Libro de resúmenes

XV REUNIÓN DE BIOLOGÍA VEGETAL

Hotel Enjoy Coquimbo

5-7 de diciembre del 2022

CHILEAN
SOCIETY OF
PLANT BIOLOGISTS

XV Reunión de Biología Vegetal

Hotel Enjoy, Coquimbo

5-7 DICIEMBRE 2022

Auspiciadores

CAVAS DEL VALLE

Agrisera Antibodies

CONFERENCE PROGRAMME

Monday, December 05

09:00 - 14:30	**Registration**
15:00 - 16:30	***[1]Oral Session 1 – *Plant Growth Promoters***
	Chair: Dr. Francisca Blanco (Universidad Andrés Bello)
	Room: Bahía 1
15:00 - 15:30	"*Plant-growth promotion by proteobacterial strains depends on the availability of phosphorus and iron in Arabidopsis thaliana plants"* - Dr. María Josefina Poupin Swinburn (Universidad Adolfo Ibáñez)
15:30 - 16:00	"*Cytokinin and auxin modulation are key to the plant development changes in response to A. nodosum biostimulants in A. thaliana*" - Dr. José O'Brien (Pontificia Universidad Católica de Chile)
16:00 - 16:30	"*A double AP2- domain transcription factor under differential alternative splicing regulation modulates root development under salt stress"* - PhD candidate Jesús Hernández (Pontificia Universidad Católica de Chile)
16:30 - 17:00	**Coffee Break**
17:00 - 19:00	**Symposium**: ***"Plant roots under stress: overcoming current climate challenges"***
	Chair: Dr. José Estévez (Universidad Andrés Bello)
	Co-Chair: Dr. José M. Álvarez (Universidad Andrés Bello)
	Room: Bahía 1
	17:00 - 17:05 **Symposium presentation**
17:05 - 17:40	"*Dissecting nitrate responses in space and time*". Dr. Rodrigo Gutiérrez (Pontificia Universidad Católica de Chile)
17:40 - 18:00	"*Face to face with underground challenges*". Dr. Lorena Norambuena (Universidad de Chile)
18:00 - 18:20	"*Gene regulatory networks controlling cell proliferation and cell expansion during plant organ growth*". Dr. Ramiro Rodríguez (Universidad Nacional de Rosario, Argentina)
18:20 - 18:40	"*WRKY-TF, a novel transcription factor that negatively regulates periderm formation*". Dr. Gonzalo Villarino (Salk Institute, USA)
18:40 - 19:00	"*To grow or not to grow": molecular mechanism of cell elongation at low temperature in single root cells*". Dr. José Estévez (Universidad Andrés Bello)

***1**. Speakers in Oral Session 1: 30 minutes, including questions and comments from the audience, will be allocated to each oral presentation*

****2.** Speakers in Oral Sessions 2,3,4,5: 20 minutes, including questions and comments from the audience, will be allocated to each oral presentation*

19:15 - 20:10	**Opening Ceremony**
19:15 - 19:20	Welcome to the XV Congress of Chilean Society on Plant Biologists
	Dr. Andrés Zurita Silva (CORFO)
19:20 - 20:00	**Opening Lecture:**

Chair: Dr. Paula Pimentel (CEAF, Chile)

Title "*Aquaporins and the soil-plant-atmosphere continuum: are there new challenges?*". Dr. Gabriela Amodeo (Universidad de Buenos Aires, Argentina)

Room: Bahía 1

20:15 - 22:00 **Welcome Dinner** at "Santerra Mercado - Enjoy"

Tuesday, December 06

09:00 - 10:30 ****[2]Oral Session 2 - *The plant cell wall***

Chair: Dr. Carlos Figueroa Lamas (Universidad de Talca)

Room: Bahía 1

09:00 - 09:20 "*Regulation of PME58 in Arabidopsis seed coat mucilage pocket*". Dr. Susana Sáez-Aguayo (Universidad Andrés Bello)

09:20 - 09:40 "*Proteomic and metabolomic integration reveals the effects of pre-flowering cytokinin applications on central carbon metabolism in table grape berries"*. Dr. Patricio Olmedo (Universidad de Chile)

09:40 - 10:00 "*Unraveling the interplay between nitrogen nutrition and cell wall metabolism for growth in Arabidopsis thaliana"*. Dr. Eleodoro Riveras (Pontificia Universidad de Chile)

10:00 - 10:20 "*Carotenoid synthesis in dark-grown carrot taproots require Phytochrome A (DcPHYA)*". Dr. Rocío Quian-Ulloa (Universidad de Chile)

10:30 - 11:00 **Coffee Break**

11:15 - 13:00 **Symposium**

Title: The Plant Cell Wall.

Chair: Dr. Alejandra Moya (Universidad de Talca)

Co-Chair: Dr. Susana Sáez

Room: Bahía 1

11:15 - 11:20 **Symposium presentation**

11:20 - 11:50 "*The role of polygalacturonase MdPG1 in apple fruit ripening and texture*". Dr. Ross Atkinson (Plant & Food Research, Auckland, New Zealand)

11:50 - 12:20 "*Camelina sativa seed mucilage polysaccharide production, structure and composition*". Dr. Helen North (Institute Jean-Pierre Bourgin- INRA, Versailles, France)

12:20 - 13:00 "*Effect of RRT1, a rhamnosyltransferase elongating the RGI backbone, on Arabidopsis seed mucilage polysaccharides*". Dr. Marie-Christine Ralet (Biopolymers, Interactions & Assemblies Research Unit, INRA Nantes, France).

13:15 - 15:00 **Lunch**

15:15 - 16:45 ****[2]Oral Session 3 - Fruit Biology**

Chair: Dr. Claudia Stange Klein (Universidad de Chile)

Room: Bahía 1

15:00 - 15:20 "*New sequenced genomes of Actinidia deliciosa var. Hayward (kiwi) and Solanum lycopersicum (tomato) var. Poncho Negro, analysis and selection of drought and salt stress tolerance negative regulators as edition targets*". Dr. Samuel Parra (Universidad de Chile)

15:20 - 15:40	*"Overexpression of the Jasmonate signaling master regulator FaMYC2 increases anthocyanin biosynthesis In Strawberry fruit"*. PhD candidate Paz Zuñiga (Universidad de Talca)
15:40 - 16:00	"*Flower bud metabolic dynamics coupled with satellite imaging as predictors for dormancy status in sweet cherry trees*". Dr. Gabriela Saavedra (Universidad Mayor)
16:00 - 16:20	"*Integration of omics datasets to study ripening desynchronization in avocado cv. Hass"*. Dr. Gerardo Ñuñez-Lillo (Pontificia Universidad Católica de Valparaíso)
17:00 - 19:15	**Poster Session I**
	Posters with even numbers and coffee break
19:30 - 20:30	**Members meeting**

Wednesday, December 07

09:00 - 10:45	****[2]Oral Session 4 – Plant Genomics**
	Chair: Dr. Jonathan Maldonado (Universidad de Santiago)
	Room: Bahía 1
09:00 - 09:20	"*Network-based integrative genomics reveals convergent transcriptional connections between drought and nitrogen signals*". Dr. José M. Álvarez (Universidad Andrés Bello)
09:20 - 09:40	"*Probing genetic cross-talk of multiple plant stresses using public data"*. Dr. Nathan Johnson (Universidad Mayor)
09:40 - 10:00	"*Plant ecological genomics at the limits of life in the Atacama Desert*". Dr. Viviana Araus Caramori (Pontificia Universidad de Chile)
10:00 - 10:20	"*The TGA transcription factors from clade II negatively regulate the Salicylic acid accumulation in Arabidopsis*". Dr. Ariel Herrera-Vásquez (Universidad Andrés Bello)
10:45 - 13:00	**Poster Session II**
	Posters with odd numbers and coffee break
13:15 - 15:00	**Lunch**
15:15 - 15:55	Red Interamericana de Sociedades de Biología Vegetal - Dr. Patricia León.
15:55 - 16:00	Short communication - Dr. Claudio Inostroza
16:00 - 16:30	Merck S.A.: "Cutting edge solutions: obtaining genetic information from difficult plant tissues".
16:30 - 17:00	**Coffee Break**
17:15 - 18:45	****[2]Oral Session 5 – Plant Breeding**
	Chair: Dr. Herman Silva Ascencio (Universidad de Chile)
	Room: Bahía 1
17:15 - 17:35	"*Identification of meta-QTLs associated with drought tolerance in durum wheat"*. Dr. Andrés Schwember (Pontificia Universidad Católica de Chile)

17:35 - 17:55	"*Precision Breeding Approaches in Fruit Trees*" Ignacia Fuentes (Meristem)
18:15 - 18:35	*"Examining physiological, water relations, and hydraulic vulnerability traits to determine anisohydric and isohydric behavior in almond (Prunus dulcis) cultivars: Implications for selecting agronomic cultivars under changing climate".* Dr. Carolina Álvarez Maldini (Universidad de O'Higgins)
18:35 - 18:55	"*The Chilean race of common beans (Phaseolus vulgaris L.) a unique genetic resource for healthy human nutrition".* Dr. Basilio Carrasco (Centro de Estudios de Alimentos Procesados CEAP - Talca).
19:00 - 21:00	**Closing Ceremony and Farewell Cocktail** includes a local winery presentation and wine-tasting experience - Cavas del Valle, Elqui valley.
21:00	**Party (not included in the registration fees)**

ORALES

Aquaporins and the soil-plant-atmosphere continuum: are there new challenges?

Pablo Caceres[1,2], Carlos Manacorda[3], Moira Sutka[1,2], Victoria Vitali[1,2], Sebastian Asurmendi[3], Irene Baroli[1,2], Gabriela Amodeo[1,2*]

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Decades ago, the role of water potential gradients was established as critical in driving downhill water flows from the soil to the plant root-shoot system to reach a water-demanding atmosphere. Through this continuum, the major hydraulic resistances can be arranged in analogy to an electrical circuit. At the shoot level, stomatal conductance tightly regulates the water exchange. On the other hand, to understand how the root system is regulated we still need to integrate recent advances at the molecular level -particularly regarding aquaporins- to assess if changes in membrane water permeability on the cell-to-cell pathway have a major impact on root hydraulic conductivity. Aquaporins are highly regulated transmembrane proteins that share common structural features regardless of their multiple isoforms, high diversity in cell localization and transport selectivity. Some members of this protein family account for high water transport capacity. Our working hypothesis is that the root cell-to-cell pathway can be a strong modulator of the plant hydraulic dynamics. Our results show that plant root hydraulic adjustment is favored if the cell-to-cell pathway is highly regulated for water exchange. This is relevant not only for the plant hydraulic dynamics but also can be demonstrated that improves the short-term response to adverse plant environmental conditions.

Acknowledgements: Supported by grants UBACyT1820 & ANPCyT PICT20-1438 to GA and PIP21-23 11220200101935CO to IB

Aquaporins and the soil-plant-atmosphere continuum: are there new challenges?

Pablo Caceres[1,2], Carlos Manacorda[3], Moira Sutka[1,2], Victoria Vitali[1,2], Sebastian Asurmendi[3], Irene Baroli[1,2], Gabriela Amodeo[1,2*]

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Acknowledgements: Supported by grants UBACyT1820 & ANPCyT PICT20-1438 to GA and PIP21-23 11220200101935CO to IB

Area: System biology

“Network-based integrative genomics reveals convergent transcriptional connections between drought and nitrogen signals”.

Nathan Johnson[1], Tomás C Moyano[2], Viviana Araus[3], Jonathan Canan[1], Ji Huang[4], Gloria M. Coruzzi[4], Elena A. Vidal[1,3], **José M. Alvarez**[2,3F]

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(3) Agencia Nacional de Investigación y Desarrollo-Millennium Science Initiative Program, Millennium Institute for Integrative Biology, Santiago, Chile.
(4) Center for Genomics and Systems Biology, New York University, New York, USA.

Nitrogen (N) and water are crucial inputs for plant survival. Given their importance, the molecular mechanisms that plants rely on to signal N or water status changes - *i.e.* drought - have been under intense scrutiny. However, how plants sense and respond to the combination of nitrogen and drought signals at the molecular level has received scant attention. By utilizing the wealth of publicly available RNA sequencing data for *Arabidopsis thaliana*, we predict and compare regulatory connections between these signals. Searching online repositories, we found over 500 sequencing libraries relating to N, drought, and abscisic acid (ABA)-hormone treatments. We completed and normalized their metadata profiles, allowing for universal comparisons of treatment details and regimes. Differentially expressed genes (DEGs) were derived for each experiment and further filtered to find consistently regulated genes for a given treatment. We found high and significant overlaps for N, ABA, and drought treatment genes. More importantly, N and drought-regulated genes show a strong negative correlation indicating these are opposite signals. To investigate, we assayed changes in the *Arabidopsis* leaf transcriptome to a combinatorial design varying N and water levels. By these means, we found that increasing the N dose negatively impacts drought-responsive transcriptome. Conversely, drought levels negatively impact genes' responses to N. In addition, we identified convergent transcriptional circuits that control both N and drought responses through network inference using machine learning tools. Using mutant plants and transcriptomics, we validated NLP7 as a transcription factor that activates N signaling and negatively regulates drought responses. Hence, NLP7 could act as a central integrator of transcriptional networks in plant stress and nutrient signaling. This is a powerful approach to interrogating the relationship between signaling pathways and identifying key regulatory components for plant responses to combinatorial environmental changes.

Financing: This work is funded by ANID FONDECYT 1210389, ANID – Millennium Science Initiative Program- Millennium Institute for Integrative Biology (iBio) ICN17_022, and National Science Foundation (NSF) grant IOS – 1840761.

Keywords: Nitrogen, Drought, Transcription, Systems biology, Networks

Area: Genomics

“Plant ecological genomics at the limits of life in the Atacama Desert”.

Viviana Araus Caramori[1,3,4], Gil Eshel[2], Felipe Sobarzo[1,3,4], Soledad Undurraga[3], Daniela C. Soto[3], Carol Moraga[3], Alejandro MonteMiguel L. Allende[9], Robert DeSalle[5], Alejandro Maass[8], Dennis W. Stevenson[10], Mauricio González[11], Claudio Latorre[3], Gloria M. Coruzzi[2], Rodrigo Gutiérrez[1,3,4]

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(10) New York Botanical Garden
(11) Laboratorio de Bioinformática y Expresión Génica, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile

Atacama Desert is one of the harshest environments on Earth. Yet, dozens of species grow there, including Atacama-endemic plants. Here we establish the Talabre-Lejía transect(TLT) in the Atacama as an unparalleled natural laboratory to study plant adaptations to extreme environmental conditions. We characterized climate, soil and plants, at 22 sites along the TLT over a 10-y period. We quantified drought, nutrient deficiencies, large diurnal temperature oscillations, and pH gradients that define three distinct vegetational belts along the altitudinal cline. We deep-sequenced transcriptomes of 32 dominant plant species spanning the major plant clades. The top-expressed genes in the Atacama species are enriched in stress responses, metabolism, and energy production. To identify genes associated with plant adaptation to harsh environments, we compared 32 Atacama species with the 32 closest sequenced species and 6 model species. We perform phylogenomic reconstruction and concatenated 15,972 ortholog groups. We identified 265 candidate positively selected genes (PSGs) in the Atacama plants. For 59 PSGs with an *Arabidopsis* ortholog, we uncovered functional evidence linking them to plant resilience.

Currently, we are deepening into the study of one edible specie form TLT, *Hoffmannseggia doelli*. This plant is able to generate a tuber that was collected and consumed by ancestral Andean communities. We goal to characterize the life cycle, as well as main factors affecting its growth and yield. This study is a demonstration of the “genetic goldmine” existing in Atacama to face climate change.

Financing: Acknowledgment. Funded by CONICYT-MPG190013. Fondecyt 1220594. Millennium Institute of Integrative Biology (iBio), ICN17_022. Millennium Institute Center for Genome Regulation (iCRG) ICN2021_044, PYT-2021-0246 Fundación para la innovación agraria

Keywords: desert, evolution, adaptation, stress

Area: Genetic resources

"The Chile race of common beans *(Phaseolus vulgaris L.)* a unique genetic resource for healthy human nutrition".

Basilio Carrasco[1], Barbara Arévalo[1], Katherine Mérquez[1], Andrea Plaza[1], José Ricardo Pérez-Díaz[1], Gerardo Tapia[2], Ricardo Cabeza[3], Meisel Lee[4], Herman Silva[5], Andrés Schwember[6]

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The common bean (*Phaseolus vulgaris* L.) is native to America and consumed worldwide due to its low cost of production and valued nutritional quality. In addition, its consumption adjusts very well to the new trends of healthy eating. Natural populations of common beans spread across America began around 186,000 BP, and its domestication would have co-occurred in North and South America at approximately 6,000 to 8,000 BP. Currently, two genetic groups are recognized, the Mesoamerican pool made up of the Mesoamerican, Jalisco, and Durango races, and the Andean pool made up of the Nueva Granada, Perú, and Chile races. The Chile race is a group of landraces cultivated through various agroclimatic conditions through Chile and shows significant variability in their nutritional composition. Therefore, the Chile race is a significant genetic resource to face the effects of climate change and meet the population's nutritional needs. This research aimed to characterize the genetic identity of the Chile race through a microarray of 12,000 "Single Nucleotide Polymorphism [SNPs]" and to analyze the nutritional composition of the most consumed local varieties in our country. The results indicate that the Chile race represents a unique genetic group compared to other races of the Andean pool and shows a suitable nutritional composition (proteins, amino acids, phenolic compounds, among others), which makes it a highly relevant resource for human nutrition.

Financing: Programa de Fortalecimiento Científico de Centros Regionales ANID, Proyecto R20F0001

Keywords: Chile race; landraces, microarray, proteins, phenolic compounds, SNPs.

Area: Biochemistry and molecular biology

“To GROW or not to GROW”: molecular mechanism of cell elongation at low temperature in single plant cells”.

Jose Estévez[1]

(1) Universidad Andrés Bello, Santiago, Chile

How cells regulate their size is one of the most fascinating questions in current biology. Root hairs (RH) are single plant cells that can expand several hundred-fold their original size and they are an excellent model system for learning about cell size regulation. Root hair size determines the surface area/volume ratio of the whole roots exposed to the nutrient and water pools, thereby likely impacting nutrient and water uptake rates. Their growth speed is determined by cell-intrinsic factors like hormones (e.g., auxin and ethylene) and external environmental signals like nutrient availability in the soil (e.g., phosphate and nitrates). Previously, we have determined that the low-temperature treatment at 10°C is able to trigger, unexpectedly, an exacerbated RH growth compared to the room-temperature control condition (22°C). This plasticity in RH growth at low temperature was linked to a reduced nutrient availability in the media. In this talk, I will explore the molecular basis of this strong RH growth response involving a cell surface receptor-like kinase named FERONIA, cell wall PEROXIDASES, downstream components of the TORC1 pathway and a complex regulation of the gene expression. Although nutrient availability in the soil is one of the key factors for a sustained plant growth, the molecular mechanisms behind the perception and the downstream signaling pathway in the roots are still far from clear.

Financing: ANID – Programa Iniciativa Científica Milenio ICN17_022, NCN2021_010 and Fondo Nacional de Desarrollo Científico y Tecnológico [1200010] to J.M.E.

Keywords: *Arabidopsis*, root biology, nutrients, TOR kinase, FERONIA, low temperature

Area: Molecular Breeding, Phenomics and new breeding technologies

“Precision breeding approaches in fruit trees”

Ignacia Fuentes[1]

(1) Meristem SpA, Santiago, Chile

Base-editing is an emerging technology that enables precise modifications in a single nucleotide base in the genome. In plants, this technology has opened the possibility to apply targeted genome modifications to improve crop varieties without being subjected to GMO regulations. Being new, not many examples have been published in fruit crops. However, the potential of base-editing in plant research has already been recognized as to be revolutionary for plant biotechnology. At Meristem, we are committed to use gene-editing to generate fruit crops for a more sustainable future. Thus, we have been working to develop tools to be able to use this technology in two particularly challenging fruit trees: Mandarin and Cherry. Firstly, we started by generating novel, customized, and optimized editors that host multiple substrate specificity and editing windows. These results allowed us to have flexibility and precision to select the optimal editor for each intended edition. Moreover, we developed a platform for rapid prototyping of efficiency and precision of our editors in plant cells. This platform allowed us to confirm the correct functioning of our editors prior to testing them in species of interest. On the other hand, we are using innovative approaches to develop proprietary protocols for the transformation and regeneration of Mandarin and Cherry species. By using a fluorescence-based reporter assay and NGS, we showed we are able to obtain *in vivo* editing in cells of two recalcitrant tree species. In the following months, we will be focusing on developing editions of relevant traits in the context of contributing to having a better and more sustainable agriculture in the future.

Financing: Meristem SpA

Area: System biology

“Dissecting nitrate responses in space and time”.

Rodrigo Gutiérrez[1,2,3]

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(2) Millennium Institute for Genome Regulation
(3) Millennium Institute for Integrative Biology

Nitrate is a nutrient and a potent signal that impacts global gene expression in plants. The regulatory factors controlling spatiotemporal nitrate responses have been poorly characterized. We assayed nitrate-responsive transcriptome changes in five major root cell types of the *Arabidopsis thaliana* root as a function of time. We found that the gene-expression response to nitrate is dynamic and highly localized. Endodermis stands out as having the largest and most connected nitrate-regulatory gene network and identified *ABF2* and *ABF3* as major hubs for transcriptional responses in the endodermis cell layer. We experimentally validated TF–target interactions for ABF2 and ABF3 by chromatin immunoprecipitation followed by sequencing and a cell-based system to detect TF regulation genome-wide. Validated targets of *ABF2* and *ABF3* account for more than 50% of the nitrate-responsive transcriptome in the endodermis. Moreover, *ABF2* and *ABF3* are involved in nitrate-induced lateral root growth. We obtained an unprecedented spatiotemporal resolution of the root response to nitrate and identified important components of cell-specific gene regulatory networks. As a complementary strategy, we evaluated the role of nucleocytoplasmic mRNA dynamics during the root nitrate response, we isolated mRNA from nucleus, cytoplasm, and whole-cells from nitrate-treated *Arabidopsis thaliana* roots and performed RNA-seq analysis. We identified 402 differentially localized transcripts in these compartments (DLTs) in response to nitrate. DLTs showed five distinctive localization patterns. DLTs also exhibited large changes in RNA polymerase II occupancy of cognate genes in response to nitrate treatments and high mRNA turnover rates, indicating these are rapidly replaced mRNAs. We propose that regulating nucleocytoplasmic mRNA distribution is a strategy for buffering cytoplasmic transcript levels due to their rapid replacement. Our results show a dynamic mRNA nucleocytoplasmic distribution in response to nitrate and suggest a relevant role for mRNA nuclear export in the plant’s adaptive response to nitrogen nutrient signals.

Financing: Center for Genome Regulation is a Millennium Institute Project ICN2021_044, Millennium Institute of Integrative Biology (iBio) ICN17_022, Fondecyt 1220594

Keywords: Nitrate, mRNA, root, gene expression, spatiotemporal

Area: Biochemistry and molecular biology

“A double AP2-domain transcription factor under differential alternative splicing regulation modulates root development in salt stress”.

Jesús Hernández Urrieta[1], Martín Pincheira[1], José Miguel Álvarez Herrera[3], José Antonio O’Brien Opazo[1,2]

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(3) Universidad Andrés Bello, Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Santiago, Chile

Increased soil salinity is one of the main threats to agriculture on a global scale. Root system architecture and plasticity are key traits for plant salt tolerance by limiting salt intake and avoiding local salt batches. In the last decade, transcriptomic information has shown that alternative splicing (AS) plays a role in the response to abiotic stresses. However, AS involvement in the root developmental response to salt stress and the underlying hormonal component has not been fully explored. Here, we present the identification of a double AP2-domain transcription factor in *Arabidopsis thaliana*, PinR4, that suffers a differential intron retention event in response to salt stress. First, we used publicly available transcriptomic data to detect differential events using the ASpli software, which helped identify PinR4. Then, in a Y1H screening, we determined that PinR4 interacts with the *PCRE7* (*PIN CYTOKININ RESPONSE ELEMENT*) in the promoter of the PIN-FORMED (PIN) efflux carrier *PIN7*. Consequently, using two insertional mutant lines, we observed that the loss of PinR4 function alters root architecture in salt stress. Overall, these results suggest that PinR4 participates in the modulation of root development in response to salt stress via controlling the expression of *PIN7* and leave the door open for further exploration on whether AS regulation could be involved in the process.

