QTL followed by validation of candidate genes by heterologous expression in *Arabidopsis thaliana* and *Nicotiana benthamiana* confirmed the identity of Wf. Our results indicate that during the first stages of development, melon fruits are white and have plastids with poorly differentiated membranous structures. In orange-fleshed melons, the CmOrHis variant induces carotenoid synthesis and chromoplast differentiation. In fruits lacking the CmOrHis variant, cultivars with the non-functional wf allele differentiate chloroplasts during ripening and eventually become green, whereas those with the Wf variant are white because they do not develop chloroplasts and instead

contain amyloplasts. Further evidence supporting the mode of action of the Wf-encoded protein and an integral model of the molecular mechanisms responsible for the coloration of the melon fruit flesh will be presented.

Keywords: *Flesh color, chloroplast differentiation, melon*

Characteristics of red-fleshed sweet oranges and their impact on gut microbiota and health

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The health and nutritional benefits of citrus fruit consumption are associated with their balance of nutrients and bioactive compounds. The pigmented citrus varieties are of growing interest to the citrus industry and consumers, and the red-fleshed oranges are a new alternative by their attractive coloration and the potential health-related benefits. These varieties displayed a contrasted carotenoid profile in the pulp with accumulation of lycopene and very high concentrations of phytoene and phytofluene. This study presents a summary of the composition in main bioactive compounds and nutrients, with a special focus on the carotenoid content and composition in fruits of different red-fleshed oranges compared to traditional varieties. Furthermore, the potential biological effects of the consumption of the red-fleshed orange (Cara Cara variety, CC) in comparison with traditional orange (Washington Navel, N) were investigated, evaluating different health-related parameters and gut microbiota. An in vitro study with the juice and pulp of N and CC varieties, following simulation of gastrointestinal digestion and colonic fermentation, and incubation with gut microbiota isolated from obese and normal-weight children, demonstrated a reduction in the population of Enterobacteriaceae. Moreover, the pulp and juice of CC exhibited a more pronounced increase in the population of Bifidobacterium and Bacteroides especially in the obese group. A randomized pilot study was conducted with obese children who receive either CC or N orange (one orange fruit per day, five days per

week for four weeks). After this intervention, the carotenoid profile in the faeces and plasma of individuals supplemented with CC was significantly higher in phytoene, whereas no discernible variations in carotenoids were observed in those supplemented with N orange. No changes in body weight were observed, but orange consumption positively modulated the composition of the microbiota, and the effects in specific populations will be discussed. Interestingly, a reduction in triglycerides and the insulin resistance index (HOMA-IR) was observed in both orange-type participants, especially in those consuming CC.

Keywords: *Red-fleshed orange, health-benefits, carotenes, microbiota*

PÓSTERS

Impact of heat stress on carotenoid content in durum wheat

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Durum wheat (*Triticum turgidum* L. var durum (Desf.) Husn.) is the 10th most important cereal in the world, with an average annual production of 40 million Tm representing the 5% of total wheat production. The Intergovernmental Panel on Climate Change (IPCC) (IPCC. 2014) has projected a global warming trend of 0.3-1.7ºC by 2100, which is a serious concern for agriculture in the medium to long term. In addition to this, short heat waves (SHW), a period of several consecutive days with high temperatures above the developmental optimum, will become more frequent. In wheat, the occurrence of very high temperatures during anthesis has a negative impact on yield by reducing spike fertility and grain filling period and impairing grain filling. In addition, high temperatures also affect grain quality by altering the biosynthesis and accumulation of many grain compounds. In this work, we investigate the effect of post- anthesis SHW on durum wheat grain under field conditions. At anthesis, all spikes at the same physiological stage were labelled and the SHW stress was applied for one week using tents covered with transparent polyethylene film. The temperature inside and outside the polyethylene tents was monitored using data loggers. At maturity, all the labelled spikes were harvested. After threshing, the grains were milled and subsequently used for the determination of the total carotenoid content and profile.

The SHW treatment was successful in simulating a heat stress with temperature increases between 10- 15ºC inside the cages. This resulted in grain yield losses due to reduced thousand kernel weight and lower seed set grain caused by grain abortion. SHW did not affected some quality traits such as protein content and vitrosity but it caused significant losses on grain carotenoid content.

