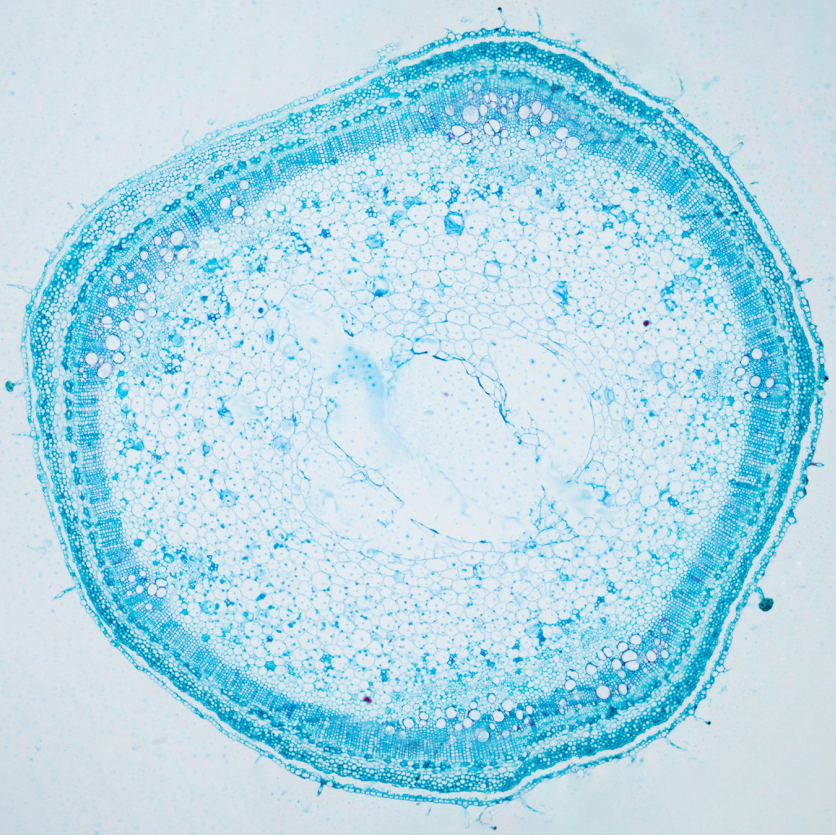
CHILEAN SOCIETY OF PLANT BIOLOGISTS

LIBRO DE RESÚMENES

XVI REUNIÓN DE BIOLOGÍA VEGETAL

28-30 de noviembre 2023

Talcahuano



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Auspiciadores

ARQUIMED INNOVACION

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Conference Program

Tuesday, November 28

12:30 - 14:30	**Registration**
15:00 - 16:30	**Oral Session 1 – Plant Physiology / System Biology** **Chair: Dra. Alejandra Moya (U. Talca)** Room: Auditorio Poeta Gonzalo Rojas
15:00 - 15:30	"Primary metabolism modulation in sweet cherry flower buds under differential water deficit conditions" - **Laura Sepúlveda** (U. Mayor / PUCV)
15:30 - 16:00	"Proteomic and low-polar metabolite profiling reveal unique dynamics in fatty acid metabolism during flower and berry development of table grapes" - **Dr. Patricio Olmedo** (PUCV)
16:00 - 16:30	"Transporter Networks form Homeostats that are the Fundamental Entities of Homeostasis in Plant Cells" - **Dr. Ingo Dreyer** (U. Talca)
16:30 - 17:00	**Coffee Break**
17:00 - 18:30	**Oral Session 2 – Genomics** **Chair: Dr. Jonathan E. Maldonado (USACH)** Room: Auditorio Poeta Gonzalo Rojas
17:00 - 17:30	"A comprehensive bioinformatic reannotation and validation of *Solanum lycopersicum* miRNAs in genome SL4.0" - **Diego Landaeta-Sepúlveda** (U. Mayor)
17:30 - 18:00	"Study of sRNA and mRNA expression profiles in two *Solanum lycopersicum* varieties with contrasting susceptibility to *Botrytis cinerea*" - **Mariola Tobar** (U. Mayor)
18:00 - 18:30	"The proanthocyanidin-related MYB700 activates the *LAR* gene associated with the biosynthesis of catechin in Japanese plum fruits (*Prunus salicina* Lindl.)" - **Benjamin Battistoni** (U. de Chile)
18:45 - 20:00	**Opening Ceremony** **Chair: Dr. Adrián A. Moreno (UNAB)** Room: Auditorio Poeta Gonzalo Rojas
18:45 - 18:50	Welcome to the XVI Congress of Chilean Society on Plant Biologists Dr. Adrián A. Moreno (UNAB)
19:00 - 19:40	**Opening Lecture:** Title "Regulatory and epigenomic networks of the sulfate deficiency response in *Solanum lycopersicum*" - **Dra. Elena Vidal** (U. Mayor)
20:00 - 21:30	**Welcome Cocktail** at External Marquee

Wednesday, November 29

09:00 - 10:30	**Oral Session 3 - Biochemistry and molecular biology / Cell Biology** **Chair: Dr. Michael Handford (U. de Chile)** Room: Auditorio Poeta Gonzalo Rojas
09:00 - 09:30	"The carrot DcFRS8 participates in photomorphogenesis and carotenoids synthesis" - **Valeria Azócar Sobrado** (Universidad de Chile)
09:30 - 10:00	"Subcellular localization of BNT1 variants and their role in response to different biotic interactions" - **Dra. Micaela Peppino Margutti** (UNAB)
10:00 - 10:30	"Unlocking the Mysteries of HG Methylesterification in *Arabidopsis* Seed Coat Mucilage" - **Dra. Susana Saez** (UNAB)
10:30 - 11:30	**Coffee Break**
11:30 - 13:00	**Poster Session I** Posters numbers from 1 to 20
13:15 - 15:00	**Lunch**
15:15 - 17:00	**Symposium**: **"Biotecnología y StartUPs en Chile"** **Chair: Dra. Ignacia Fuentes (Pontificia Universidad Católica de Chile)** **Co-Chair: Dr. Claudio Meneses (Pontificia Universidad Católica de Chile)** Room: Auditorio Poeta Gonzalo Rojas
15:15 - 15:20	**Symposium presentation**
15:20 - 15:40	**Bernardo Pollak**, Meristem, *Frutas del Futuro: Mejoramiento de precisión*.
15:40 - 16:00	**Felipe Gainza**, Concha y Toro, *Desarrollando El Viñedo del Futuro: Experiencia de Viña Concha y Toro, Desafíos y Perspectivas Innovadoras para la Industria Vitivinícola*.
16:00 - 16:20	**Felipe Aquea**, Rubisco, *Producción Sustentable de Ingredientes Activos Naturales mediante Agricultura Celular*.
16:00 - 16:20	**Jennifer Alfaro**, Botanical Solutions, *Plant Biotechnological platform for High-Value Natural Compounds Production in Industry*.
16:00 - 16:20	**Borys Chong**, Synergia Bio, *Biotechnology application to the rescue and valorisation of native species*.
16:20 - 17:00	**Mesa redonda**
17:00 – 17:30	**Coffee Break**
17:30 – 19:15	**Poster Session II** Posters numbers from 21 to 40
19:30 - 20:30	**Members meeting**

Thursday, November 30

09:30 - 10:30	**Oral Session 4 – New and emerging areas of knowledge** **Chair: Dra. Karina Ruiz (UNAP)** Room: Auditorio Poeta Gonzalo Rojas
09:30 - 10:00	"*W. murcott* tangerine as a model plant for analysis of embryogenic promoters and reporters genes by Agrobacterium-mediated transformation" - **Dra. Julia Rubio** (Meristem)
10:00 - 10:20	*Flash talks*
10:20 – 10:40	**Coffee Break**
10:45 – 13:00	**Poster Session III** Posters from 41 to 60
13:15 - 15:00	**Lunch**
15:15 - 15:45	The Global Plant Council - **Dra. Isabel Mendoza**, Communications Officer
15:45 - 16:15	"Nuevas tecnologías en PCR" - **Nicolás Nazal**, Product Manager, GeneXpress
16:30 - 17:00	**Coffee Break**
17:15 - 18:45	**Poster Session IV** Posters from 61 to 80
19:00 - 21:00	**Closing Ceremony and Farewell Dinner**

Oral

Area: System biology

Transporter networks form homeostats that are the fundamental entities of homeostasis in plant cells.

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Homeostasis in plant cells refers to the steady state of internal, physical, and chemical conditions. It is sustained by self-regulation of the dynamic cellular system. Computational cell biology dry-lab experiments proved that homeostatic control requires at least two different transporter types for the same nutrient, which are energized differently. For potassium, these are K^+ channels and H^+/K^+ sym- and/or antiporters, and for anions, these are anion channels and proton-coupled anion transporters, for instance. This gain of flexibility in adjusting a cytosolic concentration, however, was accompanied by the establishment of energy-consuming cycles at the membrane. Homeostats allowed the cells to sense external mineral nutrient concentrations and translate changes of them into cytosolic H^+ and Ca^{2+} signals. The concept emerging from the computational simulations was confirmed in wet-lab experiments using intact guard cells as model system that stably expressed the genetically encoded fluorescence sensor CapHensor. These data show that transporter networks do not only serve their primary task of transport, but can also take on the role of a receptor without requiring conformational changes of a transporter protein. Such transceptor-like phenomena may be quite common in plants.

Keywords: Homeostasis, Computational cell biology, Membrane transport, System modelling, Nutrient sensing, Wet-lab/dry-lab

Financing: This work was funded by Fondecyt grants 1210920 and 1220504 and Anillo No. ATE220043 of the Agencia Nacional de Investigación y Desarrollo (ANID) de Chile.

Area: System biology

Regulatory and epigenomic networks of the sulfate deficiency response in *Solanum lycopersicum*.

Elena A. Vidal[1,2], José David Fernández[1,2], Diego Landaeta-Sepúlveda[1,2], Felipe Arratia[1,2], Joaquín Medina[4], José Miguel Álvarez[2,3], Javier Canales[2,5]

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(2) Instituto Milenio de Biología Integrativa, Santiago, Chile
(3) Universidad Andrés Bello, Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Santiago, Chile
(4) Centro de Biotecnología y Genómica de Plantas, UPM CSIC-INIA, Madrid, Spain
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Sulfur (S) is a fundamental macronutrient for plants, supporting their growth and development. Long-term deficiency of sulfate, the primary source of S available to plants, results in diminished photosynthetic rates and amino acid content and disrupted acquisition of other essential nutrients. Plants modulate their physiology and metabolism in response to sulfate deficiency, optimizing sulfate acquisition, assimilation, and utilization. These changes are caused in part by a reprogramming of the plant's transcriptome. While these alterations in gene expression and molecular processes have been investigated in the model plant *Arabidopsis thaliana*, our understanding of the regulatory mechanisms governing the sulfate response in crop plants such as *Solanum lycopersicum* (tomato) remains limited. To gain molecular insights into the sulfate deficiency response in tomato, we performed a transcriptomic analysis that showed that tomato is highly responsive to sulfate deficiency. To identify transcriptional regulators responsible for this differential gene expression, we employed GENIE3 to infer interactions between transcription factors (TFs) and target genes, utilizing data from a comprehensive set of tomato RNA-Seq studies. This reference regulatory network was integrated with chromatin accessibility data to generate sulfate deficiency context-specific networks. Through network analysis, we identified a group of key TFs responsible for regulating tomato's response to sulfate deficiency, including a closely related homolog of SLIM1, a pivotal regulator of sulfate transport and metabolism in Arabidopsis. Furthermore, the metabolism of sulfate is intricately linked to DNA methylation as S-adenosylmethionine serves as a common substrate for methyl group transfers. We explored the methylome of tomato, revealing alterations in DNA methylation patterns within gene promoters in response to sulfate deficiency affecting critical genes involved in sulfate transport and metabolism. In sum, our work provides an integrated view of transcriptomic and epigenomic changes elicited by sulfate starvation in tomato, one of the most important crops in the world.