We acknowledge the support given by the National Agency of Research and Development of Chile (ANID), through the Fondecyt Regular Nº1221832 and the Beca Doctorado Nacional.

Keywords: Soil salinity, salt stress, root system architecture, root development, alternative splicing regulation

Area: Biochemistry and molecular biology

"The TGA transcription factors from clade II negatively regulate the Salicylic Acid accumulation in *Arabidopsis*".

Alejandro Fonseca[1], Tomás Urzúa[1], Sebastián Velozo[1], Jean Greenberg[4], Francisca Blanco-Herrera[1,2,3], **Ariel Herrera-Vásquez**[1]

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Salicylic acid (SA) is a hormone that modulates plant defenses by inducing changes in gene expression. The mechanisms that control SA accumulation are essential for understanding the defensive process. TGA transcription factors from clade II in *Arabidopsis*, which include the proteins TGA2, TGA5, and TGA6, are known to be key positive mediators for the transcription of genes such as *PR-1* that are induced by SA application. However, unexpectedly, stress conditions that induce SA accumulation, such as infection with the avirulent pathogen *P. syringae* DC3000/AvrRPM1 and UV-C irradiation, result in enhanced *PR-1* induction in plants lacking the clade II TGAs (tga256 plants). Increased *PR-1* induction was accompanied by enhanced isochorismate synthase-dependent SA production as well as upregulation of several genes involved in the hormone's accumulation. In response to avirulent *P. syringae*, PR-1 was previously shown to be controlled by both SA-dependent and-independent pathways. Therefore, the enhanced induction of PR-1 (and other defense genes) and accumulation of SA in the tga256 mutant plants is consistent with the clade II TGA factors providing negative feedback regulation of the SA-dependent and/or -independent pathways. Together, our results indicate that the TGA transcription factors from clade II negatively control SA accumulation under stress conditions that induce the hormone production. The transcriptional control could be exerted by the direct binding of TGA2 to the promoter of the *SARD1* gene, a master regulator of SA production. Our study describes a mechanism involving old actors playing new roles in regulating SA homeostasis under stress.

Financing: FONDECYT iniciación 11200944

Area: Genomics

“Probing genetic cross-talk of multiple plant stresses using public data”.

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Plants undergo many stresses during their life-cycle. Essential nutrient availability, environmental stressors like light and drought, and biotic interactions all form a mosaic of challenges, several of which might occur simultaneously. Gene-regulatory-networks (GRNs) have been used to explore the genetic components involved in stress at an individual treatment level. However, we know considerably less about how these networks interact between multiple stresses. We explored this topic in nitrogen (N), drought (D) stresses and treatments of the phytohormone ABA. Utilizing public sequencing data, we were able to identify over 1000 libraries from N, D, and ABA treatments, including more than 20 pairwise experiments for each. With differential expression analysis we were able to identify treatment dependent genes, combining the many experiments to build a core set of the most consistently regulated genes. These sets include many previously known genes related to these stresses, but also point to a much broader and distinct set of relevant genes. Comparing gene sets and expression between N and D points to a strong negative relationship between these stresses, hinting at a shared regulation pathway. By constructing GRNs we were able to identify key transcription factors that play a role in controlling this NxD relationship. These data point to an important crosstalk relationship between these stresses and provide a framework for further explorations into relationships with other stresses.

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Keywords: Big data, stress crosstalk, nitrogen, drought, regulatory networks

Area: Plant physiology

Integration of omics datasets to study ripening desynchronization in avocado cv. Hass

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Hass avocado ripening desynchronization is a recurrent problem and involves color desynchronization with softening at ready-to-eat (RTE) stage. This study aimed to unravel the mechanisms driving this desynchronization and to propose potential biomarkers by integration of transcriptomics, proteomics, and metabolomics datasets from avocado exocarp of different storage conditions and color phenotypes. Integration of omics datasets and network analysis resulted in eight transcription factors associated with differentially regulated genes between regular air (RA) and controlled atmosphere (CA) and twelve transcription factors related to avocado fruit color desynchronization control in RTE stage. CA positively correlated to genes involved in hormonal biosynthesis and signaling of auxins, ethylene, cytokinins and brassinosteroids, while RA revealed enrichment of cell wall remodeling and abscisic acid (ABA) content associated genes. At RTE higher contents of flavonoids, ABA and brassinosteroids were associated with color-softening synchronized avocados. In contrast, color-softening desynchronized fruit revealed increases of jasmonic acid, salicylic acid, and auxins levels.

Financing: This research was supported by Fondecyt N°1220223 and N°3210011 and Millennium Science Initiative Program – ICN2021_044 from ANID.

Keywords: color desynchronization, transcriptomics, proteomics, metabolomics, transcription factors, pigments, softening

Area: Biochemistry and molecular biology

"Cytokinin and auxin modulation are key to the plant developmental changes in response to *A. nodosum biostimulants in A. thaliana*".

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Area: (e) Biochemistry and molecular biology

Climate change and the growing global population is putting pressure on agriculture to increase productivity using less and more sustainable resources. Accordingly, plant biostimulants have emerged as an alternative to conventional agricultural products. It has been shown that *Ascophyllum nodosum*-derived biostimulants have been widely used as growth stimulants and to protect crops against abiotic stresses, such as freezing, salinity, and drought. *A. nodosum*-derived biostimulants have a phytohormone-like effect on plants, specifically a cytokinin-like or an auxin-like effect. However, the biostimulant composition has not been fully characterized yet. This has led to a lack of understanding of these products' underlying mechanisms of action and their role in the modulation of phytohormones throughout the plant. Here we used cytokinin-like and auxin-like commercial biostimulants produced by the fermentation of *A. nodosum* extracts to evaluate the phenotypic response and the hormonal signaling induced in *Arabidopsis thaliana* plants. Both biostimulants were able to modulate root development, particularly by promoting lateral root growth and root hair development. Moreover, we used GUS and GFP reporter lines to characterize how auxin and cytokinin signaling is modulated in the plant, confirming their antagonistic hormone modulation. Finally, we used RNAseq to further evaluate the mechanisms of action of these biostimulants.

Financing: Fondecyt Regular Nº1221832

Keywords: Biostimulants, auxin, cytokinin, root development

Area: System biology

“Proteomic and metabolomic integration reveals the effects of pre-flowering cytokinin applications on central carbon metabolism in table grape berries”.

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Enhanced table grape quality parameters influence consumer preferences worldwide. To achieve higher quality traits at ripening, grapevine producers apply different plant growth regulators. The synthetic cytokinin CPPU is widely used. However, the effect of CPPU on berry quality is poorly understood. In this study, we investigated the role of pre-flowering CPPU applications on the quality traits of ‘Thompson Seedless’ berries by integrating proteomic and metabolomic analyses. CPPU-treated grapevines showed a significant increase in berry size and firmness. Proteomic analyses indicated that CPPU-treated berries accumulated carbohydrate metabolism, glycolysis, and TCA cycle enzymes at harvest. Metabolomic analyses showed a differential abundance profile of compounds associated with carbohydrate metabolism and TCA cycle intermediates in grape berries. CPPU-treated berries showed an accumulation of primary metabolism compounds, such as organic acids and amino acids, compared to control berries. These findings suggest that CPPU applications modulate central carbon metabolism, leading to grape berry quality improvement.

Financing: Fondecyt Regular 1200260.

Keywords: *Vitis vinifera*, CPPU, proteomics, metabolomics, TCA cycle, glycolysis

Area: Biochemistry and molecular biology

"New sequenced genomes of *Actinidia deliciosa* var. Hayward (kiwi) and *Solanum lycopersicum* (tomato) var. Poncho Negro, analysis and selection of drought and salt stress tolerance negative regulators as edition targets".

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Climate change is a dramatic global event that produces droughts and soil salinization. To cope with abiotic stress, plants respond through dependent and independent abscisic acid (ABA) signaling activating adaptative responses generating tolerance. Switching to stress response requires the rapid synthesis and removal of proteins through the activity of negative regulators (NR) to promote plat abiotic stress tolerance yet, constitutive expression of NRs produce susceptibility to abiotic stress. Tomato (*Solanum lycopersicum*) and Kiwi (*Actinidia deliciosa* var Hayward) are highly consumed worldwide; however commercial varieties are very susceptible to environmental variation like droughts and soil salinization. In this work we have identified five NRs genes to drought and salt stress in different plants from available data (*OsDIS1, SlACO2, AtACS6, OsSRFP1* and *OsiSAP7*) and selected their orthologs in Chilean northern endemic tomato Poncho Negro variety and kiwi as candidates for gene editing. *OsDIS1*, *OsSRFP1* and *OsiSAP7* codes for E3 ubiquitin ligases while *SlACO2*, *AtACS6* code for ethylene biosynthesis enzymes. To design specific gRNAs for CRISPR editing we sequenced and assembled (guided by reference genomes) Poncho Negro and Kiwi var. Hayward genomes performed by PacBio and Illumina short read sequencing with 98 and 96% of completeness. We tested the expression of the selected orthologs under an acute salt and drought stress assays in tomato and kiwi and selected the best paralog for being edited in both species. The structure and complete sequence of each gene was obtained for gRNA design and explants were transformed for edition and functionality assays.

Financing: This work was supported by the National Commission for Scientific and Technological Research of Chile (Anillo ACT192073)

Keywords: Abiotic stress, CRISPR, drought, salinity, genome sequencing, Tomato, Kiwi

Area: Plant physiology

“Plant-growth promotion by proteobacterial strains depends on the availability of phosphorus and iron in *Arabidopsis thaliana plants*”.

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Rhizospheric microorganisms have crucial capacities to mobilize nutrients, release them from organic sources and increase their availability in the soil, affecting plant nutrition. Phosphorus (P) and iron (Fe) are critical nutrients in plants and can be affected by microbial activity. They are often poorly available for plants due to the low solubilities of their chemical sources or their physical reactions with soil particles. Little is known about how plant-microbial interaction changes due to P or Fe chemical compounds with different solubilities or bioavailability. Additionally, the molecular mechanisms underlying microbial modulation of P or Fe homeostasis in plants have yet to be fully understood. Here, three proteobacteria were used to inoculate *Arabidopsis thaliana* plants growing in different sources and concentrations of these nutrients (*Paraburkholderia phytofirmans* PsJN, *Pseudomonas putida* KT2440, and *Azospirillum brasilense* Sp7). All the strains promoted growth in P and Fe deficiencies in a manner that depended on the nutritional factor. A high regulation of the P content was observed in plants. Nevertheless, the inoculation with KT2440 increased P compared to the non-inoculated plants in the low-P treatment. On the contrary, inoculated plants tended to accumulate less Fe, suggesting a competition for this nutrient in the rhizosphere. In short-term experiments, the PsJN strain moved from neutral/positive to detrimental depending on the Fe source. Still, this phenomenon was not observed in the long term. The effects of the PsJN strain in a double mutant of the phosphate starvation response (PSR) were also affected by the P-source. Furthermore, depending on the nutrient source, PsJN and Sp7 strains differentially regulated PSR and IAA- associated genes. In the case of Fe, PsJN and SP7 regulated iron uptake-related genes regardless of the iron source. Overall, the results show that bacterial-induced plant growth is susceptible to the nutritional status of the soil in a strain-specific manner.

Financing: FONDECYT 1190634; MN-SAP NCN2021_010 and ANID PIA/BASAL FB0002

Keywords: Plant-growth Promoting Bacteria, phosphate, iron, PSR, beneficial bacteria, nutrient deficiency, plant microbiome

Area: Biochemistry and molecular biology

“Carotenoid synthesis in dark-grown carrot taproots require Phytochrome A (DcPHYA)”.

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Carotenoids provide yellow, orange, and red colors to flowers and fruits, participate in photosynthesis, protect molecules against photo-oxidative damage, and are precursors of plant hormones. In vegetative tissue and fruits, light promotes carotenoid synthesis through light signaling molecules, such as photoreceptors (PHYs). PHYA is both active and stable under Far-red (FR) light, where it promotes photomorphogenesis. On contrary, the orange carrot (*Daucus carota)* taproot (Nantes var.) synthetizes and accumulates high levels of carotenoids growing underground, and light impairs both carotenoid synthesis and root development. To understand the genetic regulation, we performed RNA-seq between root grown in light (R/L) and underground (R/D). Genes related to light signaling, such as *DcPHYA* are overexpressed in R/D. Also, *DcPHYA* is expressed preferably in R/D than in R/L during carrot taproot development. DcPHYA has 73% amino acid identity with AtPHYA in their functional domains. Besides, in tobacco (*Nicotiana tabacum)* expressing *DcPHYA*, present reduced hypocotyl length under FR, and reduced expression of *NtPEPCK* and *NtPar1-like*, that are genes negatively regulated by PHYA, suggesting that *DcPHYA* is functional in FR. To evaluate the role of *DcPHYA* in carotenoid synthesis in tobacco we developed treatments with shade, white and dark lights. Carotenoid content analysis of seedlings will be shown. Carrot antisense lines with over 80% reduced relative expression of *DcPHYA* present between 65%-80% reduction in carotenoid content in the taproot as well as in the relative expression of *DcPSY1, DcPSY2* and *DcLCYB1* compared to wild-type plant. These results suggest that *DcPHYA* is functional and participates in carotenoid synthesis in the carrot taproot grown underground.

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Keywords: Carotenoid, phytochrome, photomorphogenesis, Carrot, taproot

Area: Cell biology

“Unravelling the interplay between nitrogen nutrition and cell wall metabolism for growth in *Arabidopsis thaliana*”.

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Nitrate acts as a nutrient and signal that regulates plant growth and development. The shoot growth is impacted by nitrate through cell expansion and endoreplication regulation. In the transcriptomic analysis in shoots grown with either 0- or 5-mM nitrate, we found an overrepresented biological process associated with cell wall organization. However, the interaction between nitrate and cell wall metabolism during cell expansion has not been studied. To characterize the cell wall metabolism in response to nitrate, we first evaluated the role of cellulose in nitrate-mediated growth. Plants treated with Isoxaben (inhibitor of the Cellulose Synthase (CESA) Complex) showed a reduction in shoot growth and cell expansion under contrasting nitrate conditions. Interestingly, Isoxaben decoupled nitrate effect on ploidy levels and cell expansion. Similarly, we observed a reduced shoot growth in response to nitrate in mutant plants of the CESA Complex. To evaluate the impact of nitrate in the CESA complex, we measured the CESA complex velocity under contrasting nitrate conditions. We found an increment of CESA complex velocity in the presence of nitrate. Furthermore, we found that nitrate phosphorylates CESA3 protein. To evaluate the role of pectin in nitrate-mediated growth, we assessed the pectin methyl esterification levels under the nitrate regime. We found that the abundance of highly methylated pectin increases with nitrate availability. Our results indicate cellulose and pectin modification are essential for plant growth in response to nitrate and highlight the importance of studying the interplay between nitrate and cell-wall metabolism during cell expansion.

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Keywords: Nitrate, Cell wall, Shoot growth

Area: Biochemistry and molecular biology

“Gene regulatory networks controlling cell proliferation and cell expansion during plant organ growth”.

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Within meristems, the mitotic cell cycle (MCC) produces new cells that subsequently engage in cell expansion and differentiation programs. The latter is frequently associated with endoreplication (ER), an alternative cell cycle that replicates DNA without nuclear division, causing an increase in somatic ploidy.

MCC regulators have oscillating activities regulated by transcriptional and posttranscriptional mechanisms. For example, the E2F and MYB3R transcription factors (TF) control gene expression during the G1/S and G2/M transitions. Genomic studies indicated that only a subset of the genes with expression enriched at the G1/S or G2/M transitions are directly regulated by the E2F or MYB3R TFs, suggesting that other regulators remain to be identified.

To identify these genes, we first analyzed the transcriptome of cells in G2/M and found hundreds of genes whose expression is reduced or enriched in G2/M cells. This dataset enabled us to identify new potential regulators, including TFs, enzymes involved in redox and calcium metabolism, motor proteins, etc.

Here, we focus on *SCARECROW-LIKE 28* (SCL28), a GRAS transcription factor, whose mRNA accumulates to the highest levels in G2/M and is regulated by MYB3R transcription factors. Functional analysis indicates that *SCL28* promotes progression through G2/M and modulates the selection of cell division planes.

Surprisingly, gene expression studies indicated that SCL28 regulates *SIAMESE-RELATED* genes, encoding CDK inhibitors with a role in promoting mitotic cell cycle exit and endoreplication. Consistent with this, mutants in *SCL28* displayed reduced endoreplication. We also found evidence indicating that *SCL28* regulates genes related to the biogenesis, assembly and remodeling of the cytoskeleton and cell wall.

Altogether, our results suggest that *SCL28* has dual functions modulating not only cell proliferation, but also endoreplication, cell expansion and differentiation.

Keywords: development, mitosis, endoreplication, transcription factors

Area: Plant physiology

“Flower bud metabolic dynamics coupled with satellite imaging as predictors for dormancy status in sweet cherry trees”.

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Perennial trees such as sweet cherries have annual growing cycles with different phenological stages controlled by endogenous and environmental signals. In summer, active vegetative growth occurs followed by senescence of leaves and dormancy during autumn and winter, respectively. After a prolonged chilling exposure plants are released from dormancy blooming in spring. We believe that carbon dynamics within the tree and the different tree phenological stages are connected. To validate our hypothesis, we measured several physiological parameters in adult leaves of the late sweet cherry variety ‘’Regina’’. CO2 assimilation, transpiration, stomatal conductance and photochemical parameters were evaluated during the whole vegetative stage (summer), foliar senescence (autumn) and dormancy entrance (beginning of winter). To complement the analysis, floral buds were collected to analyze polar metabolite composition by GC-MS. The results showed that sweet cherry trees consume high CO2 during the vegetative stage, and this is accompanied by a greater conversion of photons to chemical energy, which decays towards dormancy. Fructose, glucose, malic, palmitic and stearic acids, as well as sorbitol and myoinositol showed a close relationship with the phenological stage of the tree and dormancy stage. We also monitored the field through remote sensing to correlate the vegetative indices derived from satellite images with physiological parameters, in order to estimate the timing of key phenological events. Our results indicate that carbon dynamic pattern and metabolite composition can be used as phenological and dormancy stages predictors in sweet cherry trees.

Financing: Fondecyt 1201010, ANID/ACT210007

Keywords: Prunus avium, Dormancy, Photosynthesis, Organic acids, Sugars, Phenology

Area: Cell biology

“Regulation of PME58 in *Arabidopsis* seed coat mucilage pocket”.

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Seed mucilage is a hydrated gel-like structure produced by seed coat tegument of *Arabidopsis* seed coat. *Arabidopsis* mucilage is mainly composed of unbranched rhamnogalacturonan-I (RG-I) and homogalacturonan (HG) pectic domains. HG domain is form by a backbone of Galacturonic acid (GalA) which can be methylesterified. The methylesterification pattern is key because it defines the fate of its domain: two demethylesterified HG with calcium could form the “egg box” structure forming micro gels into the cell wall, associated with cell wall stiffening. On the other hand, a random pattern of methylesterification could lead to HG degradation by specific enzymes causing cell wall softening. The demethylesterification is carried out by the enzymes pectin methylsterases (PME) which are regulated by its inhibitor (PMEI). It has been described that PME58 have a role in mucilage structuration. Our group identified two PMEIs (PMEI3 and PMEI13) with the same PME58 expression profile suggesting their participation in HG metabolism. Additionally, mutants in *pmei* show alterations in mucilage release and mucilage structuration. Biochemical analyses were carried out, demonstrating that both mutant lines have less methanol content compared to wild type in their mucilage layers confirming the participation of both enzymes in mucilage HG metabolism. To determine if both PMEIs can inhibit PME58, double mutants were generated, and biochemical and immunological analyses were conducted suggesting that both PMEIs are able to inhibit PME58 within mucilage pocket. Finally, structural biology was used to determine if both PMEIs inhibit PME58, the molecular docking assay showed that PMEI13 more affinity to PME58 than PMEI3.

Financing: Fondecyt Regular 1201467

Keywords: *Arabidopsis*, Mucilage, Pectin, Homogalacturonan, PME, Seed Coat

Area: Molecular Breeding, Phenomics and new breeding technologies

“Identification of meta-QTLs associated with drought tolerance in durum wheat”.

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Abiotic stress strongly affects yield-related traits in durum wheat, in particular drought is one of the main environmental factors that have effect on grain yield and plant architecture. In order to obtain new genotypes well adapted to stress conditions, the highest number of desirable traits needs to be combined in the same genotype. In this context, hundreds of QTL have been identified for yield-related traits in different genetic backgrounds and environments. Meta-QTL (MQTL) analysis is a useful approach to combine data sets and for creating consensus positions for the QTL detected in independent studies for the reliability of their location and effects. MQTL analysis is a useful method to dissect the genetic architecture of complex traits, which provide an extensive allelic coverage, a higher mapping resolution and allow the identification of putative molecular markers useful for marker-assisted selection. In the present study, a complete and comprehensive MQTL analysis was carried out to identify genomic regions associated with grain-yield related traits in durum wheat under different water regimes. A total of 724 QTL on all 14 chromosomes (genomes A and B) were collected for the 19 yield-related traits selected, of which 468 were reported under rainfed conditions, and 256 under irrigated conditions. Out of the 590 QTL projected on the consensus map, 421 were grouped into 76 MQTL associated with yield components under both irrigated and rainfed conditions, 12 genomic regions containing stable MQTL on all chromosomes except 1A, 4A, 5A and 6B. Candidate genes associated to MQTL were identified and an *in-silico* expression analysis was carried out for 15 genes selected among those that were differentially expressed under drought. These results can be used to increase durum wheat grain yields under different water regimes and to obtain new genotypes adapted to climate change.

Financing: Fondecyt Regular n. 1210092

Keywords: Meta-QTL, drought tolerance, yield component, durum wheat

Area: System biology

“WRKY-TF, a novel transcription factor that negatively regulates periderm formation”.

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During plant secondary growth the periderm arises as a protective layer in roots replacing the epidermis. Lengthwise, the periderm comprises a third of the mature taproot and it is derived from the pericycle. Composed of 3 different cell types – phellogen, phelloderm and phellem – the periderm is present in most eudicots’ plants. The transit-amplifying cells forming the meristematic phellogen tissue differentiate bidirectionally; inwardly into the phelloderm and outwardly into the phellem. Although histological and quantitative root staining studies have shed light into the molecular mechanism of the periderm development, no key regulatory gene for periderm development has been identified so far. In this work, we used for the first-time natural variation resources to study secondary growth and gain better understanding in periderm development. We employed a neural network to measure periderm, which combined with automated microscopy/cellular phenotyping and Genome-Wide Association Studies led to uncover *WRKY-TF*, a novel and undescribed gene in periderm formation. Root staining, confocal microscopy, and single-cell RNA-seq reveal that *WRKY-TF* is a key repressor in periderm development.

Keywords: Periderm, roots, plant development

Area: Biochemistry and molecular biology

“Overexpression of the jasmonate signaling master regulator *FaMyc2* increases anthocyanin biosynthesis in strawberry fruit”.

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Commercial strawberry (*Fragaria ×ananassa*) is an attractive model for studying molecular mechanisms implicated in fruit biology. It´s an important source of flavonoids for human consumption such as anthocyanins, but little is known about the upstream regulatory genes controlling anthocyanin biosynthesis. MYC2 is a core transcription factor of most aspects of the jasmonate signaling pathway in plants. Also acts to control the expression of genes that participated in the synthesis of secondary metabolites in a species-specific manner. In this research, we study the effect of agrobacterium-mediated native *MYC2* overexpression along with a mutated non-repressible *MYC2*-variant on anthocyanin biosynthesis in strawberry fruit. We analyzed differences in color, anthocyanin content, the profile of phytohormones, and changes in transcript levels of transcriptional regulators and genes associated with anthocyanin biosynthesis. Also, the promoter regions of differentially expressed genes were analyzed to identify MYC2 *cis*-regulatory elements. The overexpression of native and mutated MYC2 induced higher anthocyanin content in fruits as well as an increase in the expression levels of genes related to MBW complex (*FaMYB10*), anthocyanin biosynthesis (*FaANS*, *FaUFGT*), abscisic acid biosynthesis (*FaNCED1*, *FaNCED2*), and the transcriptional regulator *FaYAB1*. Also, we found the presence of canonical G-box (CACGTG) and previously described G-box-like elements on the promoter regions of these genes. Together, these data suggest that *FaMYC2*, the master regulator of JA signaling, could be a relevant upstream positive regulator for anthocyanin biosynthesis through *FaMYB10* controlling expression.