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Key words: *durum wheat; heat stress; short heat wave (SHW); grain quality;*

Antioxidant effect of crocin-enriched tomato extract on brain tumor growth

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Gliomas are the most frequent primary tumors of the central nervous system originated from neoplastic glial cells. Among them, Glioblastoma (GB) is the most common type of glioma, characterized by its aggressiveness and infiltration capacity. GB is an incurable disease mainly due to treatment resistance because of an increased oxidative stress and the resulting neurodegeneration, thus it is crucial to pursue new therapeutic strategies to prevent its progression. Mitochondrial dysfunction and derived oxidative stress have been described in other neurodegenerative diseases such as Alzheimer's disease, ALS, Parkinson's disease, as the neural tissue is highly sensitive. Based on this, therapeutic strategies using antioxidant molecules towards redox equilibrium are proposed as a new perspective for battling GB progression. In this work we are interested in an apocarotenoid present in saffron, that has antioxidant properties in vitro: crocins. We have introduced the crocin biosynthesis pathway using specific promoters in tomatoes through metabolic engineering, resulting in the accumulation of 15mg/g of crocins in dried tomato (Cromate). This work aims to study the effect of crocins as an antioxidant agent on neurodegeneration and tumor progression in a Drosophila melanogaster GB model. We have found that there are indeed changes in the survival rates of the treated individuals which show a less invasive and proliferative phenotype and a reduction of the neurodegeneration. In addition, we performed a RNAseq assay which shows changes in gene expression in treated individuals, these candidate genes will be further studied as well as the antioxidant properties of crocin- enriched tomato extract as a treatment for neurodegenerative diseases.

Keywords: Glioblastoma, neurodegenerative disease, crocins, oxidative stress

Liposome encapsulation of Tomafran extract as a novel sunscreen ingredient

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Liposomes are exceptional vehicles for cosmetic formulations such as sprays, sunscreens, moisturizers, and anti-aging creams due to their unique structure, which includes an aqueous reservoir capable of encapsulating carotenoids. This feature allows liposomes to retain and protect these active compounds while enhancing their bioavailability and bioaccessibility through the skin.

In this study, we formulated liposomes to encapsulate a tomato extract rich in crocins (4.84 mg of crocin/g of extract) and phenolic compounds, leveraging the development and optimization of a plant- based biofactory. The objective is to create a natural filter against UV radiation, offering an innovative approach to skin protection.

Liposomes were prepared according to the film hydration method, using L-α-phosphatidylcholine and cholesterol as lipids to form the bilipidic layer of the vesicles, and then the extrusion method was used to reduce and homogenize the size of the formed liposomes optimizing the PDI of the nanoparticles.

Characterization of nanoparticles (particle size, zeta potential and polydispersity index (PDI)) was determined by photon correlation spectroscopy through Dynamic light scattering (DLS).

Tomafran-encapsulated liposome formulations demonstrated successful encapsulation with an efficiency of 16.12%, nanoscale size ranging from 60.9 to 80.7 nm, narrow size distribution (PDI <0.3), and high stability in aqueous media, indicated by an appropriate Z-potential value of -21.5 mV.

Transmission electron microscopy (TEM) was used to assess the morphological and external characteristics of the nano-formulations. The liposomes exhibited a distinct round morphology with minor aggregation in the microscopy sample, without any ruptures. Their size was consistently smaller than 100 nm, corroborating the data obtained by dynamic light scattering (DLS). Importantly, their size remained unaffected after the freeze-drying process, suggesting that these liposomes can be stored cost-effectively while maintaining morphological stability.

Tomafran extract and liposomes containing extract were added to a cosmetic formulation to measure their photoprotective capacity. Tomafran extract increased the cream absorbance, although the photoprotection values increased to a very small extent. However, liposomes containing the Tomafran extract demonstrated enhanced stability in cosmetic formulations. This stability is attributed primarily to the lack of Tomafran release when incorporated into the cream, which contributes to the observed superior stability of the liposomes under storage conditions.