Keywords: Tomato, Transcriptomics, Gene Regulatory Networks, Methylome, Sulfate Deficiency

Financing: This work was funded by ANID-FONDECYT 1211130, ICM-ANID iBio ICN17_022 and Anillo ACT210007

Area: Genomics

A comprehensive bioinformatic reannotation and validation of *Solanum lycopersicum* miRNAs in genome SL4.0.

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MicroRNAs (miRNAs) are short noncoding RNA approximately 20 to 22 nucleotides long. They undergo processing by Dicer and play a key role in the post-transcriptional regulation of gene expression by cleaving mRNA. Tomatoes (*Solanum lycopersicum*) represent one of the most significant vegetable crops globally and have emerged as an attractive model for research in recent years, including in miRNA research. The release of Tomato Genome Version 4 (SL4.0) in 2019, along with the 2020 annotation (ITAG4.1), includes information on genes, transposable elements, and long non-coding RNAs. miRBase is a public repository containing information on miRNAs in many organisms, though it is unfortunately woefully out of date. Although the miRBase contains miRNA annotations for the SL2.5 genome (released in 2014) with 122 precursors and 147 mature miRNAs, only the mature sequences have been experimentally validated. Furthermore, more recently proven miRNAs have not been incorporated into this database. Our work has focused on upgrading the miRNA annotation to the SL4.0 genome. We implemented a simple strategy to streamline this process, involving the collection of hairpin precursors and mature sequences from various studies and databases. Our approach entailed the blasting of hairpin sequences to the SL4.0 genome, ensuring a high-confidence match. Subsequently, we assigned common names and families to the miRNAs. Furthermore, we validated the mature sequences using degradome data and thoroughly tested the new annotation with smal-RNA-seq data. The comprehensive results of our study revealed an updated annotation comprising 357 hairpins and 434 mature miRNAs. Out of these, 219 hairpin structures were successfully predicted, and 270 mature miRNAs were validated through degradome data analysis. In conclusion, our findings present a significant advancement in the understanding of post-transcriptional gene regulation in tomatoes, thereby providing a valuable resource for further research in this area.

Keywords: miRNAs, *Solanum lycopersicum*, Gene Annotation, Degradome, Post-transcriptional regulation

Financing: This work was supported by ANID-FONDECYT 1211130 and ANID-Millennium Science Initiative Program ICN17_022. DL-S is supported by ANID-Beca de Doctorado Nacional

Area: Genomics

Study of sRNA and mRNA expression profiles in two *Solanum lycopersicum* varieties with contrasting susceptibility to *Botrytis cinerea*.

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Botrytis cinerea is an important pathogen to the tomato industry. The current strategy for *Botrytis* control is the use of fungicides. However, the indiscriminate use of these chemicals has generated a growing interest in safer alternatives. The study of plant transcriptomic responses to infection has traditionally provided a better understanding of crop defense mechanisms. In addition, the recent discovery of bidirectional sRNA transfer between *Solanum lycopersicum* and *Botrytis* represents a new level of transcriptional regulation with great biotechnological potential. Therefore, in this work we sought to study how the differential expression profiles of sRNAs and mRNAs induced by *Botrytis* infections are integrated into the defense response of two commercial tomato varieties with contrasting susceptibility to this fungus: Marmande as resistant and Rosado as susceptible. For this purpose, mRNA and sRNA sequencing libraries were generated from control and infected tomato leaves. We found that the number of differentially expressed transcripts (DEGs) was higher in susceptible plants than in resistant plants. Both varieties have upregulated processes related to small molecule metabolic process, response to hormone and defense response. However, processes related to response to other organisms, to external biotic stimulus and interspecies interaction between organisms were only upregulated in Marmande samples. In relation to the changes in the sRNA transcriptome, a smaller number of differentially expressed clusters was observed in the resistant variety compared to the susceptible variety. In addition, a higher number of sRNA clusters were differentially expressed in a genotype specific manner rather than in an infection-related manner. Finally, two sRNA clusters were highly overexpressed in Marmande infected over Rosado infected samples which targeted *Botrytis* genes involved in secondary metabolism and virulence. This approach let us to identify tomato sRNA clusters with putative regulatory potential on *Botrytis cinerea* infection process.

Keywords: *Botrytis cinerea*, *Solanum lycopersicum*, sRNAs, mRNA, Defense response, Commercial varieties

Financing: This work was funded by ANID-FONDECYT 3210312 ANID-FONDECYT 1211130 and ANID Millennium Science Initiative Program - ICN17_022

Area: Genomics

The proanthocyanidin - related MYB700 activates the *LAR* gene associated with the biosynthesis of catechin in Japanese plum fruits (*Prunus salicina* Lindl.).

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Chile is the main exporter of Japanese plum fruits of the south hemisphere. Fruits of this species contain phenolic compounds from the proanthocyanidin (PA) family, including catechin and epicatechin, widely associated with bitterness and astringency. Catechin and epicatechin are biosynthesized by the LAR and ANR enzymes, respectively. Additionally, genes encoding for these enzymes are transcriptionally regulated by transcription factors (TFs) belonging to the MYB, bHLH and WD40 families, which compose the MBW complex. Previously, our group studied the phenolic composition of Japanese plum fruits of two cultivars, '98-99' and 'Angeleno', which showed a higher concentration of catechin and epicatechin, respectively. Phylogenetic analysis using flavonoid - related MYBs of *Arabidopsis* allowed us to identify ten PsMYBs putatively regulating the PA biosynthesis. Expression analysis of young and ripe fruit helped us to detect one *PsMYB* with an expression 10-fold higher in '98-99' than 'Angeleno', in the youngest developmental stage. Also, the closest homologous of this gene corresponded to the *PpMYB7*, previously reported as a regulator of the expression of *LAR* but no *ANR* in peach. Since the similarity with the *PpMYB7* was 63%, we named this TF as PsMYB700. Here, we described this novel MYB TF, which is involved in the transcriptional regulation of structural genes of the PA biosynthesis. Using dual-luciferase assays, we observed that PsMYB700 only activates the *LAR* promoter but not the *ANR* promoter, suggesting a preferential activation of *LAR* over *ANR*. This agrees with the higher accumulation of catechin in fruits of '98-99'. Moreover, unlike the previously described PpMYB7, PsMYB700 can regulate the expression of *LAR* without the presence of a bHLH as a co-factor. According to these results, we identified a novel element regulating the PA biosynthesis in Japanese plum. Also, PsMYB700 can be a factor involved in the higher catechin concentration in this fruit species.

Keywords: Flavan-3-ol, Flavonoids, Asian plum, Expression analysis, Dual-luciferase, Phenolic compounds

Financing: This study was funded by the National Agency of Research and Developm (ANID)/ the Scholarship Program/BECAS DE DOCTORADO NACIONAL/2020–21200330; Fondecyt Regular N°1191446; Fondecyt Regular N°1231614.

Area: Plant physiology

Primary metabolism modulation in sweet cherry flower buds under differential water deficit conditions.

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Perennial trees accumulate carbohydrates and other metabolites in overwinter organs before winter to survive dormancy and sustain spring emergence. Under drought stress it is known that leaves accumulate different osmolytes, but few data had been reported on flower buds. In this work, we evaluated the impact of water deficit on primary metabolites accumulation in flower buds of sweet cherry *var*. Regina. Five irrigation treatments were applied during spring and summer seasons: T0 (100%), T1 (75%), T2 (50%), T3 (25%) and T4 (0). Flower buds were collected at the beginning of autumn for primary metabolites analysis using GC-MS. Water potential decreased up to - 1.9 MPa in T3 and T4 treatments presenting significant differences compared to the rest. In the case of maximum photosynthesis efficiency (Fv/Fm), no significant differences were perceived. It was observed an increase in organic acids, amino acids, and polyols content in flower buds from treatments T2, T3 and T4, compared to T0 and T1. This differential metabolite content in response to differential water deficit treatments may affect flowering and thus fruit productivity in the following harvest season.

Keywords: *Prunus avium*; Flower buds; water deficit; photosynthesis efficiency; primary metabolites

Financing: FONDEF ID21I10297, FONDECYT 1230163, ANID/ACT210007

Area: Plant physiology

Proteomic and low-polar metabolite profiling reveal unique dynamics in fatty acid metabolism during flower and berry development of table grapes.

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Grapevine development and ripening are complex processes that involve several biochemical pathways, including fatty acid and lipid metabolism. Fatty acids are essential components of lipids, which play crucial roles in fruit maturation and flavor development. However, the dynamics of fatty acid metabolism in grape flowers and berries are poorly understood. In this study, we present those dynamics and investigate the mechanisms of fatty acid homeostasis on 'Thompson Seedless' berries using metabolomic and proteomic analyses. Low-polar metabolite profiling indicated a higher abundance of fatty acids at the pre-flowering and pre-veraison stages. Proteomic analyses revealed that grape flowers and berries display unique profiles of proteins involved in fatty acid biosynthesis, triacylglycerol assembly, fatty acid β-oxidation, and lipid signaling. These findings show, for the first time, that fatty acid metabolism also plays an important role in the development of non-oil-rich tissues, opening new perspectives about lipid function and its relation to berry quality.

Keywords: *Vitis vinifera*, Proteomics, Metabolomics, Lipids, Fatty acid metabolism

Financing: Proyecto ICN2021_044 and Fondecyt 1200260

Area: Cell biology

Unlocking the mysteries of HG methylesterification in *Arabidopsis* seed coat mucilage.

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The plant cell wall is a complex structure crucial for plant physiological functions and it is composed of cellulose, hemicellulose, and pectins. Pectins are synthesized in the Golgi apparatus and deposited into the apoplast. The major pectin component in the cell wall is Homogalacturonan (HG), which consists of a galacturonic acid (GalA) backbone that can be methylesterified, affecting the cell wall mechanical properties. In the apoplast, enzymes like pectin methylesterases (PMEs) and pectin methylesterase inhibitors (PMEIs) can control HG methylesterification. *Arabidopsis* mucilage is a gel-like structure produced by the seed coat and rich in HG and serves as a valuable model for studying HG fine-tuning. Analyses performed in our laboratory indicated that mutants in *pme31*, *pme51*, *pmei13*, and *pmei3* displayed mucilage phenotypes, suggesting their probable roles in regulating HG methylation. *PMEI3* and *PMEI13* are inhibitors expressed specifically in mucilage secretory cells, as confirmed by the detection of GUS activity in plants carrying proPMEI3-GUS and proPMEI13-GUS constructs. Immunolabeling experiments with JIM5, JIM7, and 2F4 antibodies demonstrated their involvement in the same pathway, controlling egg-box structure formation. Bioinformatic analysis suggested that PME58, a PME mucilage specific, is regulated by PMEI3 and PMEI13, indicating the involvement of these two PME58-PMEI3 and PME58-PMEI13 inhibition complex in egg-box structure formation. Sugar content remained unchanged in *pmei13*, *pmei3*, and *pmei13xpmei3* mutants, suggesting that methylation changes do not affect pectin synthesis. In contrast, *pme31*, *pme51*, and *pme31xpme51* mutants exhibited a significant increase between 20.6 and 46.12% in GalA content in mucilage, indicating that the targets of PME31 and PME51 differ from those regulated by PMEI3, PMEI13, and PME58. This underscores the complex and multifaceted nature of HG within the plant cell wall.