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Keywords: Strawberry, Jasmonates, Anthocyanins, MYC2

EFFECT OF RRT1, A RHAMNOSYLTRANSFERASE ELONGATING THE RGI BACKBONE, ON *ARABIDOPSIS* SEED MUCILAGE POLYSACCHARIDES

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During seed development in certain species, the epidermal cells of the seed coat accumulate polysaccharides. On imbibition, the hydrated polysaccharides are released and encapsulate the seed in a halo of mucilage. In *Arabidopsis*, mucilage consists of two layers; the outer layer is formed almost exclusively from the pectic domain RGI, which consists of the repeating disaccharide unit [→2)-*α*-L-Rha-(1 → 4)-*α*-D-GalUA-(1→], while the inner layer contains cellulose and small amounts of other polymers in addition to RGI (Macquet et al., Plant Cell Physiol., 2007).

Recently, a rhamnosyltransferase, which transfers the rhamnose residue from UDP-*β*-L-rhamnose to RG-I oligosaccharides, has been identified and characterized (Takenaka et al., Nature Plants, 2018). *RRT1* is highly expressed during formation of the seed coat mucilage but the amount of mucilage produced by the *rrt1* T-DNA mutant is intriguingly only slightly reduced and validation of *rrt1* phenotypes with different alleles is needed. GATL5, a potential galacturonosyltransferase, has also been shown to be associated with the production of *Arabidopsis* seed coat mucilage.

In the present work, two *rrt1,* one *gatl5*, and one double CRISPR mutants were generated. The amount of mucilage produced in the outer and inner layers and the macromolecular characteristics of the polysaccharides of the outer layer of these mutants were investigated.

CAMELINA SATIVA SEED MUCILAGE POLYSACCHARIDE PRODUCTION, STRUCTURE AND COMPOSITION

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Camelina sativa (camelina) is an oilseed crop from the Brassicaceae family whose cultivation is being revived due to its high potential in sustainable and agroecological farming. Camelina is myxospermic and on imbibition its seeds become encapsulated by a polysaccharide-rich hydrogel called mucilage. The constituents of mucilage are accumulated in the epidermal cells of the seed coat during its development on the mother plant. Mucilage is an excellent system for studying the production of polysaccharides and interactions between polymers. Here, we present a detailed characterisation of the structure and differentiation of camelina seed coat epidermal cells. Camelina mucilage is generally more abundant than that of its close relative *Arabidopsis* indicating that polysaccharide synthesis is more active in the former. In addition, results will be presented concerning the composition and structural organisation of camelina mucilage. These have been analysed in detail through a combination of cytological stains, immunolocalisation and biochemical analyses. Camelina seed coat epidermal cells are hexagonal shaped, have columella and produce mucilage where the predominant component is the pectin rhamnogalacturonan I, like *Arabidopsis*. Nevertheless, our analyses highlight specific features of camelina mucilage production and release, indicating fine-tuning of its structure and properties for function. Together, our data outline the potential of camelina mucilage as a complementary resource for studying polysaccharide synthesis and physicochemistry and as a valuable co-product from camelina cultivation.

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The role of polygalacturonase MdPG1 in apple fruit ripening and texture

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Polygalacturonases (PGs) breakdown homogalacturonan, a cell wall pectin that is a major component of the middle lamella that sticks cells together. In apple fruit, *MdPG1* is a key gene associated with fruit quality [(a)] and marker assisted selection for the gene is deployed to improve apple texture. Apple fruit from lines overexpressing *MdPG1* produced a 'silver' fruit, where cells in the epidermal and hypodermal layers are separated from each other, allowing light reflection. The fruit also show high water loss associated with cuticular cracking. Despite the physical changes, little change is seen in immunolabelling for pectins and chemical changes to the cell wall are minimal. PGox apples appeared to mature earlier than wildtype apples. Although literature suggests oligosaccharides from breakdown of pectin may induce faster fruit maturation, we found no evidence for a higher level of such molecules in PGox fruit. Transcriptomic analysis was used to investigate the molecular basis for the accelerated ripening. From this study candidate genes are being studied in a fast flowering system that allows transgenic apple fruit to be obtained in less than one year.

[(a)] Atkinson et al. (2012). Down-regulation of POLYGALACTURONASE1 alters firmness, tensile strength and water loss in apple (*Malus* x *domestica*) fruit. BMC Plant Biol. 12, 129. doi:10.1186/1471-2229-12-129

Examining physiological, water relations, and hydraulic vulnerability traits to determine anisohydric and isohydric behavior in almond (Prunus dulcis) cultivars: Implications for selecting agronomic cultivars under changing climate

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The search for drought tolerant species or cultivars is important to address water scarcity caused by climate change in Mediterranean regions. The anisohydric–isohydric behavior concept has been widely used to describe stomatal regulation during drought, simply in terms of variation of minimal water potential (9min) in relation to pre-dawn water potential (9pd). However, its simplicity has sometimes failed to deliver consistent results in describing a complex behavior that results from the coordination of several plant functional traits. While Prunus dulcis (almond) is known as a drought tolerant species, little information is available regarding consistent metrics to discriminate among cultivars or the mechanisms underlying drought tolerance in almond. Here we show a sequence of plant stomatal, hydraulic, and wilting responses to drought in almonds, and the main differences between anisohydric and isohydric cultivars. In a pot desiccation experiment we observed that stomatal closure in P. dulcis is not driven by loss in turgor or onset of xylem cavitation, but instead, occurs early in response to decreasing 9min that could be related to the protection of the integrity of the hydraulic system, independently of cultivar. Also, we report that anisohydric cultivars of P. dulcis are characterized by maximum stomatal conductance, lower water potentials for stomatal closure and turgor loss, and lower vulnerability to xylem cavitation, which are traits that correlated with metrics to discriminate anisohydric and isohydric behavior. Our results demonstrate that P. dulcis presents a strategy to avoid cavitation by closing stomata during the early stages of drought. Future research should also focus on below-ground hydraulic traits, which could trigger stomatal closure in almond

Financing: Fondecyt Iniciación n° 11200807

Keywords: drought, functional traits, xylem vulnerability to cavitation, hydroscapes, leaf water potential, stomatal conductance

PANELES

Area: System biology

Gene regulatory network of the Sulfate deficiency response *in Solanum lycopersicum*

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Sulfur (S) is an essential macronutrient and a key factor for plant growth. Deficiency of sulfate, the main source of S in soils, has profound effects on development, leading to growth retardation and a reduction in crop productivity. In tomato, sulfate deficiency causes detrimental effects on leaf growth, that are partly explained by concomitant changes in expression of thousands of transcripts. In this work, we aim to define gene regulatory networks (GRNs) underlying this massive reprogramming of the transcriptome in leaves in response to sulfate deficiency and to pinpoint key regulatory transcription factors. For this, we used available transcriptomic data and chromatin accessibility data of tomato leaf tissue, to determine regulatory interactions between transcription factors (TF) and their genome-wide targets. Our approach allowed us to produce a context-specific GRN for sulfate deficiency responses in tomato leaves. A rank of TFs with a central role in controlling sulfate-related genes was generated by analyzing centrality measures, as well as expression and potential involvement of TF homologs in *Arabidopsis* in nutrient responses and growth processes. The top 5 TFs are predicted to control a relevant proportion of sulfate-responsive genes in leaves, with an overrepresentation of terms related to sulfate deficiency responses, immune responses, leaf senescence and signaling pathways involving salicylic and jasmonic acid hormones. Our results provide important ground information about regulatory networks essential in tomato responses to sulfate deficiency stress.

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Keywords: GRN, Solanum lycopersicum, GENIE3, Sulfate, Tomato

Area: Plant physiology

UNRAVELLING THE *TRICHODERMA*-PLANT-PATHOGEN INTERACTION MODULATED BY NITROGEN NUTRITION IN TOMATO

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Nitrogen is a fundamental macronutrient for growth and developmental processes of plants as well as for obtaining high yields in agricultural productive systems. However, it corresponds to one of the most limiting nutrients in agricultural fields with an impact on plant response to abiotic and biotic stresses. Variations in nitrogen availability have been correlated to susceptibility to diseases, and its effect depends on the plant species and pathogen. Tomato (*Solanum lycopersicum*) corresponds to one of the most widely planted and consumed vegetables worldwide, becoming a reference species for plant-pathogen interaction studies. *Botrytis cinerea* (*Bc*) is one of the most damaging pathogens that attack tomato and many other plant species, and its control strategies are based in the application of periodic and large quantities of chemical fungicides with the consequent negative environmental and human health impacts.

In this context, the use of beneficial symbionts of the genus *Trichoderma* becomes a promising alternative to conventional practices for disease management. Many *Trichoderma* species grow in the rhizosphere and the associated plant responses result in an induced systemic resistance that confers tolerance to pathogen invasion.

In order to characterize the defense response against *Bc* mediated by *T. atroviride* (*Ta*) and modulated by nitrogen, *we used S. lycopersicum cv.* 'Moneymaker' plants cultured under contrasting nitrate availabilities, representing limitation and sufficiency, in hydroponics. Our phenotypical results indicate that the defense response to *Bc* infection is positively affected by *Ta* colonization of the roots and also enhanced by nitrogen nutrition in tomato plants, showing a major reduction of the lesion size of the *Bc*-inoculated leaves in plants exposed to nitrogen sufficiency and *Ta*-root colonization.

Currently, we are working at the molecular level in order to have a complete overview of this complex biological interaction and provide insights into potential crosstalk mechanisms.

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Keywords: plant-pathogen interaction, *Trichoderma*, nitrogen, *Botrytis*, tomato, plant defense response

Area: Plant physiology

BIOMECHANICS OF *OPUNTIA FICUS-INDICA* ROOTS EXPOSED TO EXTREME DROUGHT

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Succulent plants possess traits that allow them to complete physiological functions under extreme environments and roots are at the frontline of the stress: the drying soil. Previous works in succulent plants have reported the extraordinary reversible mechanism of root shrinkage that disconnects plants from drying soils, reestablishing the hydraulic connection when water availability is restored. Yet, this rectifier-like mechanism would require complex biomechanical control at organ, tissue, and cell level. In here we evaluated the changes of the mechanical behavior of *Opuntia ficus-indica* fine roots under extreme drought stress. Using a combination of tensile strength test techniques to quantify the mechanical behavior of the different treated *Opuntia* roots on the stress-strain relationship, breaking strain, tensile strength (N), breaking stress (MPa), and Young´s module as breaking E (MPa). We found that fine roots get more elastic as drought stress gets more extreme, allowing cells to modify their shape while preventing permanent damage. Furthermore, we found that extreme drought progressively increased root shrinkage over 10-, 30-, and 45-days period (6%, 10%, and 22%, respectively). Our data suggest that, in drought stressed succulent plants, the biomechanics of organs, tissues, and possible cell walls are deeply coupled with suberin deposition and structural damage inside endodermis via lacunae formation and possible cell wall folding. Nevertheless, further investigation is needed to understand how cell wall folding could be a relevant factor that alters fine root biomechanics under extreme drought.

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Keywords: Succulent plants, Root biomechanics, Drought, Mechanical behavior, Root shrinkage

Area: Plant physiology

GRAPEVINE ROOTSTOCKS SELECTED FROM HYPER-ARID ENVIRONMENTS OF CHILE: A NEW TECHNOLOGY TO CONFRONT SALINE AND HYPERSALINE ENVIRONMENTS

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Salinity is a major problem in vineyards from semi-arid to arid regions in the world, and this problem is expected to worsen due to climate change. Grapevine fine roots are the gatekeepers of water and nutrients uptake from the soil; and are challenged to interact with complex and extreme environments in the soil-plant-atmosphere continuum (SPAC). Salinity modifies the osmotic component of the root medium, producing alterations on the hydraulic relations of the medium-root interface (i.e., the exosmotic and endosmotic hydraulic conductivity of the root, Lpr_{EX} and Lpr_{EN}, respectively). Naturalized grapevines selected from hyper-arid environments of Chile (i.e., Atacama Desert; G-65 and G-70) could possess different biophysical mechanisms that can be useful to overpass the salinity problems compared to traditional rootstocks (i.e., 101-14 Mgt and 110-R). However, the hydraulic properties of fine roots under different salinity environments remains unknown. Using novel root hydraulic techniques, we measure and studied the Lpr_{EX} and Lpr_{EN} under four levels of NaCl hydroponic treatments. Here we show that salt stress produced a general reduction between 20-90% of Lpr. Lpr_{EX} of G-65 and G-70 were 10-fold higher at a concentration of 250 mM NaCl compared to 101-14 Mgt and 110-R, and Lpr_{EN} displayed a reduced Lpr (20-70%), especially for 101-14 and 110R. Furthermore, we found that the Lpr_{EX} and Lpr_{EN} (i.e., movement of water from the medium to, and through the root) exhibit similar behavior for G-65 and G-70 and similar differences were found in 101-14 Mgt and 110-R. These results could be related to the modification of cortical cell wall material deposition and/or structure (e.g., suberin and lignin, and cortical lacuna, respectively). Besides, our results suggest that hyper-arid rootstock grapevines from Atacama Desert of Chile could possess new biophysical adaptations, that should be further studied, helping to maintain the SPAC under high levels of salinity.

Financing: This study was funded by Agencia Nacional de Investigación y Desarrollo (ANID), within the Fondecyt Regular grant Nº1220235. C.B-S received funding through and ANID doctoral scholarship Nº21221119.

Keywords: Salinity, Grapevine, Rootstock, Root hydraulic conductivity, Fine roots, Root biophysics

Area: Plant physiology

PANEL

05

REDUCTION OF DAMAGES CAUSED BY UV-B RADIATION IN RICE PLANTS *(ORYZA SATIVA L.)* TREATED WITH KAOLINITE.

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The decrease in ozone level has resulted in a greater incidence of UV-B radiation on the earth's surface. Continuous exposure to UV-B radiation indirectly influences plants by inducing the formation of reactive oxygen species within the cell and consequently photo-oxidative damage. In Chile rice cultivation are exposed to high levels of UV radiation, especially in December and January. The objective of this study was to evaluate the effect of UV-B radiation on plants treated with kaolinite. Rice plants were sprayed with three applications of kaolinite (25kg/ha) and were exposed to UV-B radiation for 3 days (7 hours/day). During the UV-B treatment, the relative amount of chlorophyll was measured using the SPAD-502 plus instrument, and post-treatment chlorophylls, other photosynthetic pigments and malonaldehyde (MDA), as an indicator of membrane damage, were quantified by spectrophotometry. Our results showed a higher concentration of pigments and a lower concentration of MDA in plants treated with kaolinite compared to plants without kaolinite. In conclusion, kaolinite has a possible photoprotective role rice leaf expose to UV-B radiation.

Financing: Universidad San Sebastián

Keywords: UV-B damage, Protection UV-B, MDA, Oxidative stress

Area: Plant physiology

PERFORMANCE OF THE RICE VARIETY *(ORYZA SATIVA L.)* ZAFIRO INIA IN DIFFERENT SOWING SYSTEMS.

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In Chile, rice cultivation requires high water consumption. For reduce the water use, the planting method and agronomic practices, are determinant. Therefore, the objective of this work was to determine the performance of rice cultivation with different irrigation methodologies. During 2021-2022 season, 18 fields trials were distributed in Chilean rice area (North: Linares and Longaví; Center: Parral and Linares; South: San Carlos, Ñiquén and San Nicolás). Nine trials were established with the pre-germinated sowing and nine with direct seeding, using the rice variety Zafiro-INIA. Plant height (cm), flowering (days), paddy yield (t ha^{-1}) and industrial grain yield (% whole grain) and NDVI, were measured. The pre-germinated sowing system obtained yield values of 6.2 t ha^{-1} and the direct sowing system 7.6 t ha^{-1}. The industrial grain yield, was similar with values close to 60%. No differences between flowering dates (102-113 days) and plant height (80-89 cm), were observed. The best performance for rice production was observed in direct seeding, with a reduction in about 25% of water use.

Financing: FONDEF IT19I0023, Tucapel S.A., Nestlé Chile S.A., INIA Chile

Keywords: Rice production, Direct Seeding, Water use

Area: Plant physiology

PANEL

07

STUDY OF ANATOMICAL CHANGES ASSOCIATED WITH THE CELL WALL IN *VITIS VINIFERA* BERRIES IN RESPONSE TO CYTOKININ PRE-ANTHESIS APPLICATIONS

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Chile is a world leader in table grape exports. To obtain this position, several agronomic practices are carried out to achieve high-quality fruit. Among the products used are growth regulators that increase berry distribution in the bunch, fruit size, color, and berry firmness. Published information indicates that the cell wall is closely related to fruit firmness and the number of cells that make up the berry. Cytokinins (CKs) have been reported to induce changes in the rate of cell division. However, there needs to be more information on the effects of CKs applied at the early stages of berry development and their impact on firmness at harvest. The present work evaluated morpho-anatomical changes in Thompson Seedless table grapes at both flowering and harvest stages treated with CKs (6 ppm N-2-chloro-4-pyridyl-N-phenylurea, CPPU) at pre-anthesis (-30 and -20 days before flowering). Morpho-anatomical changes were studied by bright field microscopy (toluidine blue) and confocal microscopy using specific antibodies to detect cell wall components. The results show that berries with CPPU application presented higher cell density at both flowering and harvest. In addition, at harvest, the cell density of berries with CPPU was observed to be significantly higher in the inner mesocarp than in the outer mesocarp. Confocal microscopy showed changes in the methylation pattern in berries with CPPU, concomitant with a higher signal for egg-box calcium-binding motifs, which would partly explain the higher firmness at harvest of these treatments (Fondecyt 1200260).

Financing: Fondecyt 1200260.

Area: Plant physiology

UNDERSTANDING THE MATURITY DATE BASED ON THE INTERACTION OF HORMONE AND CELL WALL REMODELING DURING FRUIT DEVELOPMENT IN *PRUNUS PERSICA*

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Prunus persica has four fruit development stages: S1, S2, S3 and S4. On the other hand, differences in Maturity Date (MD) between early and late nectarine varieties are mainly observed in the duration of the S2 stage. Thus, early-season varieties have a short S2 and late-season varieties show this stage more extended in time. However, the molecular and physiological factors that determine the duration of S2 are unknown. It is known that peach and nectarine development and ripening are mediated by hormones such as jasmonic acid and ethylene. Additionally, cell wall remodeling is common during fruit development and ripening. The objective of this work is to compare the cell wall profile, including uronic acid, hemicelluloses and neutral sugars; in addition to the profile of critical phytohormones at stages of development (S1-S4) of an early season variety (‘Early Juan’) and a late season variety (‘Super August’). Within the cell wall profile, a higher enzyme activity related to wall degradation during ripening was found in the Super August cultivar, given by the higher solubilization detected in this cultivar from the S2 stage. In particular, the fraction of ionically bound pectins seems to be the major contributor to the higher solubilization of polymers in SA, given by the more significant decrease in the contents observed between stages S2 and S4. In the case of hormones, the main differences are found during the S2 stage, where a high content of SA, jasmonic acid and Jalle was detected in the EJ variety. Likewise, and inversely, these hormones show an increase in the SA variety. This study could be the beginning of understanding the physiology related to MD and describing the basis for developing this fruit.

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Keywords: maturity date, cell wall, hormones, nectarine development

Area: Plant physiology

The tomato hybrid salt-tolerant rootstock obtained from a cross between a cultivated and a wild tomato stabilize the antioxidant capacity and activity in “Old-Limachino-Tomato” plants under saline stress conditions

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Tomato is considered a salt-sensitive plant species, and salinity drastically reduces fruit yield and quality. The grafting procedure has been recommended as a promising strategy to improve salt resistance in this species. The hypothesis is that the salt-tolerant rootstock (F1 JUPAFOR) improves shoot growth and physiological performance in “Old Limachino Tomato” (OLT) grafted plants under saline conditions (NaCl). The purposes of this research was to stablish the effect of a salt-tolerant rootstock on antioxidant activities in OLT-grafted plants under saline stress (NaCl) at the vegetative stage. Grafted, self-grafted, and non-grafted OLT plants were cultivated at different levels of salinity stress, induced by NaCl (0 and 160 mmol L-1). Sodium chloride was added during 21 days after seven days of transplanting, which was diluted in a watering nutritive solution. The effect of rootstock and salinity was evaluated on protein, MDA and chlorophyll contents in leaves. In parallel, it was measured antioxidant capacity (FRAP and DPPH) and activity (enzymatic and non-enzymatic). The enzymatic antioxidant activity included the enzymes APX, CAT, DHAR, GST and GR, and the non-enzymatic antioxidant activity was assessed by polyphenol content. Levels of proteins and chlorophyll were increased by salt stress, with no significant differences by grafting type. The MDA contents were increased by the effect of salinity in both non-grafted and self-grafted OTL. However, it remained stable in plants grafted on the resistant rootstock, but with showing higher contents. It was observed that the antioxidant capacity (FRAP and DPPH) and antioxidant activity increased during the salinity stress, but they were less affected in OTL plant grafted on the tolerant rootstock. In conclusion, these results suggest that the salt-tolerant rootstock stabilize (less modified) the antioxidant capacity (FRAP and DPPH) and activity in OLT grafted, in comparison with non and self-grafted OLT plants under saline conditions (NaCl).

Financing: FONDECYT project Nº 1220909

Keywords: Old Limachino Tomato, roostock, salinity, antioxidant capacity, antioxidant activity, tomato hybrid

Area: Plant physiology

A MULTI-OMIC APPROACH TO STUDY THE DIFFERENCES IN ROOT, CANOPY AND FRUIT DEVELOPMENT OF HASS AVOCADO GROWN ON TWO ROOTSTOCKS IN A SOILLESS AND PROTECTED SYSTEM

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In Chile, avocado fruit production has decreased considerably in the last ten years due to: (i) the high water requirements of this crop, and (ii) the drought in the regions with the largest cultivated area for this species (Valparaíso and Coquimbo). However, the high global consumer demand for avocado fruit has maintained its market competitiveness. Recent research has shown and proposed that soilless cultivation of avocado in containers and under greenhouse conditions is feasible, making efficient use of water and fertilizers and achieving normal tree growth. However, differences have been observed at the root and canopy developmental levels between trees grown under the same cultivation conditions but on two types of rootstocks, Mexicola (propagated from seed) and Dusa® (propagated asexually). For this reason, integrating transcriptomic and metabolomic information from roots, leaves and fruits, the objective of this research is to identify changes in gene expression and/or abundance of metabolites in Hass avocados grown on two types of rootstocks, Mexicola and Dusa®, making efficient use of water and fertilizers, giving a special emphasis on the possible differences that could be found in fruit quality parameters between avocados grown on both types of rootstocks.

Financing: This research was supported by Fondecyt N°3210011 and Millennium Science Initiative Program – ICN2021_044 from ANID.