Keywords: *Tomafran, crocin, liposomes, nanoparticles, encapsulation*

Supplementation with Vitamin A or Beta- Carotene in Mice during Lactation Modulates Short- and Long-term Expression of Key Genes in Retinoid Signaling

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Introduction and Objectives: Endogenous retinoid signaling could play a role in metabolic programming and be affected by an obesogenic maternal diet and modulated by the infant's vitamin A (VA) status. Objective: To evaluate the impact of exposure to a maternal Western diet (WD) on the expression of retinoid pathway genes in the liver of offspring in both the short and long term, and the influence of supplementation with a physiological dose of VA in the form of retinyl palmitate (RP) or beta-carotene (BC) during lactation.

Materials and Methods: Suckling mice born to WD mothers were treated with vehicle, RP, or BC. Some were sacrificed at 26 days of age. The rest were fed a control or WD diet for 3 months after weaning. In parallel, animals exposed to a control diet from conception were sacrificed at PND-26 and PND-110. Liver levels of retinoids and expression (mRNA) of key genes in retinoid signaling were analysed: genes related to VA absorption, retinoid esterification, and the synthesis, metabolism, and effects of retinoic acid (VA most active form). The design allows evaluation of the effects of a maternal WD and those of a contemporary WD (vs. control) in animals previously exposed to maternal WD, and their modulation by VA supplementation during lactation.

Results: Short-term, in the liver: (i) WD reduced free retinol, while RP supplementation increased retinoid levels; (ii) WD was associated with sex-specific changes in gene expression, which were reversed by RP but not by BC; (iii) BC had a greater impact on gene expression in females. Long-term, maternal WD was associated

with lower hepatic expression of most retinoid pathway genes. Contemporary WD decreased hepatic retinoid levels and increased hepatic expression of most of these genes, and these effects were attenuated in the animals treated with RP or BC during suckling.

Conclusions: Maternal WD affects the VA signaling pathway in the liver of offspring in both the short and long term. RP or BC supplementation during lactation attenuates the response of the VA signaling pathway in the liver to an obesogenic WD diet in adulthood.

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Keywords: *Metabolic programming, obesogenic diet, retinyl palmitate, beta-carotene*

Implicación de los carotenoides en la tolerancia a las bajas temperaturas de conservación en frutos de variedades tempranas de cítricos

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La desverdización con etileno es una práctica tecnológica frecuente en Citricultura, especialmente en variedades tempranas de mandarinas y naranjas, para estimular el color de la piel en frutos que todavía tienen coloración externa verde pero que han alcanzado un índice de maduración interno aceptable para el consumo. Los frutos de diferentes variedades de cítricos pueden presentar daños por frío (DF) en la piel cuando se conservan a temperaturas por debajo de 5ºC. Sin embargo, no se conoce con precisión la influencia de la coloración de la piel (especialmente en estados tempranos) y el efecto del etileno en la tolerancia/susceptibilidad a los DF, y la posible relación con los cambios en el contenido y composición en carotenoides. Para investigar esta posible relación, en este trabajo hemos evaluado la susceptibilidad a los DF en frutos de dos variedades de mandarinas (*Citrus clementina* Hort. Ex Tan.) Orogrande y Clemenules y una de naranja, Navelina (*Citrus sinensis* L. Osbeck), en varios estados de coloración externa (verde, virando y coloreado), así como en frutos tratados con etileno y conservados a 2 ºC durante 8 semanas. En general, los frutos con menor contenido de carotenoides totales al inicio de la conservación tuvieron mayor susceptibilidad a desarrollar DF. Sin embargo, los frutos maduros de todas las variedades estudiadas fueron mucho más resistentes a los DF y presentaron niveles más elevados de carotenoides totales. El análisis global de los resultados sugiere que, los frutos en estado de transición de cloroplastos a cromoplastos, con un complemento bajo de carotenoides y con presencia parcial de clorofilas, son mucho más susceptibles a desarrollar DF. Conforme avanza la coloración de la piel o se prolonga la desverdización con etileno se estimula la carotenogénesis, modificando el perfil y el contenido de carotenoides totales, resultando en una mayor resistencia a los DF. En resumen, los

resultados sugieren que en frutos de variedades tempranas de cítricos con un deficiente complemento de carotenoides y desorganización de los plastidios, la exposición a bajas temperaturas puede tener efectos perjudiciales en su calidad externa.