Keywords: Cell wall, Homogalacturonan, Methylesterification, PME, PMEI, *Arabidopsis*, Mucilage

Financing: Fondecyt 1201467, ECOS 210032

Area: Biochemistry and molecular biology

The carrot *DcFRS8* participates in photomorphogenesis and carotenoids synthesis.

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In plants, carotenoid synthesis is induced by light in leaves, fruits and flowers. Carrots, on the other hand, synthesize big quantities of carotenoids in its taproot that grows underground. In order to elucidate the molecular mechanism behind these phenomena, a RNAseq analysis revealed that light-regulated genes are expressed in the dark-grown taproot. Amongst these we found *DcFRS8*, member of the FRS (FAR1-related sequences) family, which is crucial for development and light signal transduction in plants.

This work aims to understand the role of *DcFRS8* on the carotenoid synthesis and vegetative and reproductive development. Firstly, we confirmed that DcFRS8:GFP has a nuclear localization in *N. tabacum* leaves, which correlates with the *Arabidopsis* paralog. Then, the gene was silenced in *D. carota* producing a 94-96% in carotenoid reduction, suggesting its role on carotenoid biosynthesis. Additionally, overexpression in *N. tabacum* did not induce an increment in carotenoid level nor in the expression of *NtPSYs* (key gen for carotenoid synthesis) but resulted in shortened stems and reduced internodal distance compared to WT plants, suggesting its role in photomorphogenesis. Genes involved in FRS8 signal transduction, like *FHY* and *FHL* increased and reduced their expression, respectively. Flowering time in DcFRS8 transgenic lines is slightly reduced but the number of flowers remains constant. These results suggest that DcFRS8 is required for carotenoid synthesis in carrot and in photomorphogenic development in *N. tabacum*. This is the first time that DcFRS8 is associated with carotenoid synthesis in plants.

Keywords: DcFRS8, *Daucus carota*, Plant development, *Nicotiana tabacum*, Light signaling, Carotenoids

Financing: FONDECYT 1221399

Area: Biochemistry and molecular biology

Subcellular localization of BNT1 variants and their role in response to different biotic interactions

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Nucleotide-binding leucine-rich repeat receptors (NLRs) are key sensors of the plant immune system, conferring resistance to diseases caused by diverse pathogens. NLRs have been found to act in several compartments inside the cell. However, NLRs have never been described as functioning from plastids, essential organelles for a successful defense against pathogens. We have recently found that the *Arabidopsis* NLR receptor BNT1 displays a functional plastid targeting signal. Moreover, the BNT1 gene can generate two splice variants due to the alternative transcription start sites, with only one the presence of an N-terminal signal. Importantly, we validated the plastid envelope or cytoplasm locations for each BNT1 variant. Thus, the differential activation of the BNT1 variants could play distinctive roles in plant defense responses against pathogens. Expression analysis showed that the relative levels of the variants are affected by bacteria and aphid attacks and differentially expressed in epidermal and vascular tissues that are proposed to play an essential role in immunity. Consistently, bnt1 mutant plants have an altered resistance to *Pseudomonas sp* and *Myzus persicae*. Our results suggest a specific requirement of BNT1 isoforms (i.e., subcellular localization and site/tissue of action) in response to different biotic interactions.

Keywords: NLR proteins, *Pseudomona* sp., *Myzus persicae*, Chloroplast, Defense response

Financing: FONCYT (PICT-2017-0589, PICT-2020-01906, PICT-2020-0483; CONICET-PIP2021-2023; Fondo Nacional de Desarrollo Científico y Tecnológico [ANID-FONDECYT regular 1210320] - [ANID-FONDECYT-3200902], Programa Iniciativa Científica Milenio - ICN17_022, NCN2021_010 and ANID PIA/BASAL FB0002ANID-FONDECYT-3200902

Area: New and emerging areas of knowledge: Molecular Breeding, Phenomics and new breeding technologies; Teaching, Educational and social aspects in Plant Biology

W. murcott tangerin as a model plant for analysis of embryogenic promoters and reporters genes by *Agrobacterium*-mediated transformation.

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Woody plants *in vitro* culture and genetic transformation are widely used techniques, many protocols have been described and published for decades in a wide range of species and varieties within the *Citrus* genus. At Meristem, we have implemented the *in vitro* culture and transformation platform of *C. reticulata* x *C. sinensis cv. W. murcott*, a tangerine known for its excellent agronomic characteristics and high commercial opportunities. The plant material used for stable transformation via *Agrobacterium tumefaciens* corresponds to a pro embryogenic mass (PEMs) obtained from ovule culture, which contains cells clusters that will develop into embryos as well as other lines cells that will remain in their undifferentiated state (callus). The development of *in vitro* culture and transformation protocols for *W. murcott* callus and PEMs, followed by embryo induction, maturation and germination and plant growth allows the generation of a robust production platform to get genetic modified material. Using constitutive expression promoters, we have obtained plants expressing fluorescent reporter genes with or without antibiotic resistance. Additionally, we have explored other selection mechanisms, such as the expression of chromophores at various stages of embryonic development. All this is due to the plasticity developed by our *in vitro* procedures to induce embryos from callus or PEMs or to dedifferentiate embryos to callus. Finally, citrus stable transformation is the tool that allow us to generate the proof of concept of the genes selected by the trait's platform, with *W. murcott* tangerine being the model plant for Meristem in the study and development of new fruit editing technologies.

Keywords: *Citrus*, Callus, Transformation, *Agrobacterium*

Poster

Area: System biology

Gene regulatory network analysis to uncover conserved plant drought stress responses.

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The availability of water is a fundamental concern for plant life, with drought events posing a growing threat due to climate change. These events have severe implications for agricultural crop production. Drought stress triggers intricate gene regulatory networks (GRNs), influencing the expression of thousands of genes. While certain plant species exhibit varying degrees of drought tolerance, the common elements and variations within their GRNs remain a subject of limited understanding.

In our effort to address this knowledge gap, we harnessed the wealth of publicly available RNA sequencing data related to drought responses. We meticulously curated and normalized metadata profiles, enabling uniform treatment comparisons across diverse experiments. Differentially expressed genes (DEGs) were identified for each experimental context, and a stringent filtration process was applied to identify shared responses across multiple plant species.

Furthermore, we employed predictive modeling of GRNs based on chromatin accessibility and gene expression profiles. This allowed us to define a set of consistently activated genes in response to drought stress, observed in Arabidopsis, tomato, rice, and maize. Our research unveils the intricate transcriptional circuitry that underlies these shared responses, offering a comprehensive perspective on the transcriptional networks governing drought adaptation in plants.

The findings of our study provide a valuable reference point for understanding the regulatory networks in each of these plant species. This knowledge can guide the formulation of hypotheses concerning essential genes and regulatory components critical for drought adaptation, ultimately contributing to enhanced agricultural resilience.

Keywords: Drought, Transcriptome, Genome, Transcription factors

Financing: This work is funded by ANID FONDECYT:1210389 and ANID – Millennium Science Initiative Program- Millennium Institute for Integrative Biology (iBio) ICN17_022, and National Science Foundation NSF-PGRP:IOS-1840761. Fondecyt Postdoctoral folio 32208701.

Area: Genomics

Unraveling the primary transcriptional cascade of heat stress in wheat.

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Wheat is a crucial crop facing significant yield risks due to the increasing frequency and severity of heat stress caused by climate change. This investigation characterizes the immediate transcriptional adaptations of wheat upon exposure to heat stress. Our temporal analysis reveals a distinct and organ-specific transcriptional adaptation to heat stress, with the leaf transcriptome showing a rapid induction of gene expression as early as 5 minutes following exposure, while root tissues initiate a response at 10 minutes, highlighting differential temporal transcriptional regulation mechanisms.

We observed a rapid increase in the quantity of differentially expressed genes (DEGs) in foliar tissue, peaking at 22.5 minutes post-exposure. Roots, however, exhibited a linear increase in DEGs, lacking the initial upsurge seen in leaves. An in-depth examination of the transcriptional regulatory network identified Heat Shock Factors (HSFs) as pivotal regulators in both organs for the upregulated pathways. In contrast, the pathways downregulated by heat stress do not share common regulators.

Subsequent analyses captured temporal changes in gene expression across various pathways, with some pathways exhibiting notable changes in their transcriptional profiles over time. Gene Ontology (GO) enrichment analysis indicated an enrichment for terms associated with stress response in the case of upregulated pathways, specifically those related to protein targeting and modifications. In contrast, the pathways downregulated by heat stress showed enriched GO terms associated with regulation of gene expression and metabolism in both organs.

This research provides new insights into the earliest transcriptional regulatory events governing acute heat stress responses in wheat. The candidate TFs identified represent potential biotechnological targets to improve heat tolerance and yield stability in wheat as temperatures rise. Uncovering the hierarchical heat stress signaling cascade will advance fundamental understanding of temperature sensing and signaling in this agriculturally vital crop.

Keywords: Wheat, Heat stress, Primary Transcriptional Response, Heat Shock Factor

Financing: FONDECYT-Iniciación N°11220937 and FONDECYT-Regular N°1230833

Area: Genomics

Emerging viruses in Chile: first report of the Babaco Q virus in papaya (*Vasconcellea pubescens*).

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Mountain papaya is grown in the coastal cordon of the Maule Region and is important for commercial development in the localities where it is grown. One of the problems presented in the orchards is a decrease in production yields, which leads to presume the existence of phytosanitary problems, highlighting possible viral pathogens. Using Ilumina technology, nine transcriptomic libraries of papaya (*Vasconcellea pubescens*) were sequenced, identifying viral genomes through the application of various bioinformatics tools, one of them being Geneious prime, 2023. Among the sequences studied there was one that has a high percentage of similarity (≈98%, E. value 0 and percentage of coverage ≈99%) with the accessions of MT113181.1, MN648673.1, MT113182.1 that correspond to the Babaco Q virus polymerase, from babaco plants. Subsequently, reference assembly and prediction of the viral genome were performed. It was observed that the identified virus presents a size of 4. 585 bp, with putative coding regions such as: gene 1, putative protein of Babaco virus Q (357 amino acids); gene 3, RNA-dependent RNA polymerase (482 amino acids) and three conserved domains, one belonging to the cl02808 superfamily: RT like Superfamily and two subfamilies of RNA-dependent RNA polymerases named RdRP 3 (Accession: pfam00998) and RT dep RNA polymerase (Accession: cd01699). The importance of this research lies in the fact that it is the first report in Chile of a viral etiological agent in *Vasconcellea pubescens* orchards.

Keywords: Papaya, Bioinformatic, Viruses, *Vasconcellea pubescens*

Financing: Funding: Innovation Fund for Regional Competitiveness FIC-R “Transfer to strengthen competitiveness in the papaya industry in the Maule Region through the development of biotechnological tools” BIP:40.001.007-0.

Area: Genomics

Towards multiomic analysis in Japanese plum (*Prunus salicina* Lindl.): linking the gene expression of bZIPs-MYBs transcription factors with the synthesis of phenolic compounds.