Keywords: Fruit quality, Transcriptomics, Metabolomics, Fruit development

Area: Plant physiology

RECIPROCAL STRESS ADAPTATION IN *ARABIDOPSIS* AND BENEFICIAL RHIZOBACTERIA INTERACTIONS

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Plant microbial interactions are dynamic, ranging from deleterious (pathogenic) to beneficial (mutualistic and symbiotic). One type of plant-bacteria interactions are those between plants and growth-promoting bacteria (PGPB). These PGPB can colonize the rhizosphere or other plant tissues, and could increase plant growth, stress tolerance and/or affect plant development. These interactions have been traditionally classified as mutualistic, even in the absence of a clear fitness reward for both partners in the Darwinian sense of the term. Furthermore, the knowledge of how the abiotic environment shapes the consequences of these relationships is limited. To evaluate the reciprocal fitness costs in the interaction of plants and PGPB grown under gradients of different abiotic environmental challenges, we used *Arabidopsis thaliana* plants and the PGPB *Paraburkholderia phytofirmans* PsJN, *Pseudomonas putida* KT2440, and *Azospirillum brasilense* Sp7 as a study model. Different plant-growth parameters and bacterial density were used as fitness proxies, using wood sticks as a model of a plant-free surface. Under saline stress, plants were positively affected by the PGPB (high root area, shoot area, or modified primary root length). Still, the effects depended on the stress level. Bacterial densities were higher when plants were present, and the effects of the stress level were strain-specific. In most cases, the PGPB did not grow or maintain their population on the plant-free surface. Thus, we found a mutualistic relationship between *Arabidopsis* and these PGPB, but the relation was modulated by the abiotic environment, which differentially affected both partners of the interaction.

Financing: FONDECYT 1190634; MN-SAP NCN2021_010 and ANID PIA/BASAL FB0002

Keywords: PGPR, adaptation, plant-bacteria interaction, abiotic stress

Area: Plant physiology

PANEL

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NLP7 TRANSCRIPTION FACTOR REGULATES GENE EXPRESSION IN A TISSUE-SPECIFIC MANNER IN STOMATA OF *ARABIDOPSIS THALIANA*

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Drought is a critical environmental consequence of climate change because it limits crop productivity and reduces plant growth and development. In plants, abscisic acid (ABA) induces stomata closure in response to drought stress, reducing water loss and diminishing the photosynthetic process. Nitrogen is an essential nutrient that participates in the stomatal opening, allowing for an increase in the transpiration rate in plants. NIN-LIKE PROTEIN 7 (NLP7) is a transcription factor (TF) of the nitrogen signaling pathway, and the *nlp7* mutant exhibits a drought resistance phenotype. In this work, we studied the function of NLP7 in tissue-specific transcriptional regulation in the stomata of *Arabidopsis* thaliana. Using fluorescence-activated cell sorting (FACS) and RNA-seq, we identified NLP7-dependent genes in the stomata. We found a significant enrichment of drought-, nitrogen- and ABA-regulated genes in the list of genes differentially regulated by NLP7 in stomata. In adittion, we found that NLP7 regulates known TFs and relevant genes for the ABA signaling pathway in a stomata-specific manner. Gene regulatory network analysis reveals that NLP7 commands a regulatory cascade in response to drought in stomata. Our results suggest that NLP7 connects nitrogen and drought signaling with a tissue-specific component in stomata.

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Keywords: *Arabidopsis*, Nitrate assimilation, Drought stress response, Transcription factor, Crop improvement, Stomatal tissue

Area: Plant physiology

PANEL

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ROOTSTOCK/SCION INTERACTION IN *PRUNUS* SP II: AQUAPORIN GENE EXPRESSION IN HOMO AND HETEROGRAFTS UNDER HYPOXIA STRESS.

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Aquaporins are part of a large family of highly conserved membrane proteins that passively allow the passage of water and small uncharged solutes in response to the osmotic gradient. These transporters respond to various types of abiotic stress, such as hypoxic stress, a common adverse condition in the soils of the O'Higgins region, where waterlogging due to poor drainage in heavy or clayey soils in the territory affects multiple crops of agricultural interest, especially those associated with stone fruit trees. In the face of oxygen deficiency, aquaporins play an important role by transporting molecules related to adaptive responses. Although the role of aquaporins against oxygen deficiency in model species has been demonstrated, few studies have evaluated them in the context of woody species. This work evaluated the abundance of aquaporins PIP2;2 and TIP2;1 in roots and leaves of two rootstocks of stone fruit trees (*Prunus* sp.) contrasting to oxygen deficiency through a study model that includes homo- and hetero-grafts of 'Mariana 2624' (M26) and 'Mazzard F12/1' (F12). Gene expression was evaluated by qPCR and protein abundance by indirect ELISA in samples of 3, 6 and 10 days under oxygen deficiency. PIP2;2 showed similar trends in transcript and protein levels over time in all grafting combinations except M26/F12. On the other hand, mRNA and protein levels of TIP2;1 showed independent behaviors. At the organ level, when the graft included root of M2624 and shoot of F12, the mRNA levels of both aquaporins showed different profiles than the rest of the combinations, suggesting an intrinsic effect of this combination.

Financing: Funding by FONDECYT 1190816 and ANID R19A10003 GORE O'Higgins

Area: Plant physiology

LONG-TERM PHYSIOLOGICAL RESPONSE OF TWO SWEET CHERRY VARIETIES TO EARLY INOCULATION OF *PSEUDOMONAS SYRINGAE* PV SYRINGAE (PSS)

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Plant-biotic interaction responses are of considerable interest in agriculture and its study allows a better understanding of the impact on a crop, whether beneficial or detrimental. Pss is a major pathogen causing bacterial canker diseases that cause significant losses in stone fruit orchards. In this study we aimed to improve our knowledge on the long-term effects on vegetative growth and physiological response during the summer season in sweet cherry plants (*Prunus avium*; cv. Bing and Santina) when inoculated early during the spring with the pathogenic bacterium Pss. 14 days post inoculation (dpi) with A1M3 strain, a higher rate of necrosis symptoms and gum secretion were observed in Santina than Bing plants. 40 dpi the plants were transported to the field to evaluate the development of the new post-inoculation shoot growth. At leaf level, plant water status, stomatal conductance and whole plant hydraulic conductance were not affected by the inoculation treatments in both varieties, as well as for photosynthesis rate or chlorophylls content. As for leaf growth parameters, Pss-inoculated plants required more thermal time both for the emergence of a new leaf and to reach a fully expanded leaf but in higher extent for Santina than Bing plants. Also, plants inoculated with Pss had a higher specific leaf area and a larger leaf size but showed a lower number of leaves and a reduced shoot branching. In the long term, leaf growth parameters were considerably affected in Pss-inoculated plants although there is no evidence indicating a limitation caused by impaired water transport or carbon acquisition. Possible responses could be related to a change in the hormonal response of the plants.

Financing: Project funded by Anillo ACTO 190001 and ANID R19A10003 GORE O'Higgins

Keywords: Sweet cherry, Pseudomonas syringe pv syringae, plant-pathogen interaction, physiological response

Area: Plant physiology

STUDY OF BIOMODULATORS TO MITIGATE EFFECTS OF SALINE AND DROUGHT STRESS IN MICRO-TOM TOMATO

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Afiliaciones: Uchile, INIA, UNAP

Climate change has many harmful effects, and agriculture is one of the most severely affected activities. Various solutions have been proposed to deal with this problem, one of which is the use of biostimulants (or biomodulators). These are eco-friendly compounds that play a protective role against abiotic stresses in various plants of agricultural interest. These compounds are often easy to apply and inexpensive.

An important crop, both worldwide and in Chile, is tomato (*Solanum lycopersicum L.*). This work focuses on the effects of different biomodulators to increase their tolerance to salt stress and drought. For this purpose, the model cultivar for tomato "Micro-Tom" was used. Plants were grown under controlled conditions in a greenhouse, and their physiological, morphological and molecular parameters were measured.

Under **drought stress**, foliar spraying of **chitosan** recovered height, internode length and root fresh weight, whilst application of **melatonin** recovered root fresh weight.

Under **salt stress** (160 mM NaCl), the application of **lipoic acid** showed a tendency to increase fruit diameter. Applications of a **carotenoid** mixture showed higher fruit set at stage 6 (S6, mature). Application of the synthetic molecule **A35** resulted in a higher weight, diameter and number of seeds per treatment, as well as a higher average number of fruits per plant. The addition of **S2**, produced more S6 fruits, with a higher weight and a higher number of seeds per treatment. Application of **chitosan** recovered plant height.

Finally, the relative expression levels of transcription factors associated with salt stress (*WRKY81*) and drought (*GATA-8*) were significantly increased when **melatonin** and **chitosan** were applied respectively, however, more information needs to be collected to understand their importance.

Financing: Funding: Anillo ACT192073

Keywords: Biomoduladores, Cambio Climático, Estrés Salino, Estrés por Sequía, Tomate

Area: Plant physiology

METABOLOMIC AND PHYSIOLOGICAL RESPONSES OF CONTRASTING PRUNUS ROOTSTOCKS TO DROUGHT STRESS UNDER WATER DEFICIT CONDITION

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The adaptation of plants to the condition of drought stress becomes an effective strategy to sustaining fruit production. On *Prunus* species, it is possible to graft different genotypes of high economic importance with different degrees of drought tolerance. The aim of this study was to identify key physiological variables on whole plants and metabolite biomarkers on the roots and leaves of two Prunus rootstocks with contrasting responses to drought stress: ROOTPAC®40 (R40) as drought-tolerant and ROOTPAC®20 (R20) as drought-sensitive under water-deficit (WD) and well-watered (WW) conditions. The samples were collected at the time in which the R20 genotype reached a transpiration reduction of 15% (mild stress), 35% (moderate stress) and 70% (severe stress). On phenomic and metabolomics data PLS-DA analysis showed that the separation of the samples was driven by the genotype and the water restriction, but it did not provide clear information based on the level of stress. Circos plots showed that under severe stress the R20 WD was more sensitive to changes of the internal CO_2 concentration and leaf water potential parameters, while stomatal conductance was higher in both genotypes under WW. In roots samples, amino acids levels were higher in WD regardless of genotype. On the other hand, different sugars and amino acids downregulate in leaves under WW treatment. Total sugars were less abundant in R20 WD, however the contents of fructose, glucose, sucrose and myo-inositol on leaves seems to be dependent on the interaction between genotype and treatment. A combination of phenomics and metabolomics data could be useful for the selection of new drought tolerant rootstocks, thus, assisting breeding programs.

Financing: This research was supported by Strategic Research Fund on Drought – FSEQ210014 from ANID.

Keywords: Drought stress, Plant physiology, Metabolomic

Area: Plant physiology

STRATEGIES OF NA AND CL ACCUMULATION *IN SOLANUM LYCOPERSICUM* AND ITS WILD HALOPHYTE RELATIVE *SOLANUM CHILENSE* UNDER SALT STRESS

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Salinity is a growing global concern that affects the yield of crop species, including tomato (*Solanum lycopersicum*). In contrast to *S. lycopersicum*, its wild relative *Solanum chilense* was reported to have halophyte properties. We compared salt resistance of both species during vegetative and reproductive phases and investigated Na and Cl accumulation in the different organs. Plants were exposed to NaCl (0, 60, 120 mM) from the seedling stage up to flowering (85 days after stress). Overall, *S. chilense* accumulated more Na than *S. lycopersicum* while Cl concentration was similar in both species under salt stress. Sodium accumulated mainly in roots in *S. lycopersicum* and in stems in *S. chilense,* showing that *S. lycopersicum* had an excluder behavior and *S. chilense* an includer behavior regarding Na accumulation while such a difference was not observed regarding Cl accumulation. Both species limited the accumulation of Na and Cl in the inflorescences. The concentration of Na and Cl in the inflorescences were respectively lower and higher in *S. lycopersicum* compared to *S. chilense* under salt stress. Na localization in the flowers was analyzed by laser ablation ICP-MS. Na was mainly located in male floral organs of *S. chilense* but in non-reproductive floral organs of *S. lycopersicum* under salinity. Na and Cl accumulation and repartition could be partly explained by gene expression of transporters involved in Na and Cl distribution. Overall, our results indicated that *S. chilense* was more salt-resistant than *S. lycopersicum* and that both species differed towards strategies of Na and Cl accumulation.

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Keywords: tomato, salinity, ion accumulation, plant reproduction

Area: Plant physiology

Use of rhizobacteria *(Bacillus amyloliquefaciens)* and its effect on growth, physiological parameters and mineral content (K^+ and Na^+) in tomato plants under salinity stress conditions.

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Tomato plants are considered as a salt-sensitive plant species, and salinity drastically reduces fruit yield and quality. The use of rhizobacteria has been recommended as a promising strategy to improve resistance to abiotic stresses such as salinity. The hypothesis postulates that the presence of *Bacillus amyloliquefaciens* in the rhizosphere of tomato plants enhances the physiological response to salinity tolerance through increased K+ uptake and Na+ exclusion at the root level. The purpose is to investigate the effect of rhizobacteria (*Bacillus amyloliquefaciens*) on growth, physiological parameters and Na+ and K+ content in tomato plants under salt stress conditions (NaCl) in vegetative stage. Two varieties of tomato were used, a commercial one (7742) and a local one, Poncho Negro. Tomato plants were inoculated with two types of strains (BAS10 and INIA-2444) at two concentrations (106 CFU/g of substrate) supplied 10 days before the application of salinity treatments.(0 and 160 mmol L^{-1} of NaCl) to nutritive solution for 21 days. The effect of *Bacillus amyloliq*uefaciens and salinity were evaluated measuring shoots and roots growth, chlorophyll and MDA contents, among others. In parallel, the mineral content (Na+ and K+) in roots and leaves was measured. Na+ content in leaves increased in response to salinity, but were higher in Poncho Negro than in LV744, accentuating this behaviour with strain BAS10 compared to strain INIA 2444. Na+/K+ ratio in roots and leaves was considerably lower in the commercial variety and in the presence of both types of strains compared to the local variety. In conclusion, this response suggests that salt tolerance is enhanced by the presence of the rhizobacteria studied but depends on the type of strains and the tomato plants genotype used, when plants are subjected to salt stress (NaCl).

Financing: Anillo project (PASSA- ACT 192073) and FONDECYT project (Nº 1220909)

Keywords: Tomato, Salinity, rhizobacteria, K+, Na+, Abiotic stress

Area: Plant physiology

PANEL

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AQUAPORIN GENE EXPRESSION ANALYSIS IN NEW PRUNUS ROOTSTOCK HYBRIDS UNDER WATER DEFICIT

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Chile, like other regions of the world, faces the challenges of climate change, going through a 10-year period of drought with a sustained decrease in average annual rainfall. Under this situation, agricultural production has an important challenge, since production must be maintained and increased, considering the current and future population. This increase cannot be carried out only based on the increase in the use of inputs without the concomitant negative environmental consequences. It must necessarily be carried out under the concept of sustainability, taking into account the economic, environmental, and social areas of agricultural production. In the recent years, rootstocks have been proposed as a key factor in increasing efficiency in the use of resources in agriculture contributing to food security and some authors propose rootstocks as one valuable tool of a second green revolution in the context of the challenges imposed by the climate change and the increase of the world population. In the recent two decades, diverse research has demonstrated that rootstocks can modify the behavior of grafted plants, altering key traits such as water-use efficiency. The creation of new hybrid rootstocks adapted to this water restriction scenario is a challenge for our Rootstock Breeding Program for stone fruits. This situation pushes the searching of new methodological approaches able to improve the characterization and selection processes of the new rootstocks. The identification of biomarkers will provide relevant information for a better understanding of complex response networks underlying drought tolerance in plants and, also, knowledge applied to the selection of new rootstocks tolerant to water deficit. In this work, we evaluated de gene expression of aquaporin PIP subfamily as candidate molecular biomarkers to discriminate genotypes with better water use efficiencies in the food production context.

Financing: Project funded by FSEQ210014 and ANID R19A10003 GORE O'Higgins

Keywords: Prunus, rootstock, hybrids, Plant Breeding Program, water deficit, aquaporin

Area: Plant physiology

Exploring the potential use of quinoa (*Chenopodium quinoa* Willd.) seed by-products as biostimulants in *Arabidopsis thaliana*.

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Quinoa is a seed-producing crop, which has been expanding outside the Andes thanks to both its nutritional value and abiotic stress tolerance. Before consumption, quinoa seeds undergo a mechanical de-hulling process, which removes the outermost coat and produces a by-product rich in saponins and other bioactive compounds. For this study, two by-products were obtained from quinoa processing, one enriched in saponins (QS) and the other enriched in proteins (QP). We investigated if QS and QP could have a biostimulant effect to improve plant performance under control or salt-stress conditions in *Arabidopsis thaliana*. Extracts were prepared by adding 10 mL water to 0.5 g of dried material. After ultrasound-assisted extraction, the suspension was filtered. *Arabidopsis* (Col-0) seeds were germinated *in vitro* on half-strength MS medium added with varying amounts of QS and QP, in a growth chamber at 25 °C and a light/dark cycle of 16/8 hrs. Seed germination was monitored for 4 days, after which time seedlings were collected for biochemical and physiological analyses. Salt stress was applied by exposing 5-days-old *Arabidopsis* seedlings to 25, 50 and 100 mM NaCl treatments for 12 days before collecting samples. QS and QP were characterized in terms of both biochemical and microbiological aspects by spectrophotometric and Plant Growth Promoting Bacteria assays, respectively. Root length was the morphological parameter most affected by both QS and QP treatments. The highest salt concentration (100 mM NaCl) significantly reduced the germination and growth of *Arabidopsis*. Experiments are underway to verify the protective and/or stimulant effects of QS and QP under salt stress.

Financing: FONDECYT INITIATION INTO RESEARCH 11190834

Keywords: Quinua, byproduct, plant growth, extract, saponins, germination, seedlings

Area: Plant physiology

PANEL

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An overlook at the complex structuring of Chilean papaya mucilage.

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The fruit industry in Chile represents an important source of income, where most of the production is exported and a smaller part is marketed locally or used as raw material for other secondary products. The industrial processing of raw materials, whether they are fruits or vegetables, produces a huge quantity of waste consisting of pulp, peel and seeds. The treatment of these waste produces large quantities of insoluble carbohydrate-rich residues, which originally form the plant cell wall and other structures like seed mucilage. Here, we approached the study of the seed mucilage of the Chilean papaya (*Vasconcellea pubescens*), waste derived from the processing and use of papaya flesh. Papaya mucilage turned out to be a fairly easy extractable structure that corresponds to 20 % of the dry weight of the seed. Among the sugars that compose it, a large portion corresponds to galacturonic acid and therefore to homogalacturonan (HG) and to a lesser extent rhamnogalacturonan-I (RG-I). To specifically establish which polysaccharides are present and their distribution, we analyzed histological sections of dehydrated and embedded seeds to perform immunofluorescence assays using monoclonal antibodies against HGs with different degrees of methylation (JIM5, JIM7) or interacting with other HG through Ca^{+2} cross-linking (2F4) and against unbranched RG-I (INRA-RUI). This information becomes interesting for the design of future technologies and novel and efficient strategies in the use of these wastes and the generation of value.

Financing: Proyecto Anillo ACT210025

Keywords: immunofluorescence, histology, mucilage, Vasconcellea pubescens, Papaya Chilena

Area: Plant physiology

PANEL

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Water shortage affects differentially the leaf growth rate and grain yield component of Common Bean *(Phaseolus vulgaris)* Chilean Landraces.

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Water availability for agricultural use is currently a global problem that worsens with climate change in several regions of the world. Among grain legumes, common bean (*Phaseolus vulgaris*) is the most cultivated in the worldwide. The Chilean germplasm of common bean is characterized by tolerance to water stress. Here, we analyzed a selection of nine ancient Chilean landraces in regard to their drought tolerance, simulating optimal (OW) and restricted watering (RW) in a Mediterranean environment. Phenological, growth, and yield traits were recorded, and correlation analysis was performed. Accordingly, leaf temperature and osmotic potential were higher under RW, while the leaf chlorophyll content decreased in all landraces. Physiological maturity days and seed-filling days were lower in RW than in OW. This similarly occurred with the grain yield. The % yield reduction was negatively correlated with the % pod reduction and the relative rate of leaf expansion (RLAE) reduction. However, the 100-seed weight value was not significantly modified by water treatment ($p > 0.05$). For instance, landraces that preferred to fill the grain with a lower rate of leaf expansion showed a lower loss in grain yield under drought conditions. These results suggest that the resource partitioning between growing leaves, flowers, and developing pods in Chilean landraces is variable, affecting the common bean drought tolerance.

Financing: Subsecretaria Agricultura, Cod. 501453-70

Keywords: Common bean, Drought stress, leaf growth rate, germplasm, Chilean landrace

Area: Plant physiology

ROOTSTOCK/SCION INTERACTION IN PRUNUS SP I: PHYSIOLOGICAL RESPONSE OF HOMO AND HETEROGRAFTS AND ROOT ANATOMICAL MODIFICATION IN PRUNUS ROOTSTOCKS UNDER HYPOXIA STRESS.

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Rootstocks have been proposed as a key factor for increasing efficiency in the use of resources in agriculture contributing to food security and a valuable tool of a second green revolution in the context of the challenges imposed by the climate change and the increase of the world population. Rootstocks are selected for rooting and grafting capacity, abiotic and biotic stress tolerance, and their ability to beneficially alter scion phenotypes. Grafting provides opportunities to exploit natural genetic variation for specific root traits to influence the phenotype of the commercial aerial part. There are many studies demonstrating the interaction between rootstocks/scion and how the rootstock can influence the response of the scion to different conditions in woody plants and in vegetable crops. Shifts in key anatomic traits of rootstocks such as root cortical aerenchyma,xylem diameter, hydraulic conductance variation, endo and exodermal lignification and suberization maymodify shoot phenotype. In this work, we studied the physiological responses and anatomical characteristics of homo- and hetero-grafts between two contrasting *Prunus* rootstocks to soil hypoxia stress. The results showed an effect of rootstock on the scion, transferring the tolerance or sensitivity to hypoxia. This homo- and hetero-graft model is useful to study this type of interaction and the effect of the rootstock on scion and vice versa.

Financing: Funding by FONDECYT 1190816 and ANID R19A10003 GORE O'Higgins

Keywords: Rootstock, Prunus, hypoxia

Area: New and emerging areas of knowledge

PANEL

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BEHIND THE INTERACTION MECHANISM BETWEEN THE PLANT MODEL *ARABIDOPSIS THALIANA* AND *CUPRIAVIDUS TAIWANENSIS* LMG19424

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Nutrient deficiency in most terrestrial ecosystems constrains global primary productivity in both natural and agricultural environments, and nitrogen (N) is one of the most limiting nutrients. Many strategies are being developed to improve N availability in soils or its use efficiency in plants; among them, the use of N-fixing bacteria. Nevertheless, there is scarce knowledge on how free-living bacteria can increase growth in non-nodulating plants. *Cupriavidus taiwanensis* LMG19424 is an N-fixing β-proteobacterium inducing nodulation in *Mimosa* spp. plants. We have found that this strain promotes growth in *Arabidopsis* plants. To disentangle the mechanisms of this growth promotion we performed several experiments, finding that this phenomenon is not related to N-fixation (but is N-dependent), siderophore and auxin production, phosphate solubilization, or ACC deaminase activity. Nutritional content analysis in plants showed that the LMG19424 strain increased the N, P, and Ca concentrations and regulated Zn, Fe, and Cu concentrations. The strain did not increase the root area in *eto2, ein2-1*, and *tir1-1 Arabidopsis* hormonal single mutants, pointing to the involvement of the ethylene and auxin signaling pathways in the *Arabidopsis* responses to this bacterium. These results agree with the transcriptional regulation pattern observed in several genes related to these phytohormones in inoculated plants. Thus, this work helps to better understand the responses of non-nodulating plants to N-fixing, free-living bacteria.

Financing: This work was funded by ANID PIA/BASAL FB0002, ANID-FONDECYT 1190634, and ANID-FONDECYT 1151130

Keywords: Nitrogen Nitrogen-fixing PGPR Rhizobia

Area: New and emerging areas of knowledge

PANEL

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Physiological and molecular effects of different priming methods in *Arabidopsis thaliana* plants challenged by heat stress

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Nowadays, studying different mechanisms to mitigate the effects of global change on plants in natural or agroecological ecosystems is highly relevant because plants are susceptible to temperature changes. Temperature rises and heat-waves are expected in different parts of the planet. It has been proposed that plants can be primed when exposed to mild stress followed by a stronger one, which induces a more effective stress response in plants. Here we compared two priming methods: (I) Thermo-priming (exposing seedlings to a progressive temperature rise, from 22°C to 40°C for 6 hours) and (II) biopriming (inoculating seeds with the beneficial rhizobacteria *Paraburkholderia pytofirmans* PsJN by inoculating the invitro media or the soil). Then, non-primed and primed plants, at 14 days after sowing, were exposed to two scenarios: normal growth conditions at 22°C (control) and heat-wave conditions (5 h at 38°C for 5 days). Our results suggest that both primings and the heat wave treatment induced an elongation of the hypocotyl, where plants presented hypocotyls 25-65% larger than the non-primed plants in control conditions. Also, plants exposed to the heat-wave presented fewer seeds per silique (10-15% less). However, the weight of the seeds was increased in the bio-primed treated plants. No other significant change in the physiology of the plant was observed.