Palabras clave: *Daño por frío, desverdización, etileno, carotenoides, frutos cítricos*

Screening of the pigmentary content in different species of algae focused on consumption

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In this study, a screening of different species of algae has been carried out, in which the main carotenoids contained in them have been quantified using high-resolution liquid chromatography. Both micro and macroalgae were included, and in each group there was representation of green, red and brown algae. To prepare the extracts, freeze-dried biomass and methanol as solvent were used in all cases, applying the mortar maceration method. The chromatographic method consists of a triple gradient that combines three organic solvents in different proportions. The column used is a ZORBAX SB-C8 model.

This screening is included within the ALGAHUB project, in which the biomass of these algae species has been characterized not only at a pigmentary level, but also in terms of nutritional content (proteins, lipids, carbohydrates, etc.) as well as bioactive activities of interest, since the project is focused on the development of functional foods incorporating algae for human consumption.

Carotenoids found in algae, in addition to their obvious participation in the use of light to carry out photosynthesis, perform a function of protecting other molecules from oxidative stress. So they can be associated with applications such as anti-oxidant, anti-inflammatory or anti-tumor, among others.

It is due to this bio-activity that the study of pigments is important in this project and furthermore, we can not forget that to offer a product and for it to be well accepted by the population it must be singular and appetizing, which is directly influenced by its appearance and color.

The analyzed algae species were: *Arthrospira platensis, Porphyridium cruentum, Anabaena marina, Nannochloropsis gaditana, Scenedesmus almeriensis, Chlorella vulgaris, Tetraselmis chuii, Rugulopteryx okamurae, Ulva sp., Gracilaria bursa-pastoris, Pyropia leucosticta* and *Chondracanthus teedei*.

Red algae, both micro and macro, stood out in their β-carotene and zeaxanthin content. On the other hand, only in the macro lutein was also found. This pigment also stood out in the microalgae of Chlorophyta phylum (*Scenedesmus, Tetraselmis* and *Chlorella*), highlighting in the last one. In *Nannochloropsis gaditana*, mainly violaxanthin and β-carotene were found, pigments that were also found in the macroalga of the same phylum Rugulopteryx okamurae, but in this one appears and distinguish an interesting concentration of fucoxanthin. In both cyanobacteria (*Anabaena marina* and *Arthrospira platensis*) it is find zeaxanthin and β-carotene as the main accessory pigments. In comparison, microalgae had a higher carotenoid content in proportion to their weight than macroalgae.

Keywords: *Screening, carotenoids, microalgae, macroalgae, bio-activity*

Characterization and Evolution of CCD Enzymes in the Bryophyte *Physcomitrella patens*

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CCDs (carotenoid cleavage dioxygenases) are dioxygenase enzymes with significant relevance in the biosynthesis pathways of carotenoids, apocarotenoids, and phytohormones such as abscisic acid and strigolactones, all of which are of great economic, medical, or agricultural interest.

In this study, we analyzed the presence of these enzymes in a database of terrestrial plants to explore their diversification and examine the characteristics of the CCD1 and CCD2 subgroups by designing an ad hoc bioinformatic workflow. This analysis provided a wealth of information on the distribution of CCDs in the phylogenetic tree of terrestrial plants, notably identifying four CCDs belonging to the CCD1/CCD2 subgroup in the bryophyte *Physcomitrella patens*, a model for studying plant complexity and adaptation to terrestrial environments.We present a characterization of CCDs in this bryophyte through the identification of chromosomal locations, gene structures, and cis-promoter elements of each of these genes, as well as their phylogenetic analysis. Additionally, we performed biochemical characterization by cloning the corresponding coding sequences and expressing them in a heterologous system producing various carotenoids, which allowed us to outline their functional properties.

The results obtained constitute the first analysis of the properties of this type of carotenoid-cleaving enzymes within the Bryophyta division, enabling us to formulate hypotheses about the evolution of carotenoid metabolism at the origin of terrestrial plants.

Keywords: *Apocarotenoids, bioinformatics, bryophyte, CCD*

Evolution of Carotenoid Content in Compost: COMPO-NERPIO operative group.

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Nerpio is a village in the province of Albacete whose economy is based on the primary mountain sector with extensive livestock farming, where one of its fundamental elements is the cultivation of walnut trees and the management of Celtiberian goats. On the other hand, domestic waste management, given the size of the population, depends on joint management with other surrounding towns. The COMPO-NERPIO operative group intends to contribute to the improvement of the self-management of bio-waste by integrating local waste to obtain a quality composted product, certified, that complies with legal standards and can be marketed, finding another outlet for the waste generated, facilitating the self-management of the municipality.