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Phenolic compounds (and among them flavonoids) are ubiquitously biosynthesized in plants, playing roles in biotic/abiotic stress tolerance, fruit quality, and nutraceutical potential. Flavonoid biosynthesis is catalyzed by several enzymes whose encoding genes are transcriptionally regulated by bZIP and MYB transcription factors. Compared to other *Rosaceae* species, the Japanese plum shows a high content of phenolic compounds in fruits. In previous works, our group identified several QTLs associated with phenols in an F1 progeny <'98-99'x'Angeleno'>, whose parents showed contrasting phenolic profiles. This work aims to identify genes with expression patterns potentially associated with the phenol content. Here, we analyzed the phenolic profile of Japanese plum fruits of '98-99' and 'Angeleno' in two developmental stages (S1 and S4). Additionally, we evaluated the expression pattern of genes involved in the flavonoid biosynthetic pathway and the R2R3-MYBs and the bZIP family to check their potential association with phenols' biosynthesis. We observed that most of the early genes of the biosynthetic pathway are expressed in cv. '98-99' at S1, while the expression of the late biosynthetic genes in the pathway has a higher expression in both cultivars in S1. Also, '98-99' presented a high correlation with the expression of *ANS*, *PsbZIP54* and *PsbZIP17* genes with catechin biosynthesis. In 'Angeleno', the *PsbZIP24, PsbZIP32* and Chr1.420 genes highly correlate with epicatechin content. Therefore, these results confirm the existence of differentially expressed genes encoding *bZIP-MYB* transcription factors potentially associated with phenolic compound synthesis in different Japanese plum cultivars.

Keywords: Transcription factor, Gene expression, Flavonoid biosynthesis, Japanese plum, bZIP, MYB, Trait variation

Financing: FONDECYT Regular 1191446, FONDECYT Regular 1231614, BECAS DE DOCTORADO NACIONAL/2020–21200330, all from ANID Chile.

Area: Genomics

Deciphering Sulfur starvation response in Wheat: elucidation of TaSLIM1-5D as a central regulator through integrative genomic and transcriptomic analysis.

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Sulfur is critical for myriad biological functions in plants, ranging from the synthesis of essential compounds to the regulation of growth processes. Plants absorb sulfur primarily as sulfate and incorporate it into essential organic molecules, including proteins, chlorophyll, and various cofactors.

Sulfur deficiency in plants leads to growth inhibition, chlorosis, and compromised yield, which has significant implications for agricultural productivity and food quality. For instance, in bread wheat (*Triticum aestivum*), adequate sulfur is imperative for optimal flour quality and protein content.

Our molecular investigations have identified a suite of genes that respond to sulfur depletion, signifying an adaptive metabolic response to this nutrient's availability. In *Arabidopsis thaliana*, the transcription factor *SLIM1* (*Sulfur limitation1*), previously classified as EIL3, is a pivotal component of the sulfur response network. As a member of the ethylene-insensitive-like (EIL) family, *SLIM1* is implicated in the global regulatory response to sulfur deficiency across various plant species.

In bread wheat, homologous genes analogous to SLIM1 are situated on chromosomes 5 and 7. Our RNA-seq analysis of sulfur-deprived bread wheat across distinct developmental stages indicates an upregulation of SLIM1 homologs in both leaves and roots, which underscores their role in sulfur metabolism. Our research suggests *TaSLIM1-5D* (*TraesCS5D03G0627100*) as the likely functional ortholog, based on its conserved DNA-binding domains relative to *AtSLIM1*, predominant expression profile, and precise predicted target genes within the wheat gene regulatory network (wGRN) database for sulfur starvation responses. Moreover, quantitative PCR analysis validates the concordance of *TaSLIM1-5D* expression with established sulfate uptake and assimilation markers, reinforcing its functional significance.

Keywords: Sulfur metabolism, Transcription factor, SLIM1, *Arabidopsis thaliana*, *Triticum aestivum*

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Area: Genomics

Transcription factor EgloNAC35 and its role in cold stress tolerance in *Arabidopsis thaliana*.

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Forestry is one of the main economic sectors of Chile, in 2020, the forest sector represented 2,1% of the GDP. The total surface of plantations in the country is *ca*. to 2 million ha, of which 30% correspond to eucalypts. This exotic species has been used at a commercial level at least in the past half-century, and it has been subjected to diverse genetic improvement programs carried out by the main forest companies in Chile. *Eucalyptus globulus* Labill has an exceptional wood quality combined with fast growth in Mediterranean areas. However, low temperature stress limits their productivity and geographical distribution. Despite the predicted global warming for the next few years, climate models project that extreme cold events will continue to occur and may be more intense than in the past years. These future events can cause high economic loss for the forestry industry, especially for companies that use frost sensitive species as *E. globulus*. Transcription factors play an important role regulated the expression of target genes in plants stress responses. The NAC family has been studied in several abiotic stress, including cold stress. This work focuses in the analyzes of the expression *EgloNAC35* genes in transgenic *Arabidopsis thaliana* plants and determining survival rate under cold stress of transgenic plants compares to wild type. The results showed a production of transgenic plant with aberrant phenotypes unsuitable for cold stress assay.

Keywords: Stress, Cold, Eucalyptus, NAC, *Arabidopsis*, Gateway, Transgenic.

Financing: Facultad de Ciencias Biológicas, Universidad de Concepción. FONDECYT 1161063 from CONICYT, CONICYT PhD scholarship 21211524, Centro de Biotecnología and Bioforest S.A.

Area: Genomics

Characterization of the regulatory role of DNA methylations over the transcriptome of *Prunus persica during* fruit development in contrasting varieties for maturity date

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Maturity date (MD) is an agronomic trait defined as the time between flowering and harvest. Peach (*P. persica*) trees encompass early, medium, and late MD varieties. This is directly related to the duration of fruit development. Several hormones regulate this process. The increase of jasmonate in early fruit development report was reported, suggesting this process in peaches. Additionally, a transcriptome analysis between MD contrasting organisms showed Differentially Expressed Genes (DEGs) associated with jasmonate. Also, DNA methylation is related to fruit development regulation since there is a massive demethylation at the end of fruit development. However, more knowledge of the role of DNA methylation in fruit development needs to be gained. We aim to understand the regulatory role of the methylome over the transcriptome during fruit development and its effect on MD in *P. persica*. We characterized and measured hormone concentration (auxin, ethylene, jasmonate, salicylic acid, cytokinin and ABA) in contrasting varieties for MD (early MD: 'Early Juan' (EJ), late MD: 'Super August' (SA)) during fruit development. From phenotypic characterization, we observed that EJ develops faster than SA, which could be associated with significative differences in jasmonate and salicylic acid at the beginning of fruit development between varieties. We characterized the methylome and transcriptome profiles, obtaining that global methylation levels increase in both varieties, especially in non-CpG contexts. Using a slide windows approach, we identified over 10,000 Differentially Methylated Regions (DMRs) between varieties. For further analysis, we considered DMRs near the gene body (2Kbp upstream and downstream). Finally, using RNA-seq data, we identify DEGs associated with hormones such as jasmonate response (*PpLOX1*, *PpFAD8*) and auxin response (*PpARF5*, *PpSAUR14*, *PpNAC101*) during fruit development between varieties. This study could be the beginning of the understanding of MD in *Prunus*.

Keywords: *Prunus persica*, DNA methylation, RNA-seq, Hormones, Epigenetic, Fruit development, Maturity date

Financing: This work was supported by ANID-Fondecyt 1200804, ANID 21230969 and ANID-Millennium Nucleus for Development of Super Adaptable Plants NCN2021-010.

Area: Genomics

Genetic markers for clonal distinction in grapevines.

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Grapevine cultivars offer a variety of clonal selections, each exhibiting distinct agronomic and enological traits. These phenotypic variations among clones can be attributed to somatic mutations accumulated over millennia of asexual propagation. Nevertheless, understanding the genetic diversity within grapevine cultivars has been challenging due to the lack of tools for clone differentiation.

Our study explored the genetic and epigenetic differences within a specific set of Cabernet Sauvignon clonal selections. We aimed to identify genetic markers that can effectively differentiate between these clones. We utilized short-read resequencing technology to sequence the genomes of these clones. On average, we detected 4 million variants per clone. Of these, 74.2% were single nucleotide variants, and 25.8% were small insertions or deletions (InDels). Notably, the prevalence of these variants remained consistent across all examined clones.

Among the multitude of variants, we successfully validated eight clonal markers using high-throughput amplicon sequencing. Interestingly, two clones displayed no genetic variations. To further explore these distinctions, we conducted a methylation analysis. For this purpose, we sequenced 12 bisulfite libraries, revealing an average methylation rate of 23.6%. When broken down, the methylation percentages were 78.8% in CpG contexts, 60.4% in CHG, and 6.9% in CHH. Our data also indicated that methylation predominantly occurs in intergenic regions. Furthermore, our analysis did not identify any differentially methylated regions that were significantly distinct from others when using 100,000 base pair windows.

Our findings represent a significant advancement in grapevine genotyping strategies, offering valuable insights for the viticulture industry, particularly in terms of plant material characterization and identification.

Keywords: Grape, Clones, Genetic markers, Epigenetics

Financing: ANID-FONDECYT Regular 1200804; ANID-FONDECYT 3180306; Chilean Economic Development Agency (CORFO; Project 13CEI2-21852), Viña San Pedro, and Viña Concha y Toro

Area: Genomics

Expression pattern analysis of network transcription factors associated with fruit maturity date in *Prunus persica*.

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Prunus persica is one of the most important fruit trees of economic interest worldwide. The financial interest generated by this tree lies in its fruits, which are consumed worldwide as food. Chile is the largest exporter in the southern hemisphere, accounting for more than 80% of exports in the off-season. Peach fruits have organoleptic characteristics such as sweetness or juiciness and nutritional characteristics such as its antioxidant content, making it a desirable food. This work seeks to contribute to the knowledge of harvest time, a little-studied character, by studying fruit development from a molecular genetic perspective. We propose that the transcription factors *NAC072*, *HB12*, *HAT9* and *MYBR1* participate in the fruit development process, with a fundamental role in defining the different ripening phenotypes (early, medium and late). To test the above hypothesis, the expression levels of each transcription factor were evaluated by RT-qPCR at six different times of fruit development, using five contrasting varieties for harvest time (two early "Big Boy" and "Rebus", two mid-season "Summer Fire" and "Venus" and one late "Late Red Jim"). Additionally, this information was complemented with data from an RNA-Seq performed on two contrasting varieties at harvest date (one early "Early Juan" and one late "Super August"). The results indicate that the transcription factors *NAC072* and *MYBR1* showed a behavior with a more significant difference between varieties of different phenotypic classes, with a lower expression of these genes in the late varieties. In addition, these transcription factors showed a more significant correlation between the expression levels of RNA-Seq and qPCR between varieties of the same phenotypic class.

Keywords: *Prunus persica*, Fruit development, Harvest date, Transcription factors

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Area: Cell biology

A novel strategy for assigning ploidy levels to cells imaged by confocal microscopy for a comprehensive insight into cotyledon size specification in response to nitrate.

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Nitrogen is a critical nutrient for plant subsistence and a key commercial factor in the agronomical industry. Nitrogen fertilizers account for approximately 50% of crop yield. The primary nitrogen source for plants is nitrate. While perceived and absorbed through the roots, nitrate modulates many aspects of plant growth and development. Recently, we demonstrated that nitrate promotes cotyledon growth by increasing cell ploidy and size in *Arabidopsis thaliana*. We observed that this response depends on the regulator of the cell cycle LOST OF GIANT CELLS FROM ORGANS (LGO). The most common method for evaluating cell ploidy in leaves is measuring nuclei DNA content by flow cytometry. However, since this method destroys the tissue, it does not allow determining ploidy values at the individual cell level. Here, we present a novel and simple approach that uses the flow cytometry parameter known as forward scatter (FSC) to assign ploidy levels to individual epidermal cells imaged by confocal microscopy based on their nuclei size. This method enabled us to separate the nitrate effect on LGO expression, cell ploidy, and cell size in a single confocal microscopy experiment by using an LGO transcriptional reporter line with a Venus fluorescent protein tagged to the nucleus (*pLGO*:3xVenus-N7).