As expected, plants exposed to the heat-wave showed a significant increase in the relative expression of *HSP101* and *HSP22,* regardless of the priming treatment. Other critical genes related to hormonal pathways are also being studied to elucidate the role that different priming strategies can have in the improvement of heat tolerance in plants.

Financing: FONDECYT 1190634; MN-SAP NCN2021_010; ANID PIA/ANILLOS ACT210052

Keywords: Heat-stress, Paraburkholderia phytofirmans PsJN, Priming, thermo-priming

Area: New and emerging areas of knowledge

PANEL

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Effects of microbe-microbe ecological interactions in the plant growth promotion induced by rhizobacteria in *Arabidopsis thaliana* plants

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Effects of microbe-microbe ecological interactions in the plant growth promotion induced by rhizobacteria in *Arabidopsis thaliana* plants

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Multiple and complex intra and inter-kingdom ecological interactions occur in the rhizosphere. Some microorganisms inhabiting the rhizosphere can promote plant growth and induce stress protection in plants. Synthetic communities (SynComs) are microbial consortia designed to mimic the role of a particular community and the underlying ecological interactions. To test the role of microbe-microbe interactions in the plant growth promotion induced by rhizobacteria, we designed a SynCom of six strains representative from the rhizosphere of *A. thaliana*, including the beneficial bacteria *P. phytofirmans* PsJN. We first analyzed the microbial interactions within the SynCom in a plant-free context, finding that *Pseudomonas putida* KT2440 inhibited the growth of the other five strains in paired interactions. We also compared the bacterial growth in root exudates, where all strains grew (excepting *Flavobacterium endophyticum* 522). We then compared the effects of each strain in *in-vitro* grown plants, whether as isolated strains or in combination with strain KT2440. Here we found that most strains increased rosette area and maintained root length, excepting KT2440 and *N. plantarum* J70, which inhibited the root growth. In combination with the other strains, KT2440 decreased the root length and rosette area. However, PsJN and *A. brasilense* SP7 reversed this phenotype. Interestingly, all strains could thrive in the *Arabidopsis* rhizosphere as single inocula but not when KT2440 was present, excepting SP7 and PsJN strains. Then, we found that microbe-microbe interactions can regulate the growth promotion in *Arabidopsis* in a species-specific manner.

FONDECYT 1190634; MN-SAP NCN2021_010 ANID PIA/BASAL FB0002

Area: New and emerging areas of knowledge

PANEL

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EVALUATION OF POTENTIAL PRIMING EFFECTS OF LOW-DOSE IONIZING RADIATION IN *ARABIDOPSIS THALIANA* PLANTS CHALLENGED BY ABIOTIC STRESSORS

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Plants are sessile organisms capable of adapting and responding to environmental stressors. Priming or preconditioning is the process where a first non-severe, low-level environmental stress, induces more effective responses in an organism confronted with a subsequent severe environmental challenge. Current knowledge of the effects of ionizing radiation (IR) suggests that low-dose exposure may induce an adaptive response that preconditions plants to respond favorably against other abiotic stressors. This work aimed to evaluate the effects of low doses of IR in the adaptation of *Arabidopsis thaliana* plants exposed to stressful growth environments. To achieve this, three different plant materials (dried seed, soaked seed, and seedling) were exposed to increasing doses of IR (gray or Gy) to define IR priming doses. These preliminary results determined that *A. thaliana* would be exposed to doses lower than 40 Gy. Then, plant materials were exposed to IR up to 40 Gy. Subsequently, the plants that originated from this material were exposed to control conditions, salt-stress, mild-heat stress, and high-heat stress. Different plant-growth parameters and metabolic/transcriptional markers were analyzed in the plants from the different treatments. The results suggest that irradiating *A. thaliana* seeds and seedlings at doses lower than 10 Gy protects plants contributing to their tolerance to other stresses, particularly in soaked seeds exposed to salt stress. The beneficial effects were also observed in control conditions. No potential priming effects were observed with a pre-exposure at 40 Gy, followed by a stress challenge. Our results suggest that low-dose IR could be used in seeds as a priming method to induce salt stress tolerance in *A. thaliana*.

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Area: Molecular Breeding, Phenomics and new breeding technologies

PANEL

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ADVANCES IN FINE MAPPING OF JAPANESE PLUM (*PRUNUS SALICINA* LINDL.) FLAVAN-3-OL PROFILE: GENETIC ANALYSIS OF THREE F1 PROGENIES

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Catechin (C) and epicatechin (EC) are stereoisomers corresponding to the flavan-3-ol family, associated with sensory attributes, postharvest and health benefits for the consumers. Both C and EC have been previously determined in *Prunus salicina* Lindl. fruits from an F1 progeny <'98-99' x 'Angeleno'>, detecting segregation in the C and EC contents in two consecutive seasons. The seedlings were classified as C or EC depending on the compound with the highest content. The offspring fitted with a 1:1 ratio corresponding to the single-gene model, suggesting the presence of a major gene whose variant was transmitted by cv. 'Angeleno'. This study aims to confirm if these findings are also observed in fruits of three F1 progenies with 'Angeleno' as a common parent by evaluating the content of these flavan-3-ols in the fruit exocarp and mesocarp. Analyzing the results, we classified the seedlings according to the C and EC contents, where the progenies <'Angeleno' x 'Sapphire'> and <'Sweet Pekeetah' x 'Angeleno'> fit with a 1:1 ratio (p-value > 0.05). On the other hand, <'Angeleno' x 'Owen T'> fitted with a 1:3 ratio (p-value > 0.05), confirming that the three F1 progenies fit with mendelian segregations. These results confirm that a significant genetic component is associated with this trait and could help to better understand the flavan-3-ol metabolic pathway. It would also provide the basis for a genetic study of the genes involved in this phenotypic variation and the genetic breeding focused on the requirements of consumers and producers of Japanese plums.

Financing: FONDECYT Regular 1191446, BECAS DE DOCTORADO NACIONAL/2020 – 21200330. ANID Chile.

Keywords: catechin, epicatechin, flavan-3-ol, japanese plum, Prunus, major locus, mapping

Area: Molecular Breeding, Phenomics and new breeding technologies

PANEL

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GENOMIC PREDICTION OF TARGET BREEDING TRAITS IN *PRUNUS PERSICA* (L.) BATSCH

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The development of high-throughput genotyping techniques has allowed large-scale genomic studies to predict complex traits with high accuracy in several plant species, including commercial traits in fruit trees. In the present study, ~330 trees from 12 parental lines of three European breeding programs were genotyped with 5K single nucleotide polymorphisms (SNPs). Genomic data were used to predict three phenotypic traits: The beginning of flowering time (FD), the beginning of ripening time (MD) and the soluble solids content of fruit flesh (SSC). With the motivation of increasing the predictive ability (PA) of genomic prediction (GS) models, a genome-wide association study (GWAS) was implemented to identify loci significantly associated with the studied traits (LSA). The ability of genomic data (including LSA) to predict FD, MD and SSC were 0.72, 0.67 and 0.44, respectively, which increased up to 18% the PA obtained by the traditional GS approach. The results provide valuable information for implementing strategies based on genomics that facilitates the selection of new genotypes and the early development of new high-productivity peach varieties.

Financing: FONDECYT N°3220494, FONDECYT N°1191446 and IDeA ID22I10266.

Keywords: Genomic Selection, QTLs, Predictive ability

Area: Molecular Breeding, Phenomics and new breeding technologies

PANEL 30

Precision Breeding Approaches in Fruit Crops: Working towards a Sustainable Future

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Today, we need better and quicker ways to improve crops. At Meristem, we work aiming for a better tomorrow. We are specifically interested in developing fruit crops for a sustainable future: to withstand climate change, provide better nutrition and reduce waste. Our approach involves integrating innovative in vitro culture techniques and gene editing to develop a streamlined workflow for trait improvement in fruit.

Meristem was incorporated in 2020, advancing with team establishment, strategic planning and laboratory setup. Our first year, we focused on sequencing and annotating genomes, tuning-up in vitro protocols and developing a gene editing optimization strategy. In early 2022, we set up a rapid prototyping platform and achieved validation of Citrus and Prunus in vivo editing. For 2023 we expect to have our first proof of concept with an edited Citrus plant; for 2025 / 2027 the first edited Citrus / Prunus plants with comercial traits; and for 2028 / 2029 results of field tests and minimal viable product (MVP).

The team is composed by 25 researchers organized in three main areas: In vitro culture, Bioinformatics and Plant synthetic biology. Based in Santiago-Chile, our facilities include infrastructure and equipment to implement each area. We have an experimental station fully equipped with phytotrons and a greenhouse for experimental validation and plant propagation.

Multiplex - our first spinoff - has developed effective molecular diagnostics technologies for plant phytosanitation by applying high-through sequencing and bioinformatic analysis for viral and viroid diagnosis. Multiplex developed Viroscope.io: an open web-service to provide on-demand HTS data viral diagnosis to facilitate adoption and implementation by phytosanitary agencies to enable precise viral diagnosis. Another creation of Meristem is Weaver, an open source biotech software for biotechnology research and development, that will soon be available for deployment at a low service cost.

Financing: Meristem spa

Keywords: precision breeding, gene editing, fruit trees, protoplast culture

Area: Molecular Breeding, Phenomics and new breeding technologies

Native endophytic fungi of Solanaceae from the Atacama Desert, as plant growth promoter and drought stress tolerance enhancer in Tomato *(Solanum lycopersicum)* var. Roma

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In the Atacama Desert, the driest poly-extreme ecosystem in the world plants can establish beneficial symbiotic associations with fungi. These associations confer diverse adaptive mechanisms to aid their survival in extreme environments. Endophytic fungi provide and maximize nutrient uptake, increase soil fertility, enhance productivity and produce bioactive metabolites. In turn, plants act as hosts, providing protection and specialized microhabitats for the correct hyphal development inside the root. In this work, we identified several endophytic fungi isolated from the roots of plants of the *Solanaceae* family from the Atacama Desert and evaluated their ability to improve germination rates and shoot development under drought stress in Tomato (*Solanum lycopersicum*) var. Roma plants. Besides, we studied the synergy between strains with improved plant growth-promoting fungi (PGPF) traits for the development of microbial consortia. We found three different fungi able to coat seeds and increase germination rates and shoot length development by 50-80% under low water potential conditions. Moreover, four strains were selected by their capacity to colonize plant roots and increase plants fresh weight and leaf area by 17-50% under water stress conditions. We found two positive protocooperative interactions between different fungal samples, revealing a >10% improvement in reciprocal and contact between hyphae in dual confrontation assays. Additionally, we identified an endophyte with antagonistic activity against all the strains evaluated. Our results serve as ground information about synergy between endophytic fungi with PGPF traits under conditions of low water availability. This is a desirable trait for the development of new microbial formulates such as seed coatings, biological control products, and biofertilizers. The use of native biological resources is a potential sustainable solution to the challenge of the current water crisis in the agricultural sector in Chile.

Financing: This work was supported by FONDEF-CONICYT VIU21P0087 and ANID-Millennium Science Initiative Program ICN17_022

Keywords: PGPF, Endophytic Fungi, Tomato, Drought, Biofertilizer, Plant Growth-Promoting Fungi

Area: Molecular Breeding, Phenomics and new breeding technologies

UNIQUE GROWTH CHAMBERS IN CHILE, WHICH ALLOW THE STUDY OF PLANTS AND CLIMATE CHANGE AT UC

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The Plant Experimentation Center of the UC Faculty of Biological Sciences has a modern infrastructure (200m^2), which allows it to provide propagation, transformation, genotyping and phenotypic analysis services. It is a space designed to support national research in the plant area.

Current research in plants or in the analysis of climate change mainly involves long-term studies where environmental conditions and parameters must be replicated in the laboratory. The new walk-in chambers of 13 m^2, and unique in Chile, are designed with the highest quality standards allowing control of parameters such as:

- Temperature rage +5°C to +45°C
- T° precision ± 0.5°C
- T° uniformity ± 1°C
- Humidity rage 40% to 80%
- H precision ± 1% RH
- H uniformity ± 2% RH
- CO_2 200ppm to 1000ppm
- Radiation +30 to +300 µmoles/m^2s
- Photoperiod – ramp segments
- Growth height – Flexible interior configuration

The availability of this space will give national students and researchers the opportunity to carry out field research with controlled parameters, allowing cutting-edge science to be carried out in local territory.

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Keywords: Climete Change

Area: Molecular Breeding, Phenomics and new breeding technologies

PANEL

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In vivo editing in Fruit trees: Developing tools for Mandarin and Cherry

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Gene editing is an emerging technology that enables precise modifications in a single nucleotide base in the genome. In plants, this technology has opened the possibility to apply targeted genome modifications to improve crop varieties without being subjected to GMO regulations. Being new, not many examples have been published in fruit crops. However, the potential of base-editing in plant research has already been recognised as to be revolutionary for plant biotechnology.

At Meristem, we are committed to use gene-editing to generate fruit crops for a more sustainable future. Thus, we have been working to develop tools to be able to use this technology in two particularly challenging fruit trees: Mandarin and Cherry. Firstly, we started by generating novel, customized and optimized editors that host multiple substrate specificity and editing windows. These results allowed us to have flexibility and precision to select the optimal editor for each intended edition.

Moreover, we developed a platform for rapid prototyping of efficiency and precision of our editors in plant cells. This platform allowed us to confirm the correct functioning of our editors prior to testing them in species of interest. On the other hand, we are using innovative approaches to develop proprietary protocols for the transformation and regeneration of Mandarin and Cherry species.

By using a fluorescence-based reporter assay and NGS, we showed we are able to obtain *in vivo* editing in cells of two recalcitrant tree species. In the following months, we will be focusing on developing editions of relevant traits in the context of contributing to having a better and more sustainable agriculture in the future.

Financing: Meristem SpA.

Keywords: Fruit trees, precision editing, gene editing, protoplast transformation

Area: Genomics

PANEL

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Using Oxford Nanopore sequencing to compare DNA methylation during fruit ripening in *Prunus avium* (sweet cherry)

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Different cultivars of Sweet cherry fruits, one of the most exported fruits by Chile, have different ripening dates. The local frame time for harvesting goes from end of November to January, defining what we know as early, middle and late varieties. One of the biological questions to be addressed is what does the difference between the three varieties categories. A global loss or gain of DNA methylation is critical to fruit ripening. In Prunus species the relationship between fruit ripening and methylation is almost unexplored and show an apparent hypermethylation (10.17660/ActaHortic.2021.1322.46). Until now, is unclear whether and how DNA methylation changes during the ripening of sweet cherry fruits. Using Oxford Nanopore DNA sequencing of immature and mature fruit (24 and 80 days after full bloom) we found a global decrease in DNA methylation during ripening. For instance, some genes associated to mechanism for regulating chromatin show a hypomethylation pattern which is concomitant with transcripts abundance. Our study therefore revealed a global lost in DNA methylation during fruit ripening and suggested a critical role of DNA methylation in sweet cherry.

Financing: This work was supported by Fondecyt regular 1200718 from ANID-Chile.

Keywords: epigenetics, bioinformatics, fruit development, High-Throughput Sequencing

Area: Genomics

PANEL

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IDENTIFICATION OF PUTATIVE PROMOTERS AT TRANSCRIPTION FACTORS NAC RELATED COLD STRESS IN *Eucalyptus globulus*.

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Forestry is one of the main economic sectors of Chile, based mainly on pulp production. Among the main species used for the forest industry, Eucalyptus globulus is one of the most important hardwood species; characteristics such as high density, cellulose quality and fast growth have positioned it as the second most cultivated species in the country. The optimal temperature range for the development of this species is between 10°-15°C; temperatures below this range affect the establishment, plant survival and productivity. Spring frosts (-5°C) cause early death in juvenile crops, which makes E. globulus a species susceptible to low temperatures. Transcription factors (TFs) play an important role in regulating the expression of target genes, acting as an ON or OFF gene expression switch in plants' stress responses. Between TFs described, the NAC family is considered the largest in plants and has been studied in several abiotic stress, including cold stress. Knowing how the promoter zone of any gene is structured helps us understand its functioning and importance in relation to stress. For gene as *NAC*, identifying the cis-elements present in their promoter zone provides us opportunities when seeking solutions to current climate change since these short non-coding sequences in DNA can regulate coding regions close to them, being target zones of various proteins that control transcription levels at promoters. The main objective of this study is to determine the *cis* elements in the promotor secuence of four *NAC* genes related to abiotic stress response and analyze the expression of this genes in cold acclimated conditions for evidence this participation in the response to cold stress in *E. globulus*.

Financing: Facultad de Ciencias Forestales Centro de Biotecnología Universidad de Concepción

Keywords: Cold stress, *cis*-element, transcription factor

Area: Genomics

PANEL

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IDENTIFICATION AND CHARACTERIZATION OF THE SNRK2-PROTEIN KINASES SUBFAMILY IN THE *Fragaria* × ananassa GENOME

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The SnRK2s are plant-specific protein kinases that play essential roles in the ABA signaling transduction and response to abiotic stresses. In strawberry (*Fragaria* × *ananassa*), nine members of SnRK2 subfamily have been previously described, however, the characterization of *FaSnRK2* genes in the octoploid genome remains unsolved. In this work, a total of 33 *FaSnRK2* genes from the strawberry genome database were identified. All of them are unevenly distributed on chromosomes 1, 2, 4 and 5. Most *FaSnRK2* exhibit the nine exon- eight intron structure like other SnRK2 genes. Multiple sequence alignment of FaSnRK2 proteins showed the Ser/Thr kinase domain and the I-domain, conserved in all SnRK2s. FaSnR2.3 and FaSnRK2.6 also displayed the II-domain needed for ABA-dependent activation. Phylogenetic analysis grouped FaSnRK2s into three groups, interestingly, only FaSnRK2.7 and FaSnRK2.8 clustered together with ABA-independent activation SnRK2 members from *Arabidopsis*. Since FaSnRK2.7 is a single copy-gene and has a meager expression level in strawberry leaves, roots and fruit, the promoter analysis was performed in FaSnRK2.8 genes. Promoters of each copy of *FaSnRK2.8* were inspected for different hormone-responsive putative cis-elements. Regulatory elements involved in ABA, jasmonates (JAs) and salicylic acid response were mostly represented among the analyzed promoters. In addition, MYC- and MYB-recognized boxes were predominant on promoter sequences of the four copies of *FaSnRK2.8* suggesting a possible regulation by key transcription factors related to the JA signaling pathway. Taken together, these results could serve as a basis for future studies to elucidate the FaSnRK2 contribution in the strawberry response to environmental challenges.

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Keywords: SnRK2 gene subfamily, strawberry, SnRK2 conserved domains, SnRK2 gene structure

Area: Genomics

PANEL

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GENOME ANALYSIS OF UV-B PERCEPCION AND RESPONSE IN THE SUBANTARCTIC MOSS *POLYTRICHUM STRICTUM*

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Polytrichum strictum is a cosmopolitan brown moss with a remarkable tolerance to a wide range of harsh climatic conditions. Each spring, the atmospheric ozone hole opens above the Antarctic reaching the Sub-Antarctic ecoregion, achieving up to an 85% increase of UV-B radiation in early December. UV-B radiation acts as a critical environmental factor shaping the moss fitness, by modulating morphological and physiological processes through the activation of changes in gene expression and metabolism. A genome analysis by NGS was made to study the putative genes of the UV-B signaling pathway of *P. strictum* moss collected from a Chilean Sub-Antarctic Island, affected regularly by the seasonal ozone hole phenomenon. We aimed to identify the putative homologs of the coding genes of UVR8, COP1, RUP1 and RUP2, involved in UV-B perception and signaling; as well as putative homologs encoding some UV-B response genes such as transcriptional regulators (e.g., HY5, R2R3MYBs) and structural genes involved in the biosynthesis of polyphenolic compounds (e.g., PAL, CHS, CHI, FNS), whose accumulation have been proposed as crucial for UV-B tolerance in mosses. Our results showed a total of 22,744 genome predicted genes in *P. strictum*, of which almost a 75% were assigned through Blastp to a biological function using GO terms. A compared genome analysis of orthologous genes using ten moss genomes was made searching for those associated with phenyl-propanoid and UVR8 signaling pathways. These results shed light on the molecular response of mosses to UV-B radiation caused by the Antarctic ozone hole.

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Keywords: Moss, Genomics, Evolution, Phylogenetics, UV-B radiation

Area: Genomics

PANEL

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NEW INSIGTH INTO FRUIT DEVELOPMENT AND MATURITY DATE IN NECTARINE BY INTEGRATING mRNAS AND LONG NON-CODING RNAS

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In *Prunus persica*, the maturity date (MD) is a crucial fruit trait for a market focused on maintaining fresh fruit and extending fruit shelf-life. MD is a consequence of the fruit developmental process, and many physiological parameters associated with maturity are also defined in the early developmental stages. Currently, some candidate genes reported for this trait are hormone biosynthesis, and cell wall modification-related genes. However, the roles of these genes in the molecular regulation of MD in stone fruits are still unknown. Previously, we used a combination of RNA-seq and DNA-seq from contrasting individuals for MD at the harvest stage. We identified genes related to JA biosynthesis and cell wall metabolism. Taking this background into account, the goal was to identify the transcriptomic control involved in the expression of genes related to jasmonate biosynthesis and cell wall modifications associated with MD during fruit development. We have identified differentially expressed lncRNAs and mRNA during fruit development using contrasting varieties (Early Juan; EJ and Super August; SA) for MD by RNA-seq. We have identified 6,480 differentially expressed genes in at least one stage between EJ and SA, some of them associated with JA biosynthesis and cell wall metabolism. Of the 956 lncRNAs identified in the transcriptome, 829 lncRNAs were differentially expressed in at least one comparative. On the other hand, network analysis found transcription factors at different developmental stages associated with fruit development. Results suggest that these transcription factors are expressed in both varieties. However, their expression is conditioned to different temporalities during fruit development, explaining the contrasting phenotype for MD between EJ and SA. This study could be the beginning of understanding the MD-related genetic control.

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Keywords: Transcriptome, maturity date, nectarine development, mRNA, long non-coding RNA

Area: Genomics

PANEL

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Gene regulatory network analysis to uncover conserved plant drought stress responses

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One of the main problems for plant life is the availability of water. Due to climate change, drought events are becoming more frequent and cause severe damage to agricultural crop production. Drought influences the expression of thousands of genes through complex drought-responsive gene regulatory networks (GRNs). Although some plants tolerate drought better than others, little is known about the variation or conserved elements in their GRNs under this condition. To address this problem, we utilized the wealth of publicly available RNA sequencing data in response to drought to identify and compare differentially expressed genes (DEGs) in plants. We completed and normalized their metadata profiles, allowing for universal treatment details and regime comparisons. DEGs were derived for each experiment and further filtered to find shared responses in each plant. In addition, we predict GRNs according to chromatin accessibility and gene expression profiles. We define a group of consistent and common drought-activated genes in *Arabidopsis*, tomato, rice, and maize and reveal the transcriptional circuitry underlying these responses. Our studies provide a comprehensive view of transcriptional networks controlling drought in plants. It will serve as a reference regulatory network in each plant species that can help formulate hypotheses about genes and regulatory components essential for drought adaptation.

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Keywords: Drought, transcriptome, genome, transcription factors

Area: Genetic resources

PROTOCOL FOR IN VITRO CONSERVATION IN POPULATIONS OF *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae).