Three different types of compost (A, B and C) have been developed, integrating in different proportions elements of the locality that were characterized: goat and sheep manure, walnut wood pruning remains and organic waste from household refuse selectively collected. The procedure was carried out at the Nerpio composting plant and on-site treatment of aerobic composting piles with mechanical turning. The process lasted approximately 4 months, and the temperature, humidity, pH, and conductivity of the piles were monitored. The resulting products were physically, chemically, and microbiologically characterized. Among the analyses carried out, an analysis of the evolution of the carotenoid content in the maturation process of compost A, B, and C was conducted over 4 sampling points (February, March, May, and June 2024). In some

of the treatments, the carotenoid content is maintained throughout the maturation process, while in others it decreases drastically, probably due to the microbial content of the mixture made.

Composting components (manure, pruning residues, or household waste) are not regarded waste after they are valorized to form a product with fertilizing capabilities; rather, they are termed ingredients. In addition to these agronomic capabilities, it has been demonstrated that they can be a key source of carotenoids, which have so far been underestimated.

***Keywords**: compost, carotenoids, waste valorization Preferred participation: poster*

Plant Cell Cultures: A Strategic Approach to Enhanced Carotenoid Production

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Plant cell cultures have become a crucial tool in biotechnology for the production of high-value metabolites. This study highlights the potential of carrot (Daucus carota) cell cultures for the production of carotenoids through several strategies aimed at enhancing the yield of these compounds. These strategies include elicitation, the use of inhibitors, and modification of growth conditions.

Two carrot cell lines were used, one orange and one green, and were cultured under controlled conditions. They were subjected to different elicitation strategies to maximize carotenoid production. The elicitation methods included individual and combined treatments with cyclodextrins, methyl jasmonate, hexenol, and β-glucan.

In the green cell line, a constitutive production of several carotenoids was observed, notably lutein and β-carotene. Elicitation with cyclodextrins led to a significant increase in carotenoid accumulation within the cells. Specifically, lutein reached a concentration of 25,949.5 µg/L, while β-carotene attained 1,138.1 µg/L. Additionally, this line was enriched with other compounds such as tocopherol (8,063.8 µg/L) and chlorophylls a (1,625.1 µg/L) and b (9,958.3 µg/L), establishing it as an efficient source of these compounds.

Conversely, the orange cell line also exhibited constitutive carotenoid production, with β-carotene and lutein as the primary compounds. Elicitation with cyclodextrins enhanced the accumulation of these carotenoids within the cells, demonstrating the potential of this cell line as an innovative platform for carotenoid production.

Furthermore, the effect of specific inhibitors on the production of other carotenoids, which were produced constitutively in lower amounts, was evaluated. This approach led to increased accumulation of additional carotenoids such as phytoene and lycopene.

In conclusion, plant cell cultures represent a promising platform for carotenoid production. This biotechnological approach not only facilitates sustainable and controlled production of these valuable compounds but also offers a flexible platform for optimizing production through various strategies tailored to industrial needs.

Keywords: *carotenoids, plant cell cultures, elicitors*

Nanovehicles for encapsulation of tomafran and B-carotene inside nutraceutical industry

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Genetically modified tomatoes expressing a mini-pathway of saffron genes called Tomafran, have demonstrated the ability to produce and accumulate crocin and picrocrocin in their fruits. On the other hand, β-carotene is a provitamin A carotenoid present in tomato, which has great antioxidant capacity. Given the neuroprotective, anti-inflammatory and antioxidant properties, among others, derived from Tomafran, it is crucial to ensure the adequate assimilation of its active compounds to maximize its therapeutic benefits. Several types of nanoparticles, construction materials and methods have been developed in order to improve bioavailability besides other disadvantages for these compounds.

Nanotechnology has many advantages over delivery systems for active molecules, such as improved stability, dispersibility, solubility, sustained release over time, protection against pH variations or oxidation phenomena. One of the most widely used types of nanovehicles for the encapsulation of active compounds for their protection in digestion processes are nanocapsules of polymeric type.