Keywords: Nitrate, Shoot growth, Cell ploidy, Cell size, LGO, Flow cytometry, Confocal microscopy

Financing: ANID-FONDECYT 1220594 and 3220033, iBio ICN17022, Beca Doctorado Nacional ANID

Area: Cell biology

Bioeffector of endophytic fungi from the Atacama Desert promotes plant growth and increases drought tolerance in Tomato *var.* Micro Tom and Roma.

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The use of microbial biological resources from the Desert is a sustainable and innovative solution to the current challenge of the water crisis in the agricultural sector in Chile. Through our research, we seek to accelerate the technological transfer of bio-products based on endophytic fungi native to the Atacama Desert that confer drought tolerance in plants with high water requirements. For this, the effect of microbial consortia and their extracts in tomato varieties under irrigated and drought conditions was studied, through proof of concept and validation with laboratory tests. Our results show that the use of prototype 1, based on live fungi in tomato var Micro Tom, accelerated the emergence of buds and flowers, in addition, increased the number of fruits, and reduced the water footprint for each tomato produced compared to the untreated control and a commercial product. On the other hand, the use of prototype 2, based on extracts obtained by fermentation in co-culture, increased seed germination rates, shoot length, root system development, and the survival percentage of Tomato var. Roma in pot trials compared to the control in drought conditions. In addition, it was shown that it enhanced the growth of beneficial fungi and decreased the growth of pathogenic fungi of agricultural importance. The exogenous application of fungi and their extracts are a non-conventional alternative for the development of bio-products specialized in drought, to alleviate the effects of climate change in the agricultural industry in Chile.

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

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Area: Cell biology

A role for cell wall metabolism in nitrate-determined organ size in *Arabidopsis thaliana* cotyledons

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Nitrate is a powerful signal that regulates gene expression, metabolism, physiology, growth and development of plants. This nutrient plays a role in controlling aerial tissue growth through the induction of endoreplication and cell expansion. We found that the CDK inhibitor LGO is necessary for nitrate-mediated endoreplication during cotyledon growth. However, in *lgo* mutant plants, cells divide instead of endoreplicating, maintaining nitrate-determined organ size. Therefore, new perspectives are necessary to address the robust specification of leaf size in response to nitrate availability. To explore molecular mechanisms involved in cell expansion, we performed a transcriptome analysis on cotyledons of plants grown under 0.5 mM or 5 mM nitrate. We classified differentially expressed genes using Gene Ontology terms and found that cell wall organization is one of the over-represented biological processes among those genes that change expression levels in response to the different nitrate regimes. We explored the interaction between nitrate and cell wall metabolism during cell expansion by characterizing the cell wall sugar profile of *Arabidopsis* cotyledons grown under contrasting nitrate conditions. We analyzed differentially expressed genes in order to identify candidate processes of polysaccharide modification that respond to nitrate. Together, our results suggest that cell wall metabolism and function are important factors required for cell growth in response to external nitrate availability.

Keywords: Nitrate, Cell wall, Cell expansion, Shoot growth

Financing: CONICYT-MPG 190013, FONDECYT REGULAR 1220594, iBio ICN17_022, Beca Doctorado Nacional ANID

Area: Genetic resources

Screening of healthy food-related traits in *Prunus* genus: exploring the diversity of phenolic compounds and antioxidant activity in cultivars of stone fruit species.

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Stone fruits (*Prunus* genus, belonging to the Rosaceae family) are recognized for their wide variety of fruits, distinctive flavors, and nutritional content. Furthermore, their composition in non-nutrient metabolites with health-promoting properties must be better studied. This work aimed to make a phenotypic screening in 53 cultivars regarding the total polyphenol content (TP; Folin-Ciocalteau method), total proanthocyanin content (Pas; DMACA method), total anthocyanin content (pH-shift method), total flavonol content (FV; by $AlCl_3$ method), and antioxidant activities (by DPPH and FRAP approaches) in different varieties of seven stone fruit species. Phenolic compounds from the skin and pulp were extracted with 80% aqueous methanol solution and determined by spectrophotometry at specific wavelengths. A positive correlation ($p<0.05$) was found between TP and PAs for all species, being widely differentiated by skin and pulp tissue, with correlation coefficients of 0.79 and 0.87, respectively. Moreover, FV, Pas and TP evaluated in the skin for all cultivars positively correlated with FRAP assay with r coefficients of 0.87, 0.62 and 0.59, respectively.*Prunus salicina* skin cultivars showed significantly higher TP, PAs, and antioxidant capacity than most other species' cultivars ($p<0.01$). *Prunus texana* pulp also showed significantly higher TP, FV, Pas and antioxidant capacity than most other species' cultivars ($p<0.01$). In summary, the *Prunus* genus shows a wide range of antioxidant content among species. *Prunus salicina* and *Prunus texana* potentially represent the most abundant source of phenolic compounds in cultivated species of the analyzed accessions of the *Prunus* genus.

Keywords: Antioxidant activity, *Prunus*, FRAP, DPPH, Fruit peel, Fruit skin

Financing: FONDECYT Regular 1191446, FONDECYT Regular 12316

Area: Genetic resources

Genome – wide association study of resistance against the aphid *Brevicoryne brassicae* in *Arabidopsis thaliana* natural accessions.

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Aphids are phloem sap-feeding insects representing 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 and by virus transmission. Also, they can reduce the photosynthetic capacity by stimulating molds that grow on the honeydew. Therefore, plants rely on mechanisms of recognition and signaling to cope with the specialized mode of phloem feeding by aphids. However, the genetic mechanism underlying resistance against aphids is still in progress. In this study, we aimed to identify a new candidate gene (CG) involved in the resistance of *A. thaliana* against the specialist aphid *Brevicoryne brassicae*. A genome-wide association mapping (GWAS) was performed to identify SNP markers accounting for variation in resistance in a worldwide collection of 200 natural accessions of *Arabidopsis thaliana*. The aphid performance on each accession was evaluated as the offspring number and used as resistance parameters. Our results revealed that the *Brevicoryne* performance was significantly different among accessions. Compared to Col-0, the accessions with more resistance were Kondara and Tsu-1, which also showed a resistance phenotype to the generalist aphid *Myzus persicae*. Marker-trait association tests identified two single nucleotide polymorphisms (SNP) located in gene-coding regions on chromosomes 2 and 4, and a complementary guided analysis in this region detected significant effects on different haplotypes on offspring number. Interestingly, most of the new CG (about 36%) were related to cell wall organization and biogenesis (cellulose synthases and arabinogalactan), and this set of CG represents a starter point for their functional characterization of their biological role in aphid performance and feeding behavior.

Keywords: *Arabidopsis thaliana*, GWAS, *Brevicoryne brassicae*, Plant defense response

Financing: Fondo Nacional de Desarrollo Científico y Tecnológico [ANID-FONDECYT Regular 1210320 and Posdoctorado 3230451] - Programa Iniciativa Científica Milenio - ICN17_022, NCN2021_010, and ANID PIA/BASAL FB0002

Area: Genetic resources

Insights into wheat grain yield: meta-analysis reveals subset of Expansins associated with grain growth.

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Wheat is a globally important cereal crop, and increasing its yield is vital for future food security. Grain yield is largely determined during grain development, relying on proper cell expansion and grain filling. Cell wall-loosening Expansin proteins are involved in cell enlargement, but their specific roles in wheat grain growth are not fully understood. We performed a meta-analysis of published RNA sequencing data from developing wheat grains to uncover Expansins related to grain development. Differential expression analysis across diverse genotypes and growth conditions revealed 33 Expansins exhibiting transcriptional regulation during grain growth. Further co-expression network analysis clustered most expansins with cell wall genes, peaking at 5 days after anthesis when cell expansion occurs. We validated six Expansins through quantitative PCR, confirming decreasing expression patterns after grain expansion ceases. Grain size and weight measurements uncovered strong negative correlations between certain Expansin transcripts and these yield components. Our integrative study substantiates a subset of expansins as putative regulators of wheat grain growth, mediating grain dimensions and weight. Characterizing these Expansins expression patterns advances understanding of the molecular factors influencing yield parameters. Future functional analyses can build on these transcriptional insights to engineer wheat grains with increased size and weight, boosting yield potential. This work sheds light on Expansins that may serve as targets for improving wheat grain yield, a priority for global food security.

Keywords: Wheat, Expansin, Cell wall modification, Gene co-expression network, *Triticum aestivum*

Financing: This work was supported by FONDECYT grant number 1230833 and ANID—Millennium Science Initiative Program—ICN17-022.

Area: Bioinformatics

Analysis of the molecular interaction between anthocyanase and anthocyanins in Cranberry and their effect on the enzymatic degradation.

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Anthocyanins are bioactive compounds responsible for various physiological processes in plants and give fruits and flowers their characteristic color. While their biosynthetic pathway is well studied, their mechanism of enzymatic degradation has been poorly investigated. Some authors have reported β-glucosidase as the enzyme responsible for the enzymatic degradation of anthocyanins in eggplant and Sicilian orange. The aim of this work was to investigate the physicochemical interactions between anthocyanase and the anthocyanins found in Cranberry. This was achieved through molecular docking to identify the residues that likely involved in anthocyanin degradation. For this purpose, the three-dimensional structure of the anthocyanase enzyme was constructed based on its amino acid sequence, which was obtained through sequence alignments among sequences belonging to the Ericaceae family. The modeled structure, designated (c8c82), consists of 551 amino acids and a $(\beta/\alpha)_8$ barrel topology, classifying it within the glycoside hydrolase 1 family. The model was refined using a 100 ns molecular dynamics simulation, and the model with an average RMSD of 0.14 nm around 75 ns was chosen. Molecular docking programs were utilized to simulate the interactions between anthocyanase and the ligands. The interactions between the enzyme and the ligands cyanidin 3-arabinoside and cyanidin 3-glucoside were the most stable, with affinity energies between -9.3 and -9.2 kcal/mol. Based on the results obtained, it is expected to propose a degradation mechanism in order to study the kinetic characteristics of the enzyme and the search for inhibitors to prevent fruit discoloration.

Keywords: Cranberry, Anthocyanase, Anthocyanin, Enzimatic Degradation, Molecular Docking

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Area: Bioinformatics

MYC2 CIS-BINDING ELEMENTS (MBEs) analysis in the strawberry ascorbate peroxidase 1 promoters

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The commercial strawberry (*Fragaria* x *ananassa*) contains high levels of vitamin C (ascorbic acid) a non-enzymatic antioxidant used in the detoxification of hydrogen peroxide thanks to the activity of ascorbate peroxidase (APX). Previous results indicated that exogenous application of Methyl Jasmonate on strawberry plants caused an increase in APX activity of harvested fruit, suggesting that *APX* expression responds to jasmonate (JA) signalling through the canonical bHLH-type transcription factor MYC2. The aim of the present study was to characterize the strawberry *APX 1* (*FaAPX1*) genes and their promoter regions in terms of MYC2 cis-binding elements (MBEs). Four allelic copies for *FaAPX1*, named *FaAPX1-1, FaAPX1-2, FaAPX1-3,* and *FaAPX1-4,* were identified in the *F.* x *ananassa* genome. The promoter regions were analyzed using PlantPAN and PlantCARE software in addition to the manual MBEs search, emphasizing the conservation of sequence, frequency, and distance between cis-elements, due to the interaction capacity of the MYC2 as a dimer when it binds only one cis-element or in tetrameric form after the formation of a DNA loop when two cis-elements are 100 to 150 bp apart within the promoter region. MBEs were found in all copies of *FaAPX*s. The analysis of the frequency and distribution of MBEs indicates that the *FaAPX1-2* and *FaAPX1-4* copies promote the putative formation of MYC2 tetramers responsible for a potential strong activation of transcription. Protein-DNA interaction assays are currently performed to probe the binding and functionality of FaMYC2 on *FaAPX1-2/1-4* promoters.