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Genetic resources: Within the strategies for the maintenance, conservation, and exchange of phylogenetic resources, *in vitro* conservation protocols are an essential part, offering a series of advantages such as aseptic conditions, reduced costs for subcultures and rapidly available genetic material. An alternative to establish *in vitro* conservation strategies is the minimum growth method, which seeks to reduce the physiological and metabolic activity of plant tissues, modifying factors and conditions in the culture medium, extending the periods between subcultures from short to medium term. *Colobanthus quitensis*, a native plant from Antarctica, is also distributed in the Andes up to 17°N. It constitutes an interesting model for studies on mechanisms of tolerance to extreme abiotic conditions, among others. The goal of this research is to generate a protocol for the *in vitro* conservation of this species, manipulating factors in the culture medium such as mineral reductions, concentrations of the gelling agent, growth inhibitors, and ethylene inhibitors to extend the subculture periods while maintaining the plants in good physiological condition. For this purpose, explants obtained in vitro from two populations of *C. quitensis* were used in each stage considering the best results of the previous stages, according to the morpho-physiological variables evaluated. The reduction of mineral salts to 50%, 12 explants per flask, 4% mannitol, film or cellophane as sealing type and ABA at 0.5 mgL-1 as growth inhibitor, achieved an extension of the subculture time of 90 days, observing a decrease in growth with optimal photosynthetic efficiency, facilitating the conservation of *C. quitensis.* The effects of increasing the gelling agent concentration and the addition of STS as a growth inhibitor are under evaluation and the results will also be show.

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Keywords: Minimal growth, in vitro preservation, *Colobanthus quitensis*.

Area: Genetic resources

RESPONSE OF INIA'S TOMATO CORE – COLLECTION TO WATER STRESS UNDER IN VITRO CONDITIONS

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Water deficit is the main limiting factor affecting worldwide crop production. Tomatoes are very sensitive to water deficits at an earlier stage of growth, including during and immediately after transplanting, flowering, and fruit development. Previously, the INIA's tomato Core – Collection was identified by ddRAD genotyping. This work characterized the response of the tomato Core – Collection under drought stress conditions. First, seeds from 34 tomato accessions were germinated on pleated paper under Control and Water Stress (14% PEG) conditions, evaluating germination percentage and root length. Subsequently, the same accessions were subjected to water stress tests in MS medium supplemented with 2% PEG for 21 days at 22°C. At the end of the assay, the following morphological parameters were evaluated: plant height, root length, fresh and dry weight, root and shoots, respectively.

Through multivariate statistical analysis, genotypes were classified as high, intermediate, and low tolerant to drought stress. From the Clustering, a group of sensitive varieties was identified, made up of varieties of Chilean, North American and Asian origin. An intermediate group is made up of varieties of different origins. In contrast, the cluster of tolerant varieties is mainly made up of Chilean varieties, whose characteristics are not far from the intermediate group, being necessary new studies to identify physiological differences among accessions of different clusters.

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Keywords: in vitro, Drought Stress, Tomato, Germplasm, Core Collection

Area: Genetic resources

PANEL

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ddRAD SEQUENCING-BASED GENOTYPING FOR POPULATION STRUCTURE ANALYSIS PROVIDES NEW INSIGHTS INTO THE INIA'S TOMATO GERMPLASM COLLECTION

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Genetic resources provide valuable genes to different biotic and abiotic stresses. Specifically, due to their adaptation to local environments, landraces are a source of valuable genes for tolerance to stressful environments. Considering that tomato (*Solanum lycopersicum*) is the most consumed vegetable at the national level and highly sensitive to drought stress, our work performed a genetic characterization of available tomato germplasm. Using ddRAD-seq genotyping we analyzed the genetic diversity of 156 tomato accessions. Of them, the 42.95% and 36.54% correspond to accession originating from Chile and the United States, respectively; while the 20.51% correspond to accessions from Peru, Argentina, United Kingdom, among others. In addition, Chilean accessions consider landraces, cultivars, and breeding lines. As results, 10.040 high quality SNPs were identified, which let us classify the evaluated accessions in 6 genetic clusters (Structure software). On the other hand, 13.763 and 1.277 SNPs are intergenic and genic regions, respectively. Finally, two different Core Collection were made using the CoreHunter1 and CoreHunter3 softwares, being identified 12 and 31 accessions, respectively. Of them, 25% and 54%, correspond to Chilean landraces, respectively, highlighting the importance of these materials as source of genes to different biotic-abiotic stresses. This analysis could be used in future studies focused on genome-wide association studies related to trait of interest as well as breeding programs.

Financing: FONDECYT DE INICIACIÓN No. 11200702

Keywords: ddRAD, Germplasm, Tomato, Core Collection, Landraces

Area: Genetic resources

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Blueberry polyploidy induction: contributing to the genetic improvement of this fruit in Chile.

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Blueberry is a fruit with high antioxidant power, which generates benefits for consumers' health and with an increasing demand worldwide. Throughout the last decade, Chile emerged as the main fresh fruit exporter in the southern hemisphere but lost that privileged position to Peru some years ago. Considering the high competitiveness of the sector and the effects of climate change, it's necessary to develop varietal replacements towards more productive cultivars, with higher quality and better adaptation.

Although there are different plant improvement techniques, the most relevant one used in blueberries is conventional genetic improvement through intraspecific and interspecific hybridization. New findings suggest other biotechnological techniques can be used for the same purpose. An interesting approach is polyploidization, which can be artificially induced with chemical, physical, or biological agents. It can generate interesting agricultural advantages such as increased organ biomass and higher content of primary and secondary metabolites, among others. Colchicine is a chemical agent that inhibits mitosis and prevents the formation of microtubules, making it impossible for sister chromatids to separate during mitosis, resulting in cells with duplicated chromosomes.

In this project, the obtention of a new cultivar was approached via polyploidization, induced by exposing explants (leaves and three buds' microstems) coming from in vitro cultures of three cultivars to 0.1% colchicine for one and two days under in vitro conditions. From a total of 180 explants treated per cultivar (Biloxi, Legacy, and Duke), results obtained showed 5, 4, and 4 polyploid plants and 4 and 2 mixoploid plants, respectively.

Flow cytometry was used to confirm the ploidy level of the plants obtained. It was possible to demonstrate that the induction of polyploidy using colchicine and in vitro regeneration is an effective technique that increases germplasm availability, developing a new genetic improvement program for blueberries in Chile.

Financing: ANID-Beca Doctorado Nacional 21191617

Keywords: Blueberry, Polyploidy, Genetic improvement, Colchicine

Area: Genetic resources

PANEL

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IDENTIFICATION OF CANDIDATE GENES INVOLVED IN RESISTANCE OF *ARABIDOPSIS THALIANA* TO THE CABBAGE APHID *BREVICORYNE BRASSICAE*.

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Aphids are phloem sap-feeding insects that represent potent agricultural pests of both wild and cultivated plants. Aphids can damage plants in many ways, mainly by withdrawing nutrients and sugar from vascular plants, by virus transmission, and also can reduce the photosynthetic capacity through the stimulation of molds that grow on the honeydew. Therefore, plants rely on special mechanisms of recognition, signaling, and defense to cope with the specialized mode of phloem feeding by aphids. Genetic variation is the basis for adaptation of plants to any future challenge. Association mapping has been proposed to detect polymorphic genetic markers involved in phenotypic variations and may prove useful in identifying interesting alleles underlying plant resistance. Using this approach, a genome-wide association mapping (GWAS) was developed on a worldwide population of 200 natural accessions of *Arabidopsis thaliana* to search for genomic regions controlling resistance to the specialist aphid *Brevicoryne brassicae*. The colonization performance (aphid reproduction) on each accession was evaluated as the offspring number and used as resistance parameters. Interestingly, we found that accessions displaying different life cycle strategies differ in their response to aphid colonization. Indeed, accessions with a winter-annual life strategy were more resistance to *B. brassicae*. In addition, the new candidate genes identify will be characterized in terms of the impact on aphid performance through a no-choice test (aphid reproduction), preference, and feeding behavior.

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Keywords: phloem sap-feeding insects, Association mapping, Aphid resistance, phenotyping, Offspring number, winter-annual

Area: Genetic resources

PANEL

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APPROACH TO GERMINATION PROBLEMS IN *Colobanthus quitensis*: A THEORY FROM THE EFFECT OF ENDOPHYTIC FUNGI

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Endophytic fungi (EF) on *Colobanthus quitensis´*s roots and leaves influence the response to harsh environment and their role seems to diminish when conditions are more benign, while it is unknown if EF are transmitted to seeds or influence the germination. The objectives are (1) to detect the presence of EF in roots and seeds of plants grown under laboratory conditions and in the Antarctic (only seeds); (2) to evaluate the effect of seed inoculation with extracts of EF isolated from araucaria. Roots of six *C. quitensis´*s populations were stained with trypan blue, and seeds were stained with rose bengal to search for EF. Inoculation with extracts of four fungi isolated from araucaria was carried out to evaluate their effect on germination. The material cultivated *in vitro* lacked EF, while the populations maintained in a common garden were associated with EF of the families Chytridiomycota, Ascomycota and Ascomycota. Seeds did not show fungal structures regardless of where they were produced. Inoculation with fungal extracts did not significantly influence the percentage, mean time and germination rate. It is concluded that *in vitro* culture of *C. quitensis* is an alternative to obtain EF-free material. EF are not transmitted vertically to offspring. Inoculation of seeds with araucaria seeds with FE does not influence the germination parameters of *C. quitensis*.

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Keywords: semillas, detección de hongos endófitos, índices de germinación

Area: Genetic resources

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A PRELIMINARY STUDY OF THE TRANSCRIPTIONAL REGULATION OF GENES INVOLVED IN ENDOCYTIC VESICULAR TRAFFIC INDUCED DURING RESPONSE TO SALT STRESS

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Soil salinity restricts water and nutrients uptake and causes ionic toxicity to the plants. Sodium compartmentalization in the vacuole is an essential response during salt stress, and this may be mediated, in part, by endocytic vesicular trafficking. Different proteins are currently known to be involved in directing vesicular trafficking to the vacuole during the salt stress response, whose genes are induced by salt. To date, the posttranslational regulation of these proteins has been extensively investigated, but no transcriptional regulation. This research set out to identify transcription factors (TF) that regulate genes involved in endocytic vesicular trafficking that contribute to sodium compartmentalization in the vacuole during salt stress. Previously, a table with publications, where the overexpression or heterologous expression of a gene coding for a TF significantly increased tolerance to salt stress. These TF were subjected to several bioinformatics analyses: 1) Evaluation of spatiotemporal gene expression under salinity conditions. 2) Comparison of homolog expression between salt stress sensitive and salt stress tolerant species. Next, the promoter sequences of 5 genes related to vesicular endocytic trafficking were analyzed in search of cis-binding elements for the TFs selected and identified common binding elements that were related to 6 TFs candidates. Finally, 18-day-old *Arabidopsis* thaliana plants of wild-type genotype were submitted to a salt stress treatment for 0, 1, 2 and 4 hours, root and leaf samples were collected, from which total RNA was isolated and the expression of the TFs genes was evaluated by qPCR. These results open the possibility of advancing research on the regulation of endocytic vesicular trafficking mediated by specific TFs.

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Keywords: Transcription factors, Endocytic vesicular trafficking, Salt stress tolerance.

Area: Cell biology

PANEL

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Bioprospection of plant growth and stress tolerance promoting bacteria from *Chenopodium quinoa seeds.*

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The study of the mechanisms that plants use to adapt to extreme conditions is a useful alternative to increase tolerance in traditional crops. Quinoa is a resilient plant that produces good-quality seeds in harsh conditions such as those found in the Chilean highlands, an environment characterized by seasons of water deficiency, high salinity, and elevated temperature fluctuations. It has been suggested that plant-associated microorganisms contribute to plant adaptation to the environment and that seeds can function as an inheritable inoculum that allows transfer of the microbiome between generations. In this work, we present a characterization of the seed-associated microbiota of quinoa and their prospection as potential growth-promoting bacteria under saline stress conditions. We generated a collection of bacteria isolated from five different varieties of Quinoa (*Amarilla, Blanca, Pandela, Regalona, Paredones*) and characterized this collection using a set of different microbiological and biochemical assays. We obtained a collection of 185 isolates, 76 of them being putative endophytes. We selected five bacteria which showed capacity to solubilize K and P, fix nitrogen, synthesize auxins, and use ACC as nitrogen source (proxy of ACC deaminase activity). We generated a synthetic consortium and evaluated it as a growth promoter and salt tolerance inducer in *Arabidopsis thaliana* and *Solanum lycopersicum* L. var. MicroTom.

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Keywords: QUINOA, PGPR, SALINITY, ABIOTIC STRESS

Area: Bioinformatics

PANEL

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Integrating histone marks, DNA accessibility, And motif data to predict regulatory maps in *Arabidopsis thaliana*

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Plants are sessile organisms that change their gene expression levels under stressful conditions. To performs an adaptative response under such conditions, there exist regulatory mechanisms that govern gene expression. Among these, the binding of transcription factors (TFs) to specific motifs in the DNA is especially relevant. However, in eukaryotic cells, DNA is compacted, forming the chromatin that needs to be reorganized to allow or impede the binding of the TFs.

Chromatin is not compacted uniformly; its structure depends on the action of several modulatory proteins that, for example, introduce chemical modifications in histone tails or the DNA. How the combinations of these factors are related to the compaction of chromatin and the binding of TFs needs to be better understood in plants. So, for a better understanding of the relationship between epigenetic marks, the accessibility degree of the chromatin, and the binding of TFs, we have integrated epigenetic information derived from *Arabidopsis thaliana*. Then, we systematically integrated this information to train Random Forest algorithms to determine the importance of each feature to contribute to transcriptional regulation. Our results demonstrate how specific combinations of epigenetic modifications predict the local structure of chromatin and TF activity, performing better than Random or no-skill model in ROC and Precision-Recall curves. Our approach will help to advance the understanding of how epigenetic features determine plant adaptation to the environment.

Financing: PROYECTO FONDECYT POSTDOCTORADO 3220673PROYECTO FONDECYT REGULAR 1210389

Keywords: *Arabidopsis* thaliana, Epigenetics, Histone Marks, DNA accessibility, Machine Learning, Data integration, environment adaptation

Area: Bioinformatics

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In silico modeling of OsAAP3 transporter from *Oryza sativa* for target identification in CRISPR-Cas9 selective mutagenesis.

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In silico modeling of OsAAP3 transporter from *Oryza sativa* for target identification in CRISPR-Cas9 selective mutagenesis.

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OsAAP3 is an aminoacid transporter (AAT) gene family member. In rice OsAAP3 transport basic aminoacid especially Lys and Arg from root to sink organs. The functionality decrease of OsAAP3 transporter has been related to increased efficiency in the use of nitrogen, overgrowth of buds and increased number of tillers in rice, generating better yields. The aim of this research is identifying the main AA residues that conform the protein and involved in the selectivity and pore interaction. All of this, in term to design a strategy to CRISPR-Cas9 mutagenesis for improve the rice yield.

The OsAAP3 was aligned with other ortholog confirming that the protein is highly conserved. The crystallographic model of Transporter of Arg from *Mus musculus* (Code 6C08) was obtained from PDB. The in-silico protein model OsAAP3 (Q5Z9R9) was obtained from PROSITE. Both structures were used as input in Modeller and Alphafold for obtaining the first model. Minimization of the model was performed using NAMD3. For identify the most important AA residues that interact with the Arg in the channel, docking analysis was performed using AUTODOCK vina.

Here, we propose use in-silico modelling for selection of the better residues to modification in context to obtain loss of activity in AA transport by OsAAP3. As results, the residues Thr140, Asn135, Leu48, Tyr164, Val57, Ala281 were present in the center of the channel of OsAAP3. They conform the more straits region of the channel, where it is more conducive to block the entrance. These residues are candidate to modify mediating CRISPR-Cas9 and obtain reduction of transport activity of the protein.

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Keywords: Bioinformática, proteínas, transportadores, aminoácidos

Area: Bioinformatics

PANEL

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IDENTICATION OF THE BZIP TRANSCRIPTION FACTOR FAMILY OF *PRUNUS SALICINA* LINDL.

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Leucine basic zipper (bZIP) family transcription factors have been shown to regulate various biological processes in plant growth and development, as well as responses to abiotic and biotic stress. In *Arabidopsis thaliana*, bZIP family is classified into 13 groups based on the phylogenetic relationship of their aminoacidic sequences. Biological functions have been described for each group, e.g., response to ABA signals and defense against pathogens Group A and Group D, respectively). However, little is known about the bZIP family in *Prunus salicina*. In the present study, we carried out a genome-wide identification and systematic analysis of the bZIP in *Prunus salicina.* A total of 55 PsbZIP genes were unevenly distributed on plum chromosomes, also these proteins were divided into 13 groups according to the phylogenetic relationship. All the PsbZIP protein sequences accounted for the principal bZIP domain (N -x7-K/R -X9-L-x6-L x6-L) and additional specific motifs for each of the described groups. Groups J and D represent the groups with the largest number of additional conserved motifs, while Group C represent the group with the least number of additional conserved motifs. This characterization of the PsbZIP family of the *Prunus salicina* genome provides a useful information for future functional studies of these genes, which could be associated with biological functions

Financing: This work has been funded by grants FONDECYT Regular 1191446, FONDECYT Iniciación 11150662 and BECAS DE DOCTORADO NACIONAL/2020 – 21200330, all from ANID Chile.

Keywords: Plum, Phylogenetics, Leucine basic zipper

Area: Bioinformatics

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Identification of sulfate deficiency-responsive microRNAs in *Solanum lycopersicum*

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Sulfate is the main source of sulfur available for plants and its deficiency negatively impacts plant growth and yield. Our previous work showed that sulfate deficiency has detrimental effects on root and leaf growth in Solanum lycopersicum, one of the most important crops in Chile. This effect is explained in part by extensive changes in gene expression in both organs. microRNAs (miRNAs) are short non-coding RNAs with an important role in post-transcriptional regulation. We sought to determine whether sulfate deficiency triggers differential expression of miRNAs in tomato, and the impact of microRNA-dependent regulatory networks on the transcriptomic response of roots and leaves. We generated sRNA-seq libraries from roots and leaves of tomato plants grown in sulfate deficiency or control conditions for three and four weeks. De novo identification of smallRNA clusters identified 232 known expressed miRNAs in roots and leaves, of which 53 of them were differentially expressed by sulfate deficiency. Sulfate regulation of miRNAs was highly time and organ-specific, with only miR395 being consistently regulated across time points and organs. Using different bioinformatic and experimental data, we determined transcript targets for the differentially expressed miRNAs, obtaining 413 miRNA-target pairs. This information was integrated with transcription factor-target regulatory interactions to build a gene regulatory network controlled by sulfate-responsive miRNAs. This network contains genes involved in sulfate transport and metabolism, as well as other processes. In sum our work provides evidence for a relevant role of miRNA regulation on the reprogramming of the root and leaf transcriptome during sulfate deficiency.

Financing: This work was supported by ANID-FONDECYT 1211130 and ANID-Millennium Science Initiative Program ICN17_022

Area: Bioinformatics

PANEL

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CISTANTHE LONGISCAPA: A GENETIC LOOK AT THE FLOWERING DESERT

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The Flowering desert is a massive blossoming natural phenomenon in the Atacama Desert, one of the driest deserts with the highest solar irradiance on the planet. *Cistanthe longiscapa* is a predominant endemic plant of the flowering desert. The unique genetic and phenotypic features of this plant that allow it to survive in this unfavorable environment are unknown. To investigate, we assembled a draft genome of *C. longiscapa* using a hybrid assembly strategy combining long with short reads. In parallel, we evaluate the plant's photosynthetic capacity against light stress (above 1000 Photosynthetic Photon Flux Density (PPFD). The 797 Mbp draft genome is distributed in 739 scaffolds, with an N50 of 2.73 Mbp. The 42,760 annotated genes represent 29.65% of the genome, of which at least 36.76% are duplicated, while repetitive elements represent another 41.17% of the genome. Strikingly, *C. longiscapa* does not show a saturation point of their photosynthetic capacity even above 2500 PPFD, which can be associated with high adaptability and resistance to light stress. This work is the first step to characterizing the survival mechanisms of the predominant specie of the flowering desert phenomenon in Atacama.

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Keywords: Hibrid assembly, light stress, Atacama desert, flowering desert, Caryophyllales, betalain, Cistanthe longiscapa

Area: Biochemistry and molecular biology

PANEL

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GENERATION OF *ACTINIDIA DELICIOSA* VAR. HAYWARD PLANTS TOLERANT TO SALINITY AND DROUGHT THROUGH EDITION OF *AdSAP7*

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Climate change produce extreme climatic events, such as drought and salinity. These abiotic stresses can reduce the yield and quality of crops in about 50%. To overcome this problem, and to maintain crop yield in salinity and drought conditions, we propose to generate abiotic stress tolerant plants. Among them, Actinidia deliciosa var. Hayward (Kiwi) emerges as an interesting plant to be improved because it is one of the most important crops cultivated and commercialized in Chile and highly sensitive to salinity. Therefore, the aim of this work was to edit negative regulators of drought and salinity stress in kiwi. By in silico analysis, we selected five genes as negative regulators of drought and salinity stress tolerance in the sequenced Actinidia deliciosa var. Hayward genome. Among them, we selected AdSAP7, AdSFRP1, AdSARP1 and AdDIS1 that codify for E3 ubiquitin ligases whose function is to send transcription factors required for abiotic stress tolerance to degradation. Additionally, we included AdACS6 that codifies for an ethylene synthesis enzyme, which reduces abiotic stress tolerance. Expression analysis of the five genes in kiwi plants subjected to acute drought and salt stress, showed that only AdSAP7 was induced by salinity and drought and AdACS6 was induced by salt stress. Therefore, both genes were selected for being edited in kiwi. Currently, specific gRNAs for AdSAP7 were designed and synthesized for CRISPR/Cas9 vector construction. Agrobacterium mediated transformation of kiwi explants with CRISPR/Cas9-AdSAP7 allowed the evaluation of edition efficiency in callus, while shoot regeneration is ongoing.

Financing: Anillo PIA ACT192073

Keywords: Actinidia deliciosa var. Hayward, Kiwi, AdSAP7, drought, salt stress, CRISPR/Cas9, gene edition

Area: Biochemistry and molecular biology

PANEL

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EXPRESSION ANALYSIS OF OSDIS1 AND TAACO1 ORTHOLOGUES IN TOMATO UNDER SALT AND OSMOTIC STRESSES AND IN VITRO ORGANOGENESIS OF TOMATO VAR. PONCHO NEGRO

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Climate change is a global phenomenon that involves a rise of drought and salinity of soils, the most relevant abiotic stresses that impair crop development and yield. At the molecular level, plants respond to abiotic stress through several mechanisms which modulates gene expression to develop an adaptive response. Nevertheless, negative regulator genes produce susceptibility to abiotic stress in plants. Tomato (*Solanum lycopersicum*) is the most cultivated and important vegetable in the world and in Chile; however, drought and salinity of soils impair yield and quality. Among tomato varieties, we select the endemic Poncho Negro and propose improve to obtain a novel tomato rootstock variety more tolerant to drought and salinity by editing an abiotic stress negative regulator gene. Upon bibliographic search we identified two genes that confer drought and salt stress susceptibility when overexpressed in plants. *TaACO1* codes for an ethylene biosynthesis enzyme, a hormone that confers susceptibility to salt and drought stress, and *OsDIS1* encodes for E3 ubiquitin-ligase which signals factors conferring stress tolerance to proteosome-dependent degradation. We found 7 and 8 orthologs in the tomato Poncho Negro genome. Through phylogenetic and *in silico* expression analysis we selected *SlACO2* and the three most conserved orthologues for *OsDIS1*; *SlDIS1*,*SlSINAT2* and *SlSINA5*. Then, through qRT-PCR we determined that *SlDIS1, SlSINAT2* and *SlACO2* are expressed under acute salt stress and drought in the commercial variety “ACE” and in Poncho Negro, which allows us to select them to be edited. In addition, somatic organogenesis of tomato Poncho Negro has been standardized to obtain edited lines. Currently, effectiveness of CRISPR/Cas9 vector that carries gRNAs for *SlACO2* has been evaluated in *in vitro* Poncho Negro calli.

Financing: PROYECTO ANILLO PIA ACT-192073

Keywords: Tomato, var. Poncho Negro, OsDIS1, TaACO1, Drought, Salt stress, CRISPR/Cas9

Area: Biochemistry and molecular biology

PANEL

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Functional characterization of UDP Arabinofuranose transporters (AT3G17430 and AT1G48230) involved in the synthesis of mucilage from *Arabidopsis thaliana* seeds.