Polysaccharides of natural source present a variability of molecules in their structure that favor resistance to the acid pH of the gastric phase depending on the monomer they present. These include chitosan, alginate, pectin, hyaluronic acid or dextran and are considered biodegradable, biocompatible and non- toxic to humans. On the other hand, lipid nanoparticles are another suitable vehicle for the controlled release of drugs in the gastrointestinal system, since they are effective in increasing antioxidant activity and bioavailability. Therefore, various types of nanovehicles were investigated for the

encapsulation of Tomafran and β-carotene. These nanovehicles were characterized by different techniques such as dynamic light scattering, electron microscopy, digestion and stability assays.

Keywords: *Tomafran, β-carotene, nanovehicles, controlled release*.

Expression of apocarotenoids in β-carotene overproducer *Fusarium fujikuroi* strains

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Carotenoids are isoprenoid compounds synthesized by all photosynthetic organisms and many heterotrophic microorganisms that play a role in harvesting light energy and protecting the cell from photo-oxidation. The electron-rich polyene system of carotenoids makes them susceptible to oxidative cleavage, yielding carbonyl products called apocarotenoids (APOs). This oxidation can be catalyzed by carotenoid cleavage dioxygenases (CCDs), which constitute a ubiquitous family of non-heme iron enzymes or are triggered non-enzymatically. Plant growth and development, response to the environmental changes, stress factors, and plant communication, are regulated by a set of hormones and signaling molecules, including APOs. Certain APOs, such as vitamin A, retinoic acid, abscisic acid, strigolactones and the aromatic β-ionone, have high economic value in the feed, food, cosmetics, pharmaceutical and agricultural industries. The colored APOs, crocins and bixin, are also gaining attention due to their therapeutic properties and potential applications for a wide spectrum of diseases. APOs biosynthesis is therefore a booming field in plant secondary metabolism, and CCDs can be envisaged as a novel source of enzymes able to process carotenoids to render APOs for species fitness and human health.

Crocins are produced and stored by a restricted number of plant species, being saffron the main source. However, the high price of saffron limits the further exploitation of these metabolites. In this regard, the CCDs of the crocin pathway in saffron and other three plant species (*Buddleja, Gardenia* and *Verbascum*) have been identified, providing

the tools to produce APOs in other hosts through metabolic engineering. Competitive and sustainable production of these metabolites can be obtained by engineering filamentous fungi with these CCD enzymes. Moreover, carotenoid-overproducing mutants are easily obtained in the ascomycete fungus *Fusarium fujikuroi*. Here, we introduced different CCDs that recognize beta-carotene as substrate in a beta-carotene over-producer strain of *F. fujikuroi* (SG292) as a first step towards the development of a fungal biofactory for the production of colored APOs.

Keywords: Carotenoids, apocarotenoids, crocins, CCDs, UGTs, Fusarium

Prebiotic Potential of Tomafran Ketchup on Probiotic Bacteria

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Carotenoids have gained attention due to their effects against diabetes, obesity and certain types of cancer. These pigments, found naturally in various fruits and vegetables, are known for their antioxidant properties and their ability to modulate immune responses. Recently, some of these compounds have been shown to interact with the gut microbiota and provide benefits to the intestinal mucosa. In our study, we evaluated the prebiotic potential from ketchup extracts made with Tomafran, a genetically engineered fruit rich in crocins and carotenoids. Crocins, which can be found in saffron stigmas, are known for their neuroprotective properties, but their microbiome-modulating effects have not been studied. For this reason, in vitro fermentations with probiotic bacteria (*Lacticaseibacillus casei* and *Lactiplantibacillus plantarum*) were performed and bacterial growth was monitored against a control with ketchup made with commercial tomatoes. The enhanced growth observed suggests that crocins present in Tomafran may have a unique modulatory effect on probiotic bacteria. This may indicate that Tomafran-enriched ketchup has potential as a functional food with prebiotic properties. Further studies are needed to elucidate the molecular and genetic interactions between crocins and prebiotic bacteria. Understanding these interactions will help determine the specific mechanisms by which crocins influence bacterial growth and activity. Moreover, it is crucial to conduct in vivo studies to confirm the prebiotic benefits observed in vitro. Such studies would provide insight into the real-world implications of incorporating Tomafran fortified products into the diet. They would

also help determine the overall impact on gut health and the potential for improving human health through dietary modifications.