Keywords: Ascorbic acid; *Fragaria x ananassa*; MYC2 transcription factor; Promoter cis-elements

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Area: Bioinformatics

Exploring alternative splicing in response to salt stress: an organ-level comparative analysis using *Arabidopsis thaliana* public transcriptomic data.

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Alternative splicing (AS) is a crucial mechanism in eukaryotic organisms for both post-transcriptional regulation of gene expression and the diversification of the genome. In plants, it plays a significant role in the response to different abiotic stresses, including salt stress. However, the extent of differential AS regulation across different organs and across diverse salt stress treatments has not been fully explored so far. Here, we performed a comprehensive analysis of AS regulation in response to salt stress using publicly available short-read transcriptomic data from 30 different studies of *Arabidopsis thaliana*. Our results show that the intersection between differentially expressed genes (DEGs) and differential AS genes (DAS) is relatively small, indicating that AS acts as an independent regulatory layer in response to salt stress. We also observed that the number of AS events detected is highly dependent on the intensity of the treatment and is influenced by other technical parameters such as the sequencing depth. Moreover, we identified a significant proportion of AS events that are potentially unique or novel to salt stress. Regarding the types of AS events, our analysis shows that intron retention (IR) is the prevalent event across different tissues, followed by alternative five prime splice site (Alt5'ss) and then by exon skipping (ES). Interestingly, we observed that in the seeds, ES and Alt5'ss occur with the same frequency, while roots and rosettes tend to rely more on Alt3'ss. Finally, we found that the intersection of AS-regulated genes between different organs is small, indicating that distinct sets of genes are regulated by AS under salt stress in different organs. Overall, our study sheds light on the complex landscape of AS regulation in the response to salt stress and highlights the importance of AS as a regulatory mechanism of plant gene expression.

Keywords: Soil salinity, Salt stress, Alternative splicing, Alternative splice site

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Area: Biochemistry and molecular biology

Response of the transcription factor HY5 to salt stress in tomato and its relationship with light.

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Salinity is an abiotic stress that limits crop productivity and in response to this, plants increase the synthesis of Abscisic Acid (ABA). On the other hand, light is not only an energy source but also mediates responses to environmental stimuli, including stress. Light is perceived by photoreceptors that activate photomorphogenesis, a process in which the transcription factor (TF) HY5 (ELONGATED HYPOCOTYL 5) participates. HY5 regulates many light-responsive genes, including genes in the ABA synthesis and signaling pathway. This makes HY5 an integrative element and suggests its participation in stress tolerance responses. However, the evidence that HY5 actually regulates responses to salt stress in adult plants is scarce.

Here, the response of *HY5* to salt stress and different light conditions in Microtom tomato plants was characterized. Results indicate that *HY5* almost doubles its expression in plants subjected to 200mM NaCl, in correlation with increases in the expression of genes required for ABA synthesis (*ABI5* and *NCED*). Likewise, genes involved in light signaling, such as *COP1*, *PHYA* and *PHYB*, change their expression patterns under these same conditions. When different light conditions are added to salt stress, *HY5* expression rises in correlation with *ABI5* and *NCED* in white light, while in dark or Far-Red circumstances, their expression decreases significantly. These preliminary results suggest that HY5 could mediate the response to salt stress in adult plants and that light conditions determine the action of this TF and therefore their response to stress. In the future, the ability of HY5 to bind and regulate the promoters of tomato *ABI5* and *NCED* will be determined, given that in *Arabidopsis* both have binding sites in their promoters for HY5.

Keywords: HY5, Salt stress, Light, ABI5, NCED, Tomato

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Area: Biochemistry and molecular biology

Enabling tissue culture and gene transfer techniques for precise breeding in the local temperate rice Onix.

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ABSTRACT

Rice (*Oryza sativa* L.) is one of the world's most important food crops and is a staple for more than half of the world's population. Generated by the local Rice Breeding Program and considered a new elite cultivar, Onix-INIA is the first black rice variety, adapted to temperate climate. Rice productivity is, however, conditioned by the effects of global climate change, leading to continuous limitations in yield potential and a decrease in arable lands that will likely impact production. The use of new breeding techniques supporting the genetic improvement of this species would allow for a more accelerated development of new varieties. Somatic embryogenesis (SE) is one base methodology for new plant breeding techniques, and particularly, in gene editing mediated by CRISPR-Cas technology. To enable GE in Chilean rice varieties, this research aimed the development of SE systems that allow for the transfer of CRISPR/Cas9 components in these genotypes. We assessed *Agrobacterium*-mediated gene transfer on somatic embryos of the elite variety Onix-INIA. Based on previous protocols, 2N6 medium was applied for seed germination and for embryogenic calli (EC) induction and propagation. Gene transfer on ECs was successfully achieved using the EHA105 bacterial strain harboring pFLC-Myb/HPT/GFP vector. The occurrence of the gene transfer process was monitored by visualization of the green fluorescent protein (GFP) expression through epifluorescence microscopy. The transformation events showed GFP-positive calli, as soon as the fourth day post infection. The regenerative capacity of hygromycin-resistant ECs was evaluated using MS regeneration medium (MSR). The resulting seedlings were subsequently transferred to a rooting medium (N6F). These results show that EC induction and propagation procedures in this study can be applied in genetic transformation and other biotechnology purposes in this variety.

Keywords: Somatic embryogenesis, Rice, *Oryza sativa*, Gene transfer, *Agrobacterium tumefaciens*.

Financing: Funded by grant Núcleo INIA 502955-70 (Humberto Prieto)

Area: Biochemistry and molecular biology

Expression of *LIP1* genes in plants: a strategy for working towards abiotic stress tolerance.

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Lipoic acid is a strong antioxidant with a well-known role in abiotic stress tolerance. LIP1, an enzyme responsible for the limiting step in lipoic acid synthesis, has been identified as a promising target for creating stress-resistant plant varieties. Previous attempts to overexpress LIP1 in *Solanum lycopersicum* var. Micro-Tom have been made using a strong, constitutive promoter, however these plants exhibited stunted growth. Through novel synthetic biology strategies, we have assembled a series of constructs to overexpress plant *LIP1* genes; *AtLIP1* and *SlLIP1* in plants under the stress-inducible promoter regions of *SlAREB* and *AtRD29A*. Additionally a betalaine-producing marker under a seed-specific promoter (*AtSESA3*) was used to identify stably transformed T1 *Arabidopsis thaliana* plants. When used to transiently transform *Nicotiana benthamiana*, a visible accumulation of betalaine was also observed in agroinfiltrated tissue. To our knowledge this is the first report of gene expression induction under the *AtSESA3* promoter region, a promoter region otherwise widely considered to be seed-specific.

Keywords: LIP1, Abiotic stress, RD29A, AREB, *Arabidopsis*, *Nicotiana*, Betalains

Financing: This work was funded by the Beca Maria Ghilardi Venegas Foundation and Fondecyt Regular 1231417.

Area: Biochemistry and molecular biology

How could Pectale lyase and Pectin Methylesterase from Calafate (*Berberis microphylla*) contribute to the circular economy?.

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Agrozzi is a major player in the food industry which produces 49.000 tons of waste annually during the elaboration of tomato paste and juice. This byproduct is basically plant cell walls (CW) composed of cellulose, hemicellulose, pectins and proteins. Several studies indicate that pectins of the CW are beneficial for gastrointestinal health, but to extract them from the tethered network of polysaccharides in the CW, we need different enzymes to loosen this structure. Pectate lyase (PL) and pectin methylesterase (PME) are two enzymes that participate in remodeling the CW. PL acts on homogalacturonan making chains shorter, whilst PME reduces the degree and pattern of methyl esterification of pectins, increasing their susceptibility to degradation. To obtain beneficial pectins from food waste, both enzymes must be robust enough to survive in harsh industrial environments. Calafate (*Berberis microphylla*) is a native berry that grows at high altitude, where plants are exposed to cold and UV radiation. Their ability to withstand extreme environmental conditions means that it is likely that their enzymes could possess properties useful for biotechnological applications. In this study, we identified two ORF sequences of PL and PME of calafate by a transcriptome analysis. Calafate RNA was isolated and the CDS of both enzymes was amplified and ligated in a vector for overexpression in *Pichia pastoris*. Once purified and characterized biochemically, they will be applied to agro-industrial waste in order to obtain beneficial health products, thus contributing to the circular economy.

Keywords: Cell wall, Pectate lyase, Pectin methylesterase, Calafate, *Pichia pastoris*

Financing: Anillo project ACT210025 “Chicobio”

Area: Biochemistry and molecular biology

Apples of the Future: Boosting carotenoid content and reducing browning with CRISPR/Cas9.

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Chile is the world's fourth-largest apple exporter and the top one in the Southern Hemisphere. However, the apple industry globally and in Chile has stagnated due to a lack of innovation and limited varietal changes. Traditional breeding methods take 12-15 years to generate new apple varieties, while New Breeding Technologies (NBT) can do it in half the time. NBT offers the advantage of producing non-GMO varieties, exempt from strict regulations. Therefore, our aim is to produce new Royal Gala (RG) and Fuji RakuRaku (FRR) apple plants with higher carotenoid (pro-vitamin A) content and reduced fruit browning through CRISPR/Cas9 gene editing. At present apples present a reduced content in provitamin A, being a good opportunity to produce a functional apple; and browning impacts freshness, flavor, and nutritional value, causing economic losses and waste. The targeted genes were carotenoid cleavage dioxygenase 4 (CCD4) and polyphenoloxidases (PPOs). Specific gRNAs were designed, and vectors were generated for plant transformation. Transformed apple explants were rooted, and functional analysis revealed plants with at least 40% more carotenoids and reduced browning. A molecular strategy, including PCR, enzymatic digestion, and sequencing, was developed to identify non-transgenic and edited plants. This has allowed us to select 7% of plants that present functional attributes and that are not transgenic. We hope to generate in the future a new Chilean apple variety with higher nutritional value that contribute to the reduction of food waste.

Keywords: Apples, Carotenoids, Browning, CRISPR/Cas9

Financing: Idea Fondef ID 23i10052

Area: Biochemistry and molecular biology

An approach to JA-ABA crosstalk in strawberry: characterization of the JAZ1-ABI1 interaction.

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Jasmonates (JAs) and abscisic acid (ABA) are well-known phytohormones that play paramount roles in regulating plant response to biotic and abiotic stresses, respectively. In recent years, the JA-ABA crosstalk has aroused interest in the scientific community, however, the molecular players in the ABA-JA interplay remain to be unveiled. Here, we evaluated the interaction between JAZs proteins and the phosphatase ABI1 from strawberry (*Fragaria* × *ananassa*). Both JAZs and ABI1 are key negative regulators of JA and ABA signaling pathways, respectively. FaJAZ1 and FaJAZ9 were identified as FaABI1 interactors by yeast two-hybrid (Y2H) assay. The FaJAZ1-FaABI1 interaction was corroborated by pull-down assay. To define which domain of FaJAZ1 interacts with FaABI1, two truncated FaJAZ1 proteins, one contained the conserved TIFY and the other the Jas domains, were generated. As the Y2H assay depicted, JAZ1 interacts with ABI1 through the Jas domain. *In silico* analyses were also performed to determine the key amino acid residues for FaJAZ1-FaABI1 interaction. From these data, the substitution of essential amino acids in the FaJAZ1-FaABI1 interaction will be executed in the future. This work is a preliminary approach to disclose the molecular link between ABA and JA pathways in strawberry.