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L-Arabinose (L-Ara) is a plant-specific monosaccharide that can be found in its furanose or pyranose conformation (L-Ara*f* and L-Arap respectively). L-Araf is part of the structure of rhamnogalacturonan-I (RG-I) which in turn are the main components of Arabidospsis seed coat mucilage. The UDP-Ara*f* synthesis of is carried out in the cytosol thanks to the UDP-Arabinose mutase (UAM) complex, which interconverts UDP-Arap to UDP-Araf witch is transported to the Golgi lumen thanks to the action of UDP-Araf transporters (UAFTs). in the Golgi apparatus, glycosyltransferases use this UDP-Araf as a substrate form pectin polysaccharide. In silico data indicate that the putative UDP-Araf transporters, *UAFT5* and *UAFT6*, are specifically expressed in the seed coat throughout seed maturation, suggesting that they may play a role in the biosynthesis of pectin from mucilage. In this work, we selected one insertional mutant line for UAFT5 and two for UAFT6. These mutants showed changes mucilage monosaccharide composition, which suggests that both genes are involved in mucilage synthesis. Additionally, mutants in both genes showed changes in the methylation patterns detected by the JIM5 and LM20 antibodies, which was supported by a reduction in the content of methanol released in the MA. These results suggest that the availability of UDP-Araf can affect the synthesis of RG-I arabinans and, indirectly, produce changes in the structure of HG in response of *UAFTs* mutation.

Financing: Funding: FONDECYT 1201467

Keywords: UDP-Araf, UAFT5, UAFT6, NST, RG-I, HG

Area: Biochemistry and molecular biology

PANEL

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Genomic editing of SlMIEL1, a negative abiotic stress regulator on *Solanum lycopersicum* cv. Poncho Negro: using a chilean endemic cultivar for boosting drought and salinity tolerance in rootstocks.

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Global warming is a human-accelerated phenomenon that is causing, amongst other consequences, a rise in world temperatures and thus magnifying abiotic stress factors such as salt and drought stress. These factors impair production yield of crops that can reach up to 50% yield loss, where tomato is amongst. This crop is the second most important in the world, with a production of ~100 million tons per year. In Chile, the endemic Poncho Negro variety from the Azapa Valley is free of royalty, making it an interesting target for improvement by means of molecular biotechnology, specifically, with the CRISPR/Cas9 system, which allows the edition of genes in a very precise manner. In this work, six negative stress regulators of drought and salinity tolerance were identified in the *Solanum lycopersicum* cv. Poncho Negro genome as edition candidates. Then, we evaluated their expression under salt and drought conditions by means of RT-qPCR, from which we selected *SlMIEL1*, an E3-ubiquitin ligase that has been reported in rice as a negative stress regulator. Then, we designed two specific gRNAs for *SlMIEL1* and cloned them into the pGGK7-AtCas9 vector, aiming for the deletion of most of its sequence, rendering this negative stress regulator non-functional. Then we evaluated by PCR the edition in transient transformed leaves and started the generation of *SlMIEL1* knock-out plants via *A. tumefaciens* transformation, stablishing a protocol for regeneration of plantlets from leaves and stem tissue.

Financing: ANID, proyecto Anillo-PIA ACT192073

Keywords: tomato, Poncho Negro, Salt Stress, Drought, CRISPR/Cas9

Area: Biochemistry and molecular biology

PANEL

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Kallfu and Wenutram: Two Chilean flaxseed cultivars, with contrasting mucilage content.

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Flax (*Linum usitatissimum*) is an important annual herbaceous plant with economic importance since ancient Egypt. Flax is traditionally used to produce textiles (Linen) and flaxseed. Flaxseed is an oily seed that produces high amounts of oil, especially a-linoleic acid, which benefits human health. Flaxseed is also known for producing mucilage; a gel mainly composed of complex polysaccharides, showing several favorable properties for human health. Flaxseed mucilage has been proposed to suit the food industry and pharmaceutical applications. In this work, we studied two Chilean flaxseed cultivars, Kallfu and Wenutram, with contrasting mucilage phenotypes. Kallfu has high mucilage content per seed; meanwhile, Wenutram has a lower mucilage content. Using biochemical approaches, we analyzed mucilage extrusion area, protein content, and monosaccharides composition. Also, to get a deeper understanding of mucilage structure, monosaccharides and polysaccharides were analyzed by separating pectins and hemicellulose. Contrasting differences were found in almost all analyzes between Kallfu and Wenutram, however, general structures and monosaccharide proportions were similar in both cultivars. Both cultivars seem to have rhamnogalacturonan-I (RG-I) as the main component of pectin with small amounts of homogalacturonan (HG), homorhamnan (HR), and rhamnogalacturonan-II (RG-II). On the other hand, xyloglucan and arabinoxylan seems to be the main polysaccharides in hemicellulose.So far, the results indicate that the main difference between the two cultivars is the total content of monosaccharides.

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Keywords: Flax, Flaxseed, Mucilage, Cell Wall, polysaccharides

Area: Biochemistry and molecular biology

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UXTs an unexplored family for synthesis of polysaccharides in the seed coat mucilage in *Arabidopsis thaliana.*

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Xylose (Xyl) is a significant component of the plant cell wall and the second most abundant sugar in the secondary cell wall. UDP-Xyl es the primary donor molecule for the biosynthesis of hemicellulose and pectins. The synthesis of these non-cellulosic polysaccharides is carried out in the lumen of the Golgi apparatus, and since the synthesis of UDP-xylose takes place mainly in the cytoplasm, the nucleotide sugar transporters (NSTs) play a pivotal role in supplying the UDP-xylose necessary for this process. Three UDP-xylose transporters (UXTs) have been described in *Arabidopsis thaliana*, and they are critical players in the biosynthesis of xylan in the xylem.

On the other hand, *Arabidopsis* mucilage has become a great model for cell wall studies because it is a pectin-rich structure, mainly composed of RG-I. At the same time, Xyl is linked to pectin domains in mucilage, and a good correlation between the content of RG-I and Xyl has been shown in different reports. Furthermore, xylan may be necessary for RG-I attachment to the seed surface. Due to this, the following question arises: how does the decrease in xylan synthesis (UDP-Xyl) impact RG-I synthesis? To solve this query, we decided to analyze what happens with RG-I biosynthesis in *uxts* mutants. Biochemical analyzes in mucilage from *uxts* showed that they affect the monosaccharide composition, particularly in rhamnose, galacturonic acid, and xylose. On the other hand, the mucilage mutants present alterations in the pattern recognized by specific antibodies and in mucilage release.

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Keywords: NST, Xylose, UDP-Xylose, Pectins, Mucilage, Cell wall

Area: Biochemistry and molecular biology

PANEL

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ETHYLENE ACTS AS A KEY MEDIATOR IN *SOLANUM LYCOPERSICUM* NITROGEN-DEPENDENT DEFENSE RESPONSE AGAINST *BOTRYTIS CINEREA*

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Botrytis cinerea is a necrotrophic pathogen described as presenting a wide array of infection strategies. Likewise, plants have developed many defense pathways with complicated interactions between a variety of factors. Contrary to observation in other commonly studied species, *Solanum lycopersicum* has been identified as a particular case as it displays less susceptibility the more nitrogen availability there is. The mechanism underlying this nitrogen-dependent resistance is not yet understood. In our work, we focus on a possible crosstalk relationship between nitrogen and the gaseous hormone ethylene to explain this observation. To evaluate this possibility, both exogenous ethylene (ethephon 1mM) and an ethylene inhibitor (2,5-norbornadiene, NBD) treatments were performed in tomato plants grown under contrasting nitrogen conditions (1 and 10 mM KNO_3) and subsequently infected with *B. cinerea* (B05.10; 5 x 10^5 conidia/ml). Lesion diameter was evaluated at regular intervals during infection progression, obtaining significantly smaller wounds in plant groups treated with both ethylene and high nitrogen concentrations. Consistently, plant groups with NBD and lower nitrogen disposition presented larger lesions than control groups. Genes related to ethylene biosynthesis and signaling, nitrogen, and defense were evaluated using qPCR at 8, 24, and 48 hours post-infection to further explore this interaction. Ethylene-related genes *LeSAMS3, LeACO5, LeETR5, LeEIL4, and LeERF1* showed a significantly augmented expression in ethylene-treated and high nitrogen groups. Nitrogen-related genes *LeNRT2.2* and *LeNR,* along with *LeMYC2* defense marker exhibited a similar behavior. A Three-way ANOVA statistical test identified the nitrogen-ethylene-time combination as a significant source of variation in all of the previously mentioned genes. Taken together, our findings suggest the existence of an infection timeline-dependent crosstalk between nitrogen and ethylene that plays a relevant part in the tomato plant's defense against *B. cinerea.*

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Keywords: Botrytis cinerea, Solanum lycopersicum, biotic stress, nitrogen, ethylene response, plant-pathogen interaction

Area: Biochemistry and molecular biology

Identification of the regulatory network controlling root hair growth under inorganic phosphate deficiency combined with high salinity conditions in *Arabidopsis thaliana*.

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Plant growth is conditioned by several stresses from drought, salinity, changes of temperature, and variable levels of nutrients. Although the impact of individual stresses has been researched, in field conditions several of them might affect plant growth at the same time. Nowadays, to understanding these multi-stress interactions have become critical to achieve sustainable agriculture in a context of global climate change. We explored the link between two abiotic stresses (phosphate (Pi) starvation and high salinity) and their impact on root hair (RH) growth. RH have a vital implication for plants as they determine the surface/volume ratio of their roots exposed to water and nutrients reserves in soils. Nevertheless, the molecular mechanisms controlling RH growth under multiple abiotic stresses are still unknown. It is known that two transcription factors, PHOSPHATE STARVATION RESPONSE1 (PHR1) and PHR1-like1 (PHL1), control the low Pi response in roots but it is still unknown how they operate at the RH level. Our research made use of RNAseq and GWAS approaches in order to identify novel key genes required to regulate RH growth under low Pi with high salinity. Our understanding of how these two-stress act at the molecular level may help us to develop super adaptable crops. Our results suggest that PHR1 could be important to respond to these multi-stress conditions as *phr1-1* mutants showed inhibition of RH growth in low Pi/high salinity treatment. Also, we showed that transcript levels of *PHR1* and *PHL1* are strongly induced after 6 hours of treatment. For GWAS analysis, we have analyzed RH length from about 100 ecotypes. Interestingly, we have found contrasting behaviors between some accessions: Ge-0, Hs-0 and Ep-0. Lastly, in order to generate super adaptable plants, ongoing research is being conducted to transfer knowledge from *Arabidopsis* to the fruit model plant *Solanum lycopersicum.*

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Keywords: *Arabidopsis* thaliana, Low Phosphate, High Salinity, Multi-Stress, RNAseq, GWAS, ecotypes

Area: Biochemistry and molecular biology

PANEL

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MULTI-GENOME SEARCH: NEW REPORTS OF THE YABBY TRANSCRIPTION FACTORS FAMILY IN *Fragaria* GENUS.

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YABBY transcription factors are specific to seed plants, regulating the growth of leaves, flowers and fruit. In this study, the complete genome of six *Fragaria* species (Rosaceae) was analyzed where the evolutionary relationship of the *YABBY* genes in each species was identified. Twenty-five YABBY members were identified *in F.* ×*ananassa* and 5 YABBY members in each of the *Fragaria* species studied (*Fragaria daltoniana, Fragaria nilgerrensis, Fragaria pentaphylla, Fragaria viridis and Fragaria vesca)*. We identified that all YABBY proteins, deduced from their sequences, presented the two highly conserved domains (zinc finger and YABBY) of the previously reported YABBYs proteins. In turn, the putative FaYABBY proteins were three-dimensionally characterized, observing the presence of two alpha helices linked by a loop, which is highly conserved in all YABBY proteins. In the case of *F.* ×*ananassa*, the *YABBY* genes were located on five different chromosomes. Regarding gene expression, a higher expression of *FaYABBY* genes was observed in the development of leaves, flowers and early stages of fruit development. Finally, the presence of cis-elements of responses to different phytohormones was identified in their promoters. Thus, these results bring new information on the *YABBY* genes and proteins of six *Fragaria* species providing a basis for studying the function of YABBY family members in strawberry plant development.

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Keywords: YABBY, transcription factors, Fragaria

Area: Biochemistry and molecular biology

PANEL

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Assessment of the methylation index in *Arabidopsis thaliana*

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Methylation of DNA and histones has profound effects on the epigenetic regulation of plants. In addition, methylation of polysaccharides, such as pectin and hemicelluloses, is essential for assembling the plant cell wall. S-adenosylmethionine (SAM) is the methyl donor for the methylation of nucleic acids, proteins, polysaccharides, lipids, lignin and is also involved in the synthesis of ethylene and polyamines; thus, SAM plays a critical role in plants. One of the products of the methylation reactions is S-adenosylhomocysteine (SAH); which acts as an inhibitor of methylation reactions; thus, methylation reactions are regulated by the concentration of the methyl donor (SAM) and the concentration of the inhibitor (SAH). Hence the SAM/SAH ratio has become known as the methylation index. However, little is known about how this index is regulated in plants. *Arabidopsis thaliana* is a model plant that became a valuable tool for genetic analysis. *Arabidopsis* has a low mass, a fully developed plant weighs less than 1 g, and different organs weigh a few milligrams; therefore, highly sensitive assays are required to work with a low amount of material. In order to develop a quantitative, highly sensitive assay to assess the methylation index in *Arabidopsis*, we develop a UPLC-MS assay to measure SAM and SAH that allows us to analyze individual organs from a single plant. Differences in SAM content were detected among different organs; however, the methylation index remains relatively constant. Despite this, some changes were observed between day and night. Finally, a valuable tool to assess the methylation index has been developed, which should help us to identify genetic and environmental cues that affect methylation in plants.

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Keywords: SAM, SAH, LC-MS, methylation, methylation index, *Arabidopsis* thaliana

Area: Biochemistry and molecular biology

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The rhomboid proteases AtRBL13 and AtRBL15 participate in the plant response to osmotic stress

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Rhomboid proteases are membrane bound proteases that can cleavage substrate proteins within its transmembrane domains. These proteases are conserved across life kingdoms from bacteria to plants. Although members of this family of proteins from bacteria, yeast, fly and mammals has been studied during the recent years, few is known about the function of these proteases in plants. From previous analyses, we identified two rhomboid proteases that could participate in the processing of a membrane bound transcription factor known as the unspliced form of the AtbZIP60 (AtbZIP60U) upon plant exposure to osmotic stress. These two rhomboid proteases are known as AtRBL13 and AtRBL15. In order to understand the role of AtRBL13 and AtRBL15 during osmotic stress, we analyze the expression of AtbZIP60U targets on T-DNA insertional mutant plants for each of the genes encoding for these proteases. Also, we evaluate the tolerance and survival of these mutants under different osmotic stress inducing agents like sorbitol or mannitol. From these analyses, we observed that mutant plants on AtRBL13 exhibit a deregulation of the expression of AtbZIP60U target genes under osmotic stress, whereas mutant plants on AtRBL15 only show a modest change on expression compared to wild type plants. Similar results were observed in the tolerance and survival analysis, since *atrbl13* mutants shown a higher survival rate and to be tolerant to higher concentrations of mannitol and sorbitol than wild type plants. In the other hand, *atrbl15* mutant plants shown a higher survival rate but only modest increment in tolerance to higher concentrations of mannitol and sorbitol than wild type plants. Overall, these results contribute to the understanding of the function of the rhomboid proteases in plants and offer an opportunity to study the role of these proteins in the plant response to osmotic stress.

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Keywords: Osmotic Stress, Endoplasmic Reticulum, Rhomboid Protease, Abiotic Stress

Area: Biochemistry and molecular biology

PANEL

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Searching for gene sequences codying for cell wall degrading enzymes within a *Vasconcellea pubescens* fruit transcriptome

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Mountain papaya (Vasconcellea pubescens) is an exotic fruit, native from the Andean region of South America. The fruit follows a climacteric ripening behavior characterized by a reduction in fruit firmness, the development of yellow color and ethylene rise. Softening is related to the action of cell wall degrading enzymes. A complete transcriptome study was performed due to the fact that little information is available about the molecular mechanisms involved in softening. For that, total RNA was extracted from softening fruit and sequenced using Roche/454 technology. From the database obtained, the search for cell wall disassembly genes was performed. Full length codying sequences were obtained: 4 Pectin methylesterases (PME), 2 Pectin methylesterase inhibitors (PMEI), 4 Xyloglucan endotransglycosylase/hydrolases (XET/XEH), 1 Polygalacturonase (PG), 1 Expansin (EXP) and 2 Pectate lyases (PL), among others. Phylogenic analyses were performed for PME-PMEI sequences allowing its classification into three different groups: 2 PMEI, 1 PME/PMEI and 3 containing the methyl transferase domain. In the case of XET/XEH, the 4 sequences were classified in group I/II. Finally, the accumulation of transcripts was analyzed by RT-qPCR, and for most of the genes tested, an increment in their expression was quantified as fruit softening progresses, suggesting their participation in papaya cell wall disassembly. These cell wall disassembly genes will be cloned in order to perform their biochemical characterization.

Financing: This work was supported by ANID-ACT210025 project

Keywords: Fruit softening, Transcriptome, XTH/XEH, PME/PMEI, Mountain papaya, cell wall disassembly genes

Area: Biochemistry and molecular biology

PANEL

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STUDYING THE ROLE OF UDP-RHAMNOSE/ GALACTOSE TRANSPORTER 3 (URGT3) IN *ARABIDOPSIS THALIANA* DEVELOPMENT.

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The cell wall is a matrix that plays an essential role in the growth and structure of plant cells. This cell wall is mainly composed of cellulosic and non-cellulosic polysaccharides. The non-cellulosic polysaccharides (hemicelluloses and pectins) are synthesized in the Golgi apparatus lumen. Nucleotide sugar transporters (NSTs) are Golgi-membrane localized proteins that play a crucial role in incorporating NDP-sugars into the Golgi apparatus. The NDP-sugars, such as UDP-rhamnose (UDP-Rha), are the building blocks for synthesizing polysaccharides such as Rhamnogalacturonan-I and Rhamnogalacturonan-II. The *URGTs* gene family are NSTs and comprises six genes involved in transporting UDP-rhamnose and UDP-galactose. UDP-rhamnose is a crucial substrate to the backbone of RG-I and RG-II side chains. In addition, the UDP-rhamnose is a substrate to flavonol rhamnosylation, a process carried out in the cytoplasm whose imbalance can disrupt auxin accumulation and thus affect plant growth. To date, there is no evidence of morphological consequences associated with the lack of some URGTs, on the availability of UDP-rhamnose inside and outside the Golgi. In this work, we find that *URGT3* plays a key role in rosette leaf growth and auxin accumulation pattern. Additionally, we found that by blocking flavonol-7-rhamnosylation using a mutant in *UGT89C1*, a gene coding for the flavonol-7-rhamnosyltransferase, it is possible to restore the dwarf phenotype exhibited by *urgt3*, by creating double mutants between *ugt89c1* and *urgt3*. These data show that it is possible to redirect the flux of UDP-rhamnose towards pectin synthesis in detriment of flavonol-7-rhamnosylated production.

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Keywords: UDP-RHAMNOSE, CELL WALL, FLAVONOL, AUXIN, *ARABIDOPSIS* THALIANA

Area: Biochemistry and molecular biology

PANEL

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THE FUNCTION OF *NSP1* AND *NLP9* GENES IN THE ASSOCIATION of *Arabidopsis thaliana* WITH THE N-FIXING BACTERIUM *Sinorhizobium meliloti*

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Nitrogen (N) is an essential macronutrient for plant development. When this element is limiting, some plants can establish beneficial associations with nitrogen-fixing bacteria (NFB) that fix atmospheric nitrogen (N_2) into N forms that plants can assimilate/use. These interactions are well described in legumes, which form specialized organs called nodules that harbor the bacteria for N-fixation. In contrast, associations described in plants that do not form nodules are much less understood. And the molecular mechanisms involved in plant-bacteria association have not been characterized. We found that the widely used *Arabidopsis thaliana* can establish beneficial associations with the NFB *Sinorhizobium meliloti*. We observed that *S. meliloti* colonizes plant roots and supports *Arabidopsis* growth under N-deficient conditions. Furthermore, we found that the *Arabidopsis thaliana NSP1* and *NLP9* genes are induced after inoculation with bacteria. We characterized *nsp1* and *nlp9* mutants and found that these genes are required for a functional association between both organisms. Interestingly, *NSP1* and *NLP9* are necessary for *nifH* induction in *S. meliloti.* Moreover, the N-fixing activity of *S. meliloti* was reduced in *nsp1 and nlp9* mutant plants. This work demonstrates potentially regulatory conserved elements in non-nodulating plants to interact with NFB and survive under low N conditions.

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Keywords: biological N-fixing, plant-bacteria interations, non-nodulating N-fixation, molecular plant-microbe interactions, diazotrophs, *Arabidopsis* microbiome

Area: Biochemistry and molecular biology

PANEL

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Evaluation of the negative regulatory gene *SAP7* in *Nicotiana tabacum.*

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The effects of climate change have worsened in recent years. Along with reduction of greenhouse gas emissions, we must generate new technological strategies for agriculture. In this sense, new gene editing and genome study technologies play a leading role, since they allow us to generate new crop varieties adapted to drought and salt stress. These abiotic stresses trigger signals in plants that led to their tolerance. Within this response there are genes that negatively regulate the abiotic stress response, affecting the plant tolerance under stress conditions, hence studying their functionality for possible editing could favor plant tolerance.

Based on bibliographic data we selected SAP7 (Stress-associated protein) an E3 ubiquitin ligase, since in rice *OsiSAP7*, acts as a negative regulator leading to salt stress susceptibility when overexpressed in *Arabidopsis*. SAP7 ubiquitinates transcription factors bZIP required for abiotic stress tolerance in plants. The *OsSAP7* orthologous were identified by bioinformatic analysis in *Actinidia deliciosa* var. Hayward (kiwi) and AdSAP7 was selected due to their high percentage identity to *AdSAP7* expression is induced at 6 and 48 hrs. in *in-vitro*-grown kiwi seedlings subjected to acute drought and salinity stress. Then, *AdSAP7* was cloned in the expression vector pGWB5 producing pG*AdSAP7: GFP*. Subcellular localization was determined by transient transformation of *N. tabacum* leaves. Stable transformation in tobacco produces several T0 transgenic plants that will be subjected to salt stress to determine *AdSAP7* role as negative regulator in abiotic stress tolerance.

Financing: ANID, proyecto Anillo-PIA ACT192073

Keywords: Salt stress, drought, AdSAP7, Actinidia deliciosa var. Hayward, kiwi, Nicotiana tabacum

Area: Biochemistry and molecular biology

PANEL

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CALMODULIN-BINDING TRANSCRIPTION ACTIVATOR (CAMTA) GENES IN RASPBERRY: GENOME-WIDE IDENTIFICATION AND EXPRESSION DURING FRUIT DEVELOPMENT AND 1-METHYLCYCLOPROPENE APPLICATION

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Raspberry (*Rubus idaeus* L.) is an economic interest species due to the nutritional attributes of its fruit. Calmodulin-binding Transcription Activator (CAMTA) is a family of transcription factors in eukaryotic organisms, and it is regulated by calmodulin, the most studied calcium-sensing protein in calcium signaling. In plants, CAMTAs are involved in development, growth, and stress tolerance, but their participation in fruit development is unknown. Therefore, this study aims to identify the *CAMTA* genes and analyze their expression during raspberry fruit development and after applying an ethylene perception inhibitor (1-methylcyclopropene/1-MCP). Four *CAMTA* genes (*RiCAMTA1/3/4/5*) were identified in the raspberry genome, and their expression was evaluated in two fruit tissues, drupelets and receptacle, during fruit development. The *RiCAMTA1/3/4/5* genes increase in the over-ripe stage, with differences between tissue. Moreover, after two days of the application in the plant of 1-MCP (193 g a.i./ha of Harvista 1.3SC), the expression of *RiCAMTA3/5* genes increased in white drupelets and *RiCAMTA5* decreased in green drupelets. These results provide valuable information for future investigations of the role and regulation of CAMTAs transcription factors in raspberry fruit ripening.