Keywords*: Tomafran, kepchup, prebiotic*

Vitamin A supplementation during lactation modulates long-term muscle transcriptome in mice fed a lifelong obesogenic diet

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Introduction and objective: Nutrition during perinatal stages is critical for maternal and offspring health. An obesogenic diet can have significant adverse effects on metabolic health, programming permanent changes. Skeletal muscle activity is key in defining energy expenditure and glucose control. This study examines the impact of supplementation during lactation with a physiological dose of Vitamin A in the form of retinyl palmitate (RP) or beta-carotene (BC) on the transcriptome of skeletal muscle in adulthood in animals exposed to a Westernized diet (WD) from conception and throughout life.

Methods: Female lactating mice born to WD fed mothers were treated with vehicle, RP or BC. After weaning they continued on the WD diet until 3 months of age when they were sacrificed. In parallel, animals exposed from conception to a control diet were kept. Skeletal muscle transcriptome changes were analyzed by RNAseq and differential gene expression levels and functional enrichment analyses were assessed using KEGG and GO.

Results: RP supplementation during lactation, but not BC, attenuated the worsening of glycemic control associated with maternal and lifetime WD feeding. WD animals supplemented with RP showed higher rectal temperature, a surrogate measure of metabolic expenditure, compared with BC. No effects on body weight or adiposity were observed. In skeletal muscle, WD and RP supplementation modulated at the transcriptional level key metabolic pathways associated with insulin and cytokine action. Transthyretin was identified as a possible inducer, via inhibition of AMPK activity, of the insulin resistance and hyperglycemia observed in the WD group and its

improvement by RP supplementation. RP supplementation showed a differential effect compared to BC, with a higher expression of “myogenic” genes and a lower expression of “adipogenic” genes in the RP animals. The higher expression of sarcolipin, a regulator of the activity of the sarcoplasmic/endoplasmic reticulum calcium pump (SERCA), could explain the differential effects of RP compared to BC on the animals´ thermogenesis.

Conclusion: RP supplementation during lactation can mitigate the adverse health effects of an obesogenic diet throughout life, improving control of insulin action and glycemia. On the other hand, BC did not show the same benefits. This study underlines the importance of the form of vitamin A used in metabolic programming and its impact on long-term health.

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Keywords: *Metabolic programming, obesogenic diet, retinyl palmitate, beta-carotene, skeletal muscle*

Phenotype and relationship to carotenogenesis of putative catalase-deficient mutants in the fungus *Fusarium fujikuroi*

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Fusarium fujikuroi is a filamentous fungus that produces carotenoids in response to different stimuli such as light, heat shock, and oxidative stress. Expression of the genes involved in carotenoid synthesis i.e., carB, carRA, carX, carD and carT, is induced by exposure to light and hydrogen peroxide causing oxidative stress. The expression of these five genes is repressed by the regulatory protein CarS, as shown by the strong increases in their mRNA levels in CarS mutants. Studies of the transcriptome of CarS loss of function mutants showed that CarS regulates a large number of genes, including FFUJ_05128 (cat4) and FFUJ_11472 (cat5), which encode catalase-domain proteins. In addition, RNA-seq data showed that, among all genes containing any catalase domain, cat4 and cat5 had the highest induction after illuminating *F. fujikuroi* for one hour.

Further RNA-seq analyses were performed to study the effect of oxidative stress on the transcriptome. Exposure of wild-type mycelia for one hour to 16 mM hydrogen peroxide resulted in a different induction pattern of the catalase domain protein-coding genes to that observed for the carS mutation. However, the highest induction of expression also corresponded to cat4, which is suspected to play a relevant role in the response to oxidative stress. Using the CRISPR-Cas9 technique, Δcat4 and Δcat5 mutants have been obtained in *F. fujikuroi* as confirmed by PCR and Southern blot analyses. In this communication, the molecular and phenotypic characterisation of these mutants, including their sensitivity to oxidative stress and their carotenoid production, will be presented.

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Keywords: *Catalases, Fusarium, oxidative stress, neurosporaxanthin*

Carotenoids compounds from peel and pulp of mango kent: high- pressure assisted extraction

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Introduction: High pressure processing (HPP) is a technology that can result in increased extractability and bioavailability of bioactive compounds in food (1,2). However, little is known about its impact on the individual carotenoid profile of mango by-products (3,4).