Keywords: *Fragaria x ananassa*, JAZ repressors; Protein phosphatase 2C (PP2C); Protein-protein interactions

Financing: National Research and Development Agency (ANID, Chile) through FONDECYT/ Regular 1210941, Millenium Science Initiative Program - NCN2021_010, and BECAS/DOCTORADO NACIONAL/2019 2190806 grants

Area: Biochemistry and molecular biology

Heterologous expression of VPPME10869 and VPPME20846 enzymes from *Vasconcellea pubescens* in *Pichia pastoris.*

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The Chilean agroindustry plays a pivotal role in fruit processing, generating substantial plant residues, primarily plant cell wall fibers. This study investigates enzymes involved in plant cell wall degradation, specifically pectin methylesterase (PME) types I and II, from Chilean mountain papaya (*Vasconcellea pubescens*). To accomplish this, the first objective was constructing the vector pCR8, housing the genes for VpPME and VpproPME, for subsequent subcloning in *E. coli*. CDS of *VpPME* and *VpproPME* were meticulously identified from RNA-seq data. To ensure the authenticity of the identified sequences, comprehensive bioinformatic characterizations were performed using tools such as NCBI, Omega Cluster, Swiss Model, and phylogenetic tracing. With nucleotide and amino acid sequences well defined, it was possible to design specific primers for VpPME10869 and VpPME20846. Amplicons were cloned into the pCR8 vector, confirmed through rigorous restriction enzyme assays, and sequenced. Remarkably, VpPME10869 exhibited a notable amino acid change, substituting phenylalanine with valine, a conservation observed in PMEs across various species. The subsequent objective involved constructing the vector pPICZA for VpPME expression in *Pichia pastoris*. Purified VpPME10869 and VpPME20846 fragments were ligated into the pPICZ alphaA vector, and confirmed through enzyme digestion. *Pichia pastoris* cells were successfully transformed via electroporation, yielding colonies and affirming the transformation's success. Future steps encompass enzyme purification, paving the way for potential applications in agroindustrial waste management. This study showcases a promising approach, leveraging Chilean mountain papaya enzymes, to address sustainable agroindustrial waste management challenges.

Keywords: Pectin methylesterase (PME), Heterologous expression, *Pichia pastoris*, Agroindustry, *Vasconcellea pubescens*, proPME

Financing: Funding: Anillo project ACT210025 "Chicobio".

Area: Biochemistry and molecular biology

Boosting carotenoid content and salt tolerance: the role of *Daucus carota* Lycopene β-cyclase 2 (*DcLCYB2*).

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Carotenoids are isoprenoid molecules essential for various plant processes, including photosynthesis, protection against reactive oxygen species (ROS) and being precursors of abscisic acid, a hormone responsible for abiotic stress tolerance. They also impart vibrant orange, red and yellow colors to flowers and fruits. The synthesis of carotenoids in plants is tightly regulated, with lycopene β-cyclase (LCYB), a key enzyme, playing a central role in converting all-trans-lycopene into α- and β-carotene.

Carrot (*Daucus carota*) has two *LCYB* genes, with *LCYB2* predominantly expressed in the taproot, the organ known for its high accumulation of α- and β-carotene. In this study, we functionally characterized the *DcLCYB2* gene. By means of heterologous complementation into *E. coli* we confirmed the enzyme's functionality. Moreover, our investigation confirmed that DcLCYB2 is localized within the chloroplast, aligning with its presumed function. Additionally, we expressed the ORF in *Nicotiana tabacum* and *Actinidia deliciosa* plants, resulting in increased total carotenoid content and enhanced performance under chronic salt treatments. Collectively, these findings validate the enzyme's functionality and position this gene as a promising candidate for enhancing carotenoid levels in commercially significant crops.

Keywords: Carotenoids, Lycopene-b-ciclase, Salt tolerance, Carrot, Kiwi

Financing: FONDECYT Regular 1221339

Area: Biochemistry and molecular biology

Data mining approach reveal new insights into plant responses to combined stress of low phosphate and salt.

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Climate change has generated significant variations in soils, leading to nutrient deficiencies and abiotic stresses that affect the normal development of plants. An example of this is phosphorus (Pi) deficiency, a macronutrient essential for plant life because it participates in the synthesis of primary metabolites such as DNA and proteins. Salt stress, on the other hand, causes significant cellular damage, affecting plant fitness. As nutrients diminish and the salt component of the soil increases, the spatial arrangement of the root system becomes crucial for optimal resource use. This spatial arrangement of the root, referred to as root system architecture (RSA), responds actively to environmental stresses. The mechanisms of mitigation and signaling that generate low Pi and salt stresses are known, but studies on these mechanisms have been carried out independently. Although these stresses often occur simultaneously in plants, little information is known about the molecular pathways that regulate this multi-stress response. To investigate this, an experimental strategy was designed based on data mining of transcriptomics and experiments databases from plants grown under low Pi and salt stresses. Based on this, novel transcription factors (TFs) that participate in both stresses were identified, and this information was integrated through transcriptional networks to identify new regulatory pathways. At the same time, these data were validated by comparing them to an RNA-seq analysis performed under dual stress conditions. On the other hand, RSA phenotype analyses were performed on mutants for three novel transcription factors (TFs) identified from data mining information under combined stress conditions. Additionally, a gene network related to these TFs suggests that RSA development is finely regulated by phytohormones.

Keywords: Transcriptions factors, Data mining, Root architecture, Root hair, Low Phosphate, Salt Stress

Financing: ANID-Millennium Science Initiative Program-Millennium Institute for Integrative Biology (ICN17_022), FONDECYT Postdoctorado 322013, FONDECYT Postdoctorado 32208701, FONDECYT Regular 1200010, FONDECYT Regular 1210389, ANID-Programa Iniciativa Científica Milenio (Grants ICN17_022 and NCN2021- 010).

Area: Biochemistry and molecular biology

Regulation of phosphate starvation (PiS) stress – associated anthocyanin biosynthesis by the MYC2 transcription factor in tomato: preliminary results.

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Phosphate starvation (PiS) is one of the nutritional constraints that most significantly impedes plant growth, reduces crop productivity, and induces changes in morphology and molecular signaling processes. At the transcriptional level, anthocyanin biosynthesis is primarily regulated by transcription factors (TFs), which act at different levels of the biosynthesis pathway. The regulation of the anthocyanin biosynthesis by the MYC2 TF provides a mechanism for plant adaptation to PiS since finally antioxidants are produced to protect cells from oxidative damage. This study aims to evaluate the molecular response of tomato (*Solanum lycopersicum*) plants subjected to PiS through the evaluation of the relationship between SlMYC2 and PiS signaling- and anthocyanin biosynthesis-associated genes. The total anthocyanin content and gene expression were evaluated in the 'Indigo Rose' and 'Moneymaker' cultivars subjected to PiS (0 mM Pi). The results reveal that tomatoes exposed to PiS exhibit higher expression of *SlMYC2* and *SlPHR*/*PHL*, the master TF in the PiS response, as well as the genes involved in anthocyanin synthesis such as *SlAN2*, *SlUFGT*, and *SlDFR*. Accordingly, a greater purple coloring in vegetative tissues and a higher accumulation of total anthocyanins were observed in 'Indigo Rose'. In summary, our findings suggest a coordinated action of SlMYC2 and SlPHR in response to PiS stress-associated anthocyanin accumulation in tomato, bringing us pathways to get a deeper understanding of the underlying molecular mechanism of the regulation of PiS stress-associated anthocyanin biosynthesis by MYC2.

Keywords: Anthocyanin accumulation, PHR transcription factor, Phosphate shortage, *Solanum lycopersicum*

Financing: National Research and Development Agency (ANID, Chile) through FONDECYT/Regular 1210941, Millenium Science Initiative Program NCN2021_010, and BECAS/DOCTORADO NACIONAL/2023 21231118 grants.

Area: Biochemistry and molecular biology

Characterization of a monoterpene synthase (MTPS) differentially expressed in response to drought in *Eucalyptus*.

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Trees respond to drought by activating genes and regulating metabolic pathways. In *Eucalyptus*, essential oils of the monoterpene type are abundant in foliage and economically valuable for their use in pharmaceuticals and industry. Changes in the level and profile of monoterpenes to water stress in plants have been reported. Gene members of the monoterpene synthase (mTPS) family are responsible for their synthesis. *mTPS* genes from one species of the *Eucalyptus* genus were studied, examining the expression levels in the transcriptome of *Eucalyptus globulus* subjected to water deficit. On the other hand, transcription factors involved in the regulation of mTPS were identified, in addition, a structural analysis of mTPS was performed. 16 putative functional genes were identified and characterized in *Eucalyptus*, whereas only mTPS077 is differentially expressed in response to water stress in leaves. The phylogenetic tree grouped these genes into 3 distinctive clades, in addition to present an individual group of taxa by species. Three transcription factors were identified: MYB, NAC and WRKY, which would participate in the regulation of monoterpene biosynthesis and stress response. Analysis of mTPS promoters for these TFs shows an average of 3 binding sites for NAC, 5 sites for WRKY, and 2 for MYB. Finally, TPS077 features 20 alpha-helices, 11 helix 3/10, 2 turns and 1 β-sheet. Structural comparative analysis of the 16 mTPS showed a RMSD of 8.85, being a RMSD of 5,12 for TPS077 compared to the template. In summary, mTPS077 could be involved in the differential synthesis of monoterpenes in response to drought.

Keywords: *Eucalyptus*, Monoterpene, Monoterpene synthase, Gene expression, Drought

Financing: ANID - project ATE220043 and Fondecyt 1201011–

Area: Biochemistry and molecular biology

Cloning and expression of *XTH* genes from *Vasconcellea pubescens.*

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The fruit undergoes changes in fruit texture during the ripening process. Softening is required to reach consumer expectation of quality. Fruit softening is related to the action of cell wall degrading enzymes acting on the plant cell wall structure. The remodeling of plant cell wall polysaccharides is facilitated by the presence of enzymes such as glycosyl hydrolases and/or lyases. Mountain papaya (*Vasconcellea pubescens* A. DC) fruit is an exotic fruit, native from the Andean region of South America and cultivated in several locations in Chile. Ripening of this fruit follows a climacteric behavior characterized by a reduction in fruit firmness, the development of yellow color and fruity aroma, in parallel with ethylene rise. A transcriptomic library was prepared from ripening mountain papaya fruit using Roche/454 technology. From the data, six sequences related to xyloglucan endotransglycosylase/hydrolase (XTH) family were identified. These enzymes with endo-transglycosylation (XET) and/or hydrolitic (XEH) activities facilitate the reorganization of linear β(1-4) chains of xyloglucans within the plant cell wall. Until now, the open reading frame of two putative XTHs, *VpXTH568* and *VpXTH1408*, were cloned and then expressed heterologously in *Pichia pastoris*. Both genes displayed an increment in expression during mountain papaya softening. Phylogenetic analysis grouped VpXTH568 into clade III-B and *VpXTH1408* into the ancestral group. *VpXTH568* translated protein has a molecular weight of ~37 kDa and an isoelectric point of 5.63, meanwhile VpXTH1408 translated protein has a molecular weight of ~50,7 kDa and an isoelectric point of 6.25. Finally, the three-dimensional models of both proteins were obtained through comparative modeling, describing their predicted active sites and the conserved protein domains.

Keywords: XTH, cell wall, heterologous expression, mountain papaya, Enzyme

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Area: Biochemistry and molecular biology

Deciphering the intricacies of UDP- Arabinofuranose/UDP-Arabinopyranose biosynthesis and their transport in plants.

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Arabinose is a monosaccharide that occurs primarily as arabinofuranose in plant cell wall polysaccharides. Their biosynthesis relies on UDP-arabinofuranose, derived from UDP-arabinopyranose by a cytoplasmic mutase. UDP-arabinofuranose is transported into the Golgi lumen by specific transporters. On the other hand, the synthesis of UDP-arabinopyranose takes place within the Golgi apparatus. However, there is no evidence for the exit of nucleotide sugars from the Golgi lumen into the cytoplasm.

To unravel the intricacies of UDP-arabinofuranose biosynthesis, we introduced the mutase into the Golgi lumen with the aim of synthesizing UDP-arabinofuranose directly in this compartment. Surprisingly, this approach hindered rather than accelerated arabinofuranose biosynthesis, suggesting a critical role for nucleotide sugar transporters in moving UDP-arabinopyranose from the Golgi lumen to the cytoplasm.

Using Arabidopsis seed mucilage as a model, we identified several nucleotide sugar transporters encoding genes expressed in mucilage-producing epidermal cells. Among these genes, we detected uronic acid transporter 1 (UUAT1), whose mutants exhibited reduced arabinose levels in various organs, suggesting that this gene family may play a role in UDP-Arabinopyranose transport. Furthermore, analysis of mutants in At1g77610, a gene expressed in seed coat epidermal cells, showed reduced arabinose levels in mucilage. Coexpression analysis revealed that At1g77610 co-express with genes encoding UDP-Arabinofuranose/UDP-Arabinofuranose mutase. Interestingly, another UUAT family member (At1g12500) shows high co-expression with genes involved in the biosynthesis of arabinogalactan proteins.

These findings suggest that the UUAT gene family members facilitate UDP-Uronic acid entry and UDP-Arabinopyranose exit from the Golgi lumen into the cytosol.

Keywords: Arabinose, UDP-Arabinofuranose, UDP-Arabinopyranose, Nucleotide sugar transporters, Plant cell wall

Financing: Fondecyt 1230859; ANID – Millennium Science Initiative Program – ICN2021_044

Area: Biochemistry and molecular biology

The UDP-Rhamnose/Galactose transporter 4 (URGT4): a key NST for biosynthesis and dimerization of RG-II.

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The plant cell wall is the extracellular matrix surrounding plant cells, primarily composed of polysaccharides. Within this complex structure, the monosaccharide rhamnose plays a pivotal role in the formation of the pectic domains known as rhamnogalacturonan I and II (RG-I and RG-II). The Golgi apparatus utilizes UDP-rhamnose to incorporate rhamnose into these polysaccharides, a process facilitated by the action of the nucleotide sugar transporters (NSTs), URGT1-6. RG-II consists of four side chains, each composed of different monosaccharides, and has been implicated in the development of various plant organs. In this study, we aimed to identify the URGT transporters involved in RG-II synthesis in plant roots.

Through mutational analysis of each URGT gene, we identified the *urgt*4-2 mutant, which exhibited shorter roots. Molecular rescue of *urgt*4-2 using the URGT4 gene under the control of its endogenous promoter restored the wild-type phenotype. Monosaccharide composition analyses revealed a reduced rhamnose content in toots from the mutant. Furthermore, in the *urgt*4-2 mutant, we also observed decreased levels of other monosaccharides in RG-II. RG-II forms dimers, which are important for its biological function; however, the mutant also displayed monomers in addition to dimers. Collectively, our findings indicate the involvement of URGT4 in the synthesis of RG-II, shedding light on the crucial role of this transporter in plant cell wall dynamics and development.

Keywords: UDP-Rhamnose, Nucleotide Sugar Transporters, Rhamnogalacturonan II, Root Development

Financing: Fondecyt 1230859; ANID – Millennium Science Initiative Program – ICN2021_044

Area: Biochemistry and molecular biology

The influence of silicon supply on phosphorus use efficiency and the gene expression of phosphate transporters in ryegrass plants.

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Silicon (Si) can improve the tolerance to phosphorus (P) deficiency in plants, but the mechanisms are still not fully understood. In order to extend our knowledge about the influence of Si on P acquisition of ryegrass plants grown under P limitation, the P use efficiency and the gene expression of phosphate transporters (*LpPHT1;1* and *LpPHT1;4*) were analyzed. Two ryegrass (*Lolium perenne* L.) cultivars (cvs. 24Seven and Ansa) were hydroponically cultivated with P (0, 0.01 or 0.1 mM) in combination with Si (0 or 2.0 mM) for 21 days. Although both cultivars did not show differences in P content and P acquisition efficiency (PAE) as a consequence of Si application, a significant improvement of P utilization efficiency (PUE) was induced by Si under P limitation. This effect was more evident in 24Seven, which increased PUE by about 71% at 0 P added and 33% at 0.01 mM P, when Si was applied. The enhancement of PUE induced by Si was a consequence of the increased biomass production by unit of P acquired at either 0 P or 0.01 mM P conditions. In addition, P deficiency increased the gene expression level of *LpPHT1;1* and *LpPHT1;4* in roots of *cv.* Ansa. Similarly, an up-regulation of *LpPHT1;4* was observed in roots of 24Seven grown under low P, whereas the transcript levels of *LpPHT1;1* were decreased in this cultivar at the same P condition. Interestingly, Si supply down-regulated the expression of *LpPHT1;1* and *LpPHT1;4* in both cultivars grown under low P availability. Accordingly, changes in the expression of this P transporters genes could be implicated in the improvement of PUE triggered by Si.

Keywords: Silicon, Phosphorus use efficiency, Phosphate transporters, Phosphorus deficiency, Ryegrass

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Area: Biochemistry and molecular biology

Up-regulation of Aluminum – tolerance genes in response to Aluminum toxicity and Phosphorus deficiency interaction in ryegrass plants.

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Soil acidity, associated with aluminum (Al) toxicity and phosphorus (P) deficiency are the major mineral stresses for grassland systems established on Chilean Andisols. The development of plants with greater Al-tolerance and P use efficiency has become in a crucial strategy for sustainable agriculture in acid soils. Although interconnected tolerance mechanisms for P deficiency and Al toxicity in plants have been found, the molecular basis related to both constraints are still poorly understood. In order to elucidate the insights into the mechanisms underlying P-Al interaction in ryegrass (*Lolium perenne* L.) plants, the expression pattern of key Al-responsive genes for internal Al detoxification were analyzed. Ryegrass cv. 24Seven was hydroponically grown with different Al and P doses, and the expression level of *LpNRAT1* (Nramp aluminum transporter 1) and *LpALS1* (Aluminum sensitive 1) in roots was evaluated by quantitative RT-PCR in a time-dependent experiment. The expression level of both genes was up-regulated by Al suggesting that these transporters are also involved in Al uptake and Al sequestration in ryegrass. The transcript levels of *LpNRAT1 and LpALS1* showed a higher and early induction when plants grown under P deficiency and Al toxicity. Interestingly, at P limitation in absence of Al supply, the expression level of both genes was also enhanced compared to normal P conditions. These results could indicate that P deficiency improved Al detoxification by a common signaling mechanism between limited P and Al stress.

Keywords: Aluminum toxicity, Phosphorus deficiency, Aluminum transporters, Ryegrass

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Area: Biochemistry and molecular biology

Characterization of a Dof transcription factor involved in the regulation of *Fragaria chiloensis* fruit ripening.

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Softening is a key event during fruit ripening. *Fragaria chiloensis* is a native species from Chile and its white-pink fruit has attractive organoleptic qualities, nevertheless it softens rapidly and intensively that limits its shelf-life. Several transcription factors (TFs) should regulate the ripening and softening of this fruit. DNA binding with one finger (DOF) TFs constitute a multigene family of plant-specific TFs that regulate the expression of genes of complex metabolic pathways, participating in varied functions such as plant development, response to stress and ripening. From a transcriptomic library *FchDOF1* was isolated (1314 bp), which encodes a protein of 301 amino acid residues and shows a conserved DOF domain at the N-terminus. The protein displayed a nuclear localization. Relative expression analysis revealed that *FchDOF1* is mainly expressed in fruit and its expression increases with ripening development. Phylogenetic analysis indicated that *FchDOF1* is evolutionary close to *FaDOF2* from *F. x anannasa*, which has been reported to participate in fruit ripening, specifically in the regulation of the volatile phenylpropanoid pathway. The promoter sequence of *FchDOF1* was obtained (2304 bp) and *in silico* analysis identified *cis* elements able to respond to hormones such as ABA and auxins. On the other hand, DOF recognition elements were identified in the promoters of genes that participate in cell wall disassembly associated to softening of *F. chiloensis*. These results suggest that *FchDOF1* could participate in the transcriptional regulation required during ripening of *F. chiloensis* fruit, however more studies are required to determine its function.

Keywords: Fruit ripening, DOF transcription factor, Chilean strawberry.

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Area: Biochemistry and molecular biology

Characterization of a potassium transporters from *Pinus radiata* in response to the loss of trunk verticality.

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Pinus radiata is a valuable gymnosperm for the forest industry due to its rapid growth, adaptability, and high wood quality. However, it is susceptible to loss of trunk verticality caused by abiotic stresses. High-affinity potassium (*HKT*) genes are a family of ion transport proteins related to this type of stress. They specialize in absorbing and distribute potassium ions in plant cells. This study characterized a potassium transporter differentially expressed in response to loss of trunk verticality in *P. radiata*. The gene was identified in transcriptome libraries built from RNA of 1- and 6-year-old pines. A 2,757 bp sequence was obtained, with a 1,932 bp CDS encoding a 644 amino acid protein (Pi 8.81/Mw 75,159.31 Da). The expression of *PrHKT1* was analyzed in the trunk of 6-year-old pines tilted by qPCR. Differential expression of *PrHKT1* was observed, with overexpression at 10 hours in the lower trunk side and repression at 24 hours in the upper trunk side in 1-year-old plants. In contrast, overexpression was observed in the apex and middle zone of 6-year-old trunks. Phylogenetic analysis grouped HKT sequences from different plant species, with *P. radiata* found in the same clade as other coniferous species. X-ray spectrometry showed a higher concentration of Na^+ and K^+ in cleaned samples from inclined pines compared to controls. A protein-protein interaction network was generated, constructing a map of gene and protein interactions associated with the potassium transporter. Finally, PrHKT1 three-dimensional model was obtained using comparative modeling, revealing a structure with alpha helices and 4 conserved pore domains forming a selectivity channel.

Keywords: *Pinus radiata*, High-affinity potassium (HKT), Potassium transporter, Abiotic stress

Financing: Fondecyt #1201011 and PIA# ATE220043

Area: Biochemistry and molecular biology

Deciphering the thermotolerance in Wheat via CRISPR/Cas9-mediated targeting of Rotamase homeologs.

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Elevated temperatures represent a critical abiotic stress, negatively impacting wheat physiology and crop yield. Prior transcriptomic analysis identified a pronounced upregulation of Rotamase, an enzyme catalyzing the cis-trans isomerization of peptide bonds, within the grains of wheat subjected to high-temperature stress (HS). This study aimed to elucidate the contributory role of Rotamase in thermotolerance. We employed the CRISPR/Cas9-system to induce targeted mutations within the A, B, and D homeologs of the Rotamase gene in wheat. From screening 50 transgenic lines, we obtained 3, 6, and 0 mutants in the A, B and D homeologs respectively. Taking advantage, of the accelerated growth condition by Speed Breeding, we crossed A and B mutant lines to generate 7 independent double A/B mutants. Comparative thermotolerance assays in seedling plants across non-mutated controls, A single, and A/B double mutants yielded several key findings: 1) Under HS without acclimation, single mutant does not show differences with respect to the control lines. On the contrary, the double mutants showed high sensitivities to HS, with a significant reduction of aerial biomass and recovery capacity after HS, suggesting to Rotamase how