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Keywords: Raspberry, Calmodulin-binding Transcription Activator, Fruit development, Ethylene, 1-MCP

Area: Biochemistry and molecular biology

PANEL

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EXPRESSION OF ABSCISIC ACID (ABA) BIOSYNTHESIS AND SIGNALING GENES AFTER 1-METHYL CYCLOPROPENE TREATMENT DURING RASPBERRY FRUIT DEVELOPMENT.

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Abstract: Raspberry (*Rubus idaeus* L.) is a fruit with high nutritional value, but it has a short shelf life because it softens quickly. The raspberry drupelets have a firmness decrease depending partially on the endogenous ethylene during cold storage. On the other hand, abscisic acid (ABA) plays a significant role during strawberry ripening. So, it has been demonstrated the role of genes related to biosynthesis (9-cis-epoxycarotenoid dioxygenase, abscisic acid 8'-hydroxylase 1-like/NCED) and abscisic acid PYR1-like receptors (PYL) during ripening of strawberry and grape. In this study, we applied 1-methyl cyclopropene (1-MCP, Harvista 1.3SC, using the equivalent of 193 g a.i./ha.) to 64 plants using a nebulizer and the control fruits were protected for 30 minutes with a sealed bag. After two days of treatment, the green and white stages of the fruits were harvested and evaluated. No significant differences in the production of ethylene and CO_2, soluble solids and acidity were observed, except for a significant decrease in firmness after 1-MCP treatment. After this treatment, the *RiNCED1* significantly increased in the drupelets of the white stage and *RiPYL1/8* significantly increased in the drupelets of both stages. These results suggest that during the inhibition of ethylene perception, the ABA could maintain the ripening process.

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Keywords: RiNCED1, RiPYL1/8, Ethylene perception, Fruit ripening, Fruit quality, Drupelets and Receptacle

Area: Biochemistry and molecular biology

PANEL

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A new twist to industrial waste

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The food industry produces a great amount of waste that goes direct to the garbage dump. Most of these waste products are organic compounds from fruits and vegetables, which are an excellent source of non-digestible carbohydrates, mostly derived from the plant cell wall (CW). CW composition varies from plant to plant, which is why different sources are composed of different structures. It has been described that many CW components have beneficial effects such as lowering, for example, cholesterol and blood sugar, or have anti-inflammatory activity. Our aim is to use food industrial waste and underestimated fruit as a source of compounds with nutraceutical value. Biochemical analyses were carried out on seven different sources of carbohydrates: papaya seed mucilage, white strawberry, and industrial pulp waste from the processing of apple, pear, juice preparation, blueberry and tomato. The overall composition of the monosaccharides showed differences between the samples, however, to obtain data about specific polysaccharides, we analysed pectins and hemicelluloses separately. Analysis of pectins showed that all samples have high levels of galacturonic acid, meaning they are rich in homogalacturonan. Some specific pectins had high levels of monosaccharides associated with rhamnogalacturonan-I branching. The hemicellulose analysis revealed a high content of xylose and glucose in all samples suggesting the presence of xyloglucan. In order to complement these analyses, we used immunoblotting with antibodies that recognise specific epitopes related to methylesterification content, egg-box structure and hemicelluloses. The diversity of content and proportion of sugars makes our samples an interesting study material for the research of carbohydrates with potential value for human nutrition.

Financing: Proyecto Anillo ACT210025 CHICOBIO

Keywords: Cell wall, pectins, hemicelluloses, industrial waste

Area: Biochemistry and molecular biology

PANEL

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CRISPR/Cas9 mediated edition of SINA5, ACS1a negative regulatory genes of abiotic stress in *Solanum lycopersicum* var. Poncho Negro.

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Climate change carries multiple problems such as the reduction of rainfall, which produces agricultural and socioeconomic problems, due to the loss of cultivable land and the increase in water need caused by drought. Genetic improvement appears in recent years as a valuable tool to find solutions to these problems. One of the trending tools for this purpose is the CRISPR/Cas system, which can edit genes of interest in a precise fashion. Negative regulators of abiotic stress genes present in many plants and crops have been established and studied, which when silenced or mutated, improve the organism's response to drought and salinity stress. One of the worlds most produced fruit and widely affected by this context is the tomato (*Solanum lycopersicum*). In this study we used the "Poncho Negro" tomato, an endemic northern Chilean variety, which has been selected with the aim of generate tomato rootstocks that improve the natural response of this crop to drought and salinity stress. For this, we identified and selected Sl*SINA5* and Sl*ACS1a* negative regulators genes in tomato for being edited by CRISPR/Cas9. Sl*SINA5* codifies a E3 ubiquitin ligase that sends to degradation specific transcription factors required for abiotic stress tolerance. Sl*ACS1a* codes for an enzyme in ethylene biosynthesis, that repress abiotic stress response. Binary vectors were generated via GoldenGate containing CRISPR/Cas9 together with two gRNAs designed to edit both genes in Poncho Negro. To validate their functionality, Agrobacterium mediated transient infiltration in tomato was standardized with 35S:Ruby reporter and edition of CRISPR/Cas9 vectors was determined by end-point PCR. Edition efficiencies on calluses will be also shown.

Financing: ANID, proyecto Anillo-PIA ACT192073

Keywords: Tomato Poncho Negro, SINA5, ACS1a, Salt stress, Drought, CRISPR/Cas9

Area: Biochemistry and molecular biology

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Identification of *Solanum lycopersicum* small RNA transferred to *botrytis cinerea* during the infection process.

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Botrytis cinerea is one of the most important pathogens in the tomato industry. The current strategy for *Botrytis* control is the use of chemical fungicides. However, the indiscriminate use of these chemicals has generated more resistant pathogens and a growing interest in safer alternatives. The study of plant transcriptomic response to infection has provided a better understanding of the crop defense mechanism. Additionally, the recent discovery of bidirectional small RNA transfer between *Solanum lycopersicum* and *Botrytis cinerea* represents a new level of transcriptional regulation with great biotechnological potential. Therefore, in this work we sought to study and identify small RNAs transferred from tomato to the fungus in response to infection and explore new ways to combat the disease. For this purpose, a susceptibility study of twelve commercial tomato varieties to *B. cinerea* was conducted. Marmande and Rosado varieties were the most resistant and susceptible cultivars, respectively. Money Maker showed an intermediate susceptibility. Subsequently, sRNAseq libraries were generated from a set of tomato leaves control and inoculated with B05.10 *B. cinerea* and a list of small RNAs differentially expressed in response to the fungus was obtained. These results will allow us to identify sRNA candidates that are potentially transferred by the plant to the pathogen and that may affect the virulence of the fungus, thus allowing us to explore new alternatives for *Botrytis cinerea* control.

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Keywords: smallRNA, cross kingdom RNAi, Solanum lycopersicum, botrytis cinerea, plant defense

Area: Biochemistry and molecular biology

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RSL4 and downstream HB13-GTL1 mediated regulatory gene network acts as an early driver of root hair growth at low temperature in *Arabidopsis thaliana*

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Root hairs (RH) are optimal model systems to study cell size regulation, as they can elongate up to hundreds of times their original size in a short period of time. Their growth is determined by intrinsic and environmental signals that are integrated into a coherent response, where the transcription factor ROOT HAIR DEFECTIVE 6-LIKE 4 (RSL4) plays a key role in the early regulation of genes associated with RH growth. Previously, we observed that in *Arabidopsis thaliana* plants treated with low temperature conditions (10ºC), RHs elongate to about twice their original size, while the growth of the rest of the plant is diminished. Nutrient bioavailability in soil is directly affected by temperature, which suggests a possible link to this exacerbated growth. However, the molecular mechanisms underlying its sensing and downstream signaling pathways remain unclear. Here, we sought to identify and characterize transcriptional networks controlled by RSL4 at low temperature by using transcriptomic and gene networking approaches. Thus, by combining RNA-seq and DAP-seq analyses we identified early responsive gene networks dependent on RSL4 under low temperature. Furthermore, we identified three major nodes downstream of RSL4 in the low-temperature response, a homeodomain leucine zipper of group I 13 (*HB13*), the trihelix transcription factor *GT-2-LIKE1* (*GTL1*), and a previously undescribed MYB-like TF (AT2G01060). Overall, our work found that the RSL4-dependent transcriptional regulatory network in response to temperature has a clear impact on HR growth.

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Keywords: Root hair, Cold response, Molecular genetics, Root physiology, Gene networks, Transcriptomics

Area: Biochemistry and molecular biology

PANEL

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DYNAMICS OF FaMYC2 AND FaNCED1/2 EXPRESSION UNDER OXIDATIVE STRESS IN LOCAL AND SYSTEMIC STRAWBERRY LEAVES.

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Oxidative stress is a common effect of biotic and abiotic stresses that affect crops. Crosstalk between jasmonate (JA) and abscisic acid (ABA) could be relevant for defense against oxidative stress. Thus, it is interesting to uncover how plants can face oxidative stress with the JA-mediated response led by the key transcription factor MYC2, and how it could be regulating the ABA biosynthesis pathway. In this preliminary study, we aim to characterize the dynamics of expression of *FaMYC2*, and the ABA-biosynthetic genes *FaNCED1* and *FaNCED2* in local and systemic strawberry (*Fragaria* x *ananassa*) leaves in oxidative stress conditions. We perform oxidative stress treatments with methyl viologen (MeV) using methyl jasmonate (MeJA) as a positive inductor for *FaMYC2* expression. Through 3,3′-diaminobenzidine (DAB) staining on treated leaves, we observed the presence of hydrogen peroxide (H_2O_2) in both MeV and MeJA treatments showing 40 µM MeV the highest H_2O_2 detection. Therefore, we performed the quantification of *FaMYC2 FaNCED1*, and *FaNCED2* transcripts in local and systemic leaves. *FaMYC2* transcription was upregulated 120 min after application (maa) in systemic MeV-treated leaves. On the other hand, *FaNCED1* and *FaNCED2* showed upregulation with 40 µM MeV and MeJA treatment 30 maa both in local and systemic tissues. *FaNCED1* and *FaNCED2* showed a higher expression under MeJA treatment at 60 maa in local and 30 maa in systemic leaves, respectively. These results showed that these genes are upregulated under oxidative stress conditions triggered by MeV with early activation of *FaNCED1* and *FaNCED2* in local strawberry leaves.

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Financing: ANID – Millenium Science Initiative Program – NCN2021_010 and Fondecyt 1210941 projects.

Keywords: Oxidative stress, jasmonate, strawberry, abscisic acid, MYC2, NCED

Area: Biochemistry and molecular biology

PANEL

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Characterization of a novel formiminotransferase ciclodeaminase differentially expressed in inclined *Pinus radiata* D.Don

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One-year-old seedlings of Pinus radiata D. Don were tilt, and differential accumulation of transcripts at 2.5 hours were analyzed. A RNAseq library for these inclined young seedlings plant was built considering a time course of 2,5, 10 and 24 hours which generated 101,575 contigs. Within the gene encodes the enzyme formiminotransferase cyclodeaminase (FTCD), which has 367 amino acids was found. This protein also has a molecular weight of 40,052 Da and a pI of 7.67. The multiple sequence alignment allowed the identification of FTCD-N domain, the conserved domain for the active site, in which the H residue is located. Additionally, the putative site for the binding folate (GPxxxxxxxG) was identified. Sequences of several plant species, *Homo sapiens* (KAI2596505.1) and *Gallus gallus* (NP_990234.1) enriched the phylogenetic analysis, from which PrFTCD grouped together with *Picea sitchensis* and close to the clade of *H.sapiens* and *G.gallus*, all of them emerged from a common ancestor *A. trichopoda*. The transcript analysis based on the FPKM values showed a greater accumulation of transcript at 2.5 hours of inclination in the upper stem side, in addition there is a decrease at 10 and 24 hours after inclination. The function of the *PrFTCDD* gene, which exhibits differential expression in response to inclination at early times, is unknown. Although it has been described that the orthologue of A. thaliana would play a role in the classification and/or transport of TGN vesicles in cover root cells. Additional experiments are required to corroborate the function of this gene in inclined stem.

Financing: Thanks to ANID-FONDECYT # 1201011

Keywords: formiminotransferase ciclodeaminase, radiata pine, stem inclination, RNAseq

Area: Biochemistry and molecular biology

PANEL

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A real time fluorimetric method for cell death detection in response to stress in plant protoplasts.

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Cell death is an essential process for the development of an organism. The cell death can result from an incidental collapse of cell functions by adverse conditions, but it could also be triggered due to a cellular process genetically controlled. In vegetal organisms, the methods described to evaluate cell death are mainly semi-quantitative and involve image analysis, including the inner biases that image methodology concerns. Here we describe a Sytox green-based methodology that allows a quantitative real-time measure of cell death in plant protoplast. Sytox green is a dye that binds DNA and emits fluorescence. It is impermeable to the cell membrane, but when it is disrupted, the dye enters the cell, binds to DNA, and emits fluorescence which is measured by a detector. The assay can be performed in a microplate system, simultaneously analyzing up to 96 samples with different treatments, including various plant genotypes.

In protoplasts isolated from *Arabidopsis* leaves, we determine that the method allows the lineal detection of cell death in a range between 1k and 15k cells.

Treatments of Salicylic Acid (SA) and H_2O_2 , known stress inductors in plants, trigger cell death in a concentration-dependent fashion. We also evaluated the cell death of protoplast from tga256 mutant plants. These plants are more susceptible to SA and oxidative stress than WT plants. The measure of protoplast cell death comparing both genotypes confirms the phenotype described in seedlings. This methodology allows the mid-throughput evaluation of cell death triggered by diverse stress conditions in different genotypes or ecotypes with a quantitative, efficient, and robust output.

Financing: Acknowledgment to FONDECYT Iniciación 11200944.

Keywords: protoplasts, cell death, methodology

Area: Biochemistry and molecular biology

PANEL

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How to reign the driest desert in the world? A look at the physiological secrets of *Cistanthe longiscapa*

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The Atacama Desert is the driest place on the planet, with temperature variations of 40 °C in one day and extreme radiation levels. These characteristics make it one of the most challenging ecosystems for the development of life. In this place, similar to the Martian surface, an annual event called “flowering desert” occurs. In this magnificent event, extensive desert territories are flooded with striking colors resulting from the flowering of different plant species. However, despite the diversity of flora that develops, *Cistanthe longiscapa*, a Chilean endemic succulent plant, is dominant over the others.

The development of *C. longiscapa* in such a challenging ecosystem, added to the territorial superiority that this species encompasses, raises the question: What makes it unique above the rest? Moreover, how does it do it? These questions have led us to unravel the mysteries of this plant, carrying out a physiological and biochemical characterization that could lead us to understand the organization and biological strategies that this plant has to reign over the Atacama Desert.

To carry out our analyses, we standardized a method for growing this plant in the laboratory. This approach allowed us to carry out immunofluorescence analyses using specific antibodies for pectin domains, a polysaccharide known for its capacity to trap water. The results revealed that cells specialized in storing a structure rich in sugars stand out. These cells could be related to the plant’s availability of water and nutrients. At the same time, the biochemical analysis highlights a high content of galactose and galacturonic acid in mesophyll cells, which could be related to the previously observed idioblastic cells. Our work aims to pave a way to position *C. longiscapa* as a model organism for succulent plants from extreme ecosystems.

Financing: This work was supported by ANID – Millennium Science Initiative Program – ICN2021_044, Fondecyt 1190695, Proyect ACE 210011.

Keywords: Desert, Atacama, Flowery, *Cistanthe longiscapa*, Extreme, Superiority

Area: Biochemistry and molecular biology

PANEL

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The transcription factors FchSHP and FchSEP3 activate gene promoters of enzymes involved in cell wall disassembly

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Transcription factors (TF) have a key role during fruit softening. It has been observed that in non-climacteric fruit they could regulate the transcription of genes codying for enzymes that participate in cell wall disassembly. The promoter sequences of cell wall disassembly genes from *Fragaria chiloensis* (*FchXTH1, FchEXP2, FchEXP5, FchRGL, FchPG* and *FchPL*) were isolated, and *cis* elements were recognized for the MADS-box family. On the other hand, two MADS-box TFs were identified in *F. chiloensis* fruit: FchSHP (Shatterproof) and FchSEP3 (Sepallata) as differentially expressed during fruit ripening. Given this background, the transcriptional regulation of cell wall disassembly genes by these TFs was studied. To do this, *FchSHP* and *FchSEP3* sequences were overexpressed in *F. chiloensis* fruits at the C2 stage (small fruit, with green achenes), and the expression of the aforementioned enzyme genes was analyzed. It was observed that fruits overexpressing *FchSHP* presented an increase in the levels of transcripts of *FchXTH1*, *FchPL*, *FchEXP2* and *FchEXP5,* while fruits overexpressing *FchSEP3* shown an increment in *FchEXP2* and *FchEXP5* transcripts. To corroborate the role of these TFs, a dual luciferase assay was performed using the promoter sequences of cell wall disassembly genes. It was shown that FchSHP protein activates the promoters of *FchXTH1*, *FchRGL*, *FchPG* and *FchEXP2*, meanwhile FchSEP3 protein activates the promoters of *FchRGL* and *FchPG*. These results indicate that FchSEP3 and FchSHP can regulate the expression of key cell wall disassembly genes involved in softening of *F. chiloensis* fruit.

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Keywords: transcription factors, fruit softening, cell wall disassembly, MADS-box, dual luciferase assay, overexpression

Area: Biochemistry and molecular biology

PANEL

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MOLECULAR AND GENOMIC CHARACTERIZATION OF THE *PSEUDOMONAS SYRINGAE* PHYLOGROUP 4: AN EMERGING PATHOGEN OF *ARABIDOPIS THALIANA* AND *NICOTIANA BENTHAMIANA*

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Environmental fluctuations such as increased temperature and water availability triggered by climate change influence plant disease dynamics by affecting hosts, pathogens, and their interactions. Here, we describe a newly discovered *Pseudomonas syringae* strain found in a natural population of *Arabidopsis thaliana* collected from the southwest of France. This strain, called *Psy* RAYR-BL, is highly virulent on natural *Arabidopsis* accessions, *Arabidopsis* Columbia-0, and tobacco plants. Despite the severe disease phenotype caused by the *Psy* RAYR-BL strain, we identified a reduced repertoire of putative Type III virulence effectors by genomic sequencing compared to *P. syringae* pv *tomato* DC3000. Furthermore, hopBJ1$_{Psy}$ effector is found exclusively on the *Psy* RAYR-BL genome and its expression on plants induces ROS accumulation and cell death. In addition, HopBJ1$_{Psy}$ participates as a virulence factor in this plant-pathogen interaction, likely explaining the severity of the disease symptoms. This research describes the characterization of a newly discovered plant pathogen strain and possible virulence mechanisms underlying the infection process.

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Keywords: Pseudomonas syringae, *Arabidopsis* thaliana, disease emergence, pathogenic reservoirs, HopBJ1, virulence factor, T3SS

Area: Plant physiology

PANEL

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QUINOA AND HIS FRIENDS: A CHANGING RELATIONSHIP FROM NORTH TO SOUTH

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Desertification negatively affects plant growth and has become one of the main abiotic factors that puts food security at risk worldwide. Although many studies have shown that plants have intrinsic mechanisms to tolerate these conditions, the root-associated microbiome can also contribute to mitigate abiotic stress in plants. Here, we investigate the role of the root microbiome in the physiological and molecular mechanisms involved in environmental tolerance, as well as seed quality and productivity, of 5 quinoa genotypes, distributed along a latitudinal gradient. For each of the 5 genotypes, the condition was generated with and without its microbiome, then were exposed to 2 saline conditions (200 and 400 mM NaCl) plus a control treatment (0 mM NaCl). As environmental tolerance variables, photochemical efficiency (Fv/Fm), expression of a transporter gene (NHX1) and survival were measured. As quality measures, the percentage of total proteins and the average weight of the seeds were measured. Under salinity conditions, the environmental tolerance variables were significantly higher in the genotypes with the presence of their microbiome, being more evident in the genotypes distributed to the north. On the other hand, the quality of the seeds was significantly higher in genotypes with their microbiome and distributed to the south. The results showed that microbiomes associated with genotypes distributed to the north have a greater contribution to environmental tolerance, while microbiomes associated to genotypes distributed to the south contribute to a greater extent on quality and productivity.

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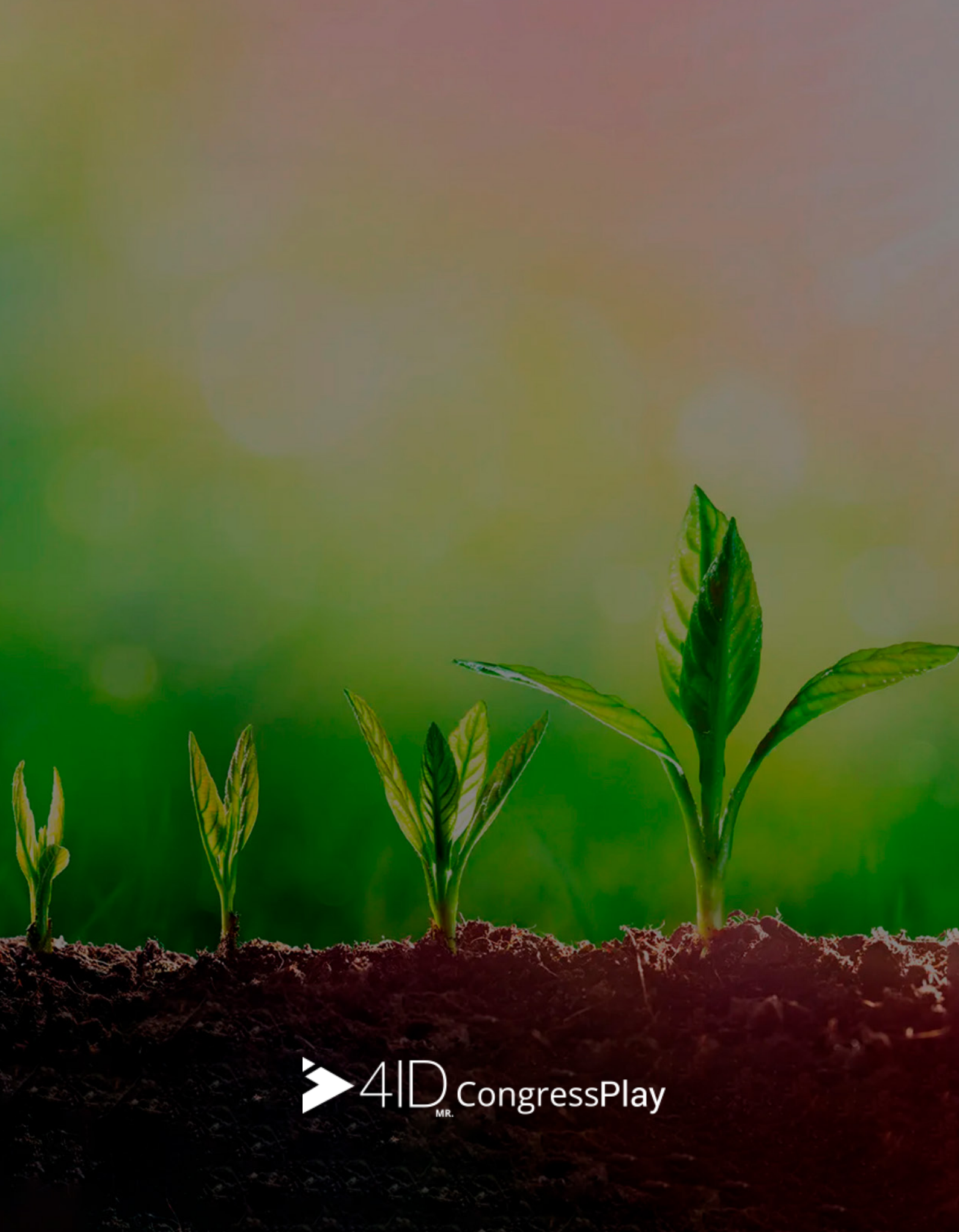
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