Material and Methods: Mangoes (Kent variety), degree of ripeness 5. The pulps and peels received high- pressure treatment (HPP) at 200, 400 and 600 MPa (25ºC, 5 min). Carotenoids from lyophilized HPP samples and untreated samples (HPPo) were extracted using a traditional method, using diethyl ether: petroleum ether (50:50). The extracts obtained were saponified. Carotene and xanthophyll content was determined by HPLC-DAD, considering the sum of both as total carotenoids (TC).

Results: Lutein (29.1), zeaxanthin (4.6), ß-cryptoxanthin (6.4), α-carotene (6.0) and ß-carotene (109.6) were identified in the peel of Kent HPPo mango (µg/g mango). Zeaxanthin (2,1), ß-cryptoxanthin (5,2) and ß-carotene (66,8) in the pulp. ß-carotene was the predominant carotenoid in peel and pulp. Lutein and α-carotene were only found in peel. The same profile was observed in the samples treated with HPP. The content of TC, with a predominance of provitamin A carotenoids (80%), was higher in the peel (45%)

than in the pulp in all samples (0, 200, 400 and 600 MPa). The content of TC (µg/g mango) extracted in peel and pulp was: HPP0: 155.7 and 74.2; 200MPa: 279.4 and 102.3; 400 MPa: 179.5 and 84.4; 600 MPa: 147.6 and 71.6.

Conclusion: Kent mangoes have a high percentage of provitamin A carotenoids and lutein and zeaxanthin, especially in the peel. Pre-treatment of pulp and peel samples at HPP 200 or 400 MPa (especially 200) resulted in an increase in extracted carotenoids compared to HPPo samples.

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Keywords: High pressure processing, mango Kent, carotenoids.

Photoprotective effects of crocin-rich tomato extract (TomaFran) and its liposome encapsulation

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Crocin and picrocrocin are carotenoids that give saffron its characteristic color and have demonstrated a range of bioactivities both in vitro and in vivo, such as antioxidant and anti- inflammatory effects. Despite their benefits, the high cost and culinary acclaim of saffron limit its application in industries like pharmaceuticals and cosmetics. To address this, a genetically engineered tomato variant capable of synthesizing crocins and picrocrocin, named "Tomafran," has been developed. This study analyzed Tomafran extract's potential photoprotective effects using spectrophotometric methods and cell culture. The extract displayed significant antioxidant capabilities through three assays: ABTS, DPPH, and □-carotene bleaching, achieving values near 80 µmol Trolox equivalents (TE) g-1 dry extract (DE) or an EC50 of 0.2 mg DE mL-1. Additionally, the extract significantly reduced the formation of AGEs (advanced glycation end- products) in human fibroblasts exposed to UV-B radiation and decreased ROS (reactive oxygen species) production in human keratinocytes exposed to UV-A radiation. The extract was also incorporated into a base cream to study its ability to filter UV radiation by calculating SPF (sun protection factor) and UVAPF (UV-A protection factor). However, the effective absorption of UV radiation was very low and consequently SPF (1.7) and UVAPF (1.2) values in creams with 15% of extracts were very low. Additionally, the photo and thermal stability of the extracts were assessed, showing a decline in crocins' stability when exposed to UV radiation and high temperatures, which in turn decreased its antioxidant activity. To counteract this degradation, enhance its benefits, and apply it in a cosmetic product, the extracts were encapsulated in

liposomes. These liposomes were stable against UV radiation and temperature, protecting the extract until its release after being applied in the skin. In conclusion, liposome- encapsulated Tomafran extract could be a good candidate for cosmetic applications due to its protective properties against the negative effects of UV radiation due to its antioxidant capacity

Keywords: *Antioxidant, Cosmetic, UV radiation*

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Stange Claudia	cstange@uchile.cl	Unraveling Carotenoid Biosynthesis in Carrots: The Roles of DcHY5 and DcPIF3	Nicolás Arancibia., Triana Dàlencon., Christian González., Patricio Mora., Claudia Stange.
Valverde Carvajal, Laura	laurav19c@hotmail.es	The flesh color of ripe melon (Cucumis melo) fruit from green and white cultivars is determined by a protein that prevents Chloroplast differenciation	Laura Valverde Carvajal., Jordi Garcia-Mas., Manuel Rodríguez-Concepción.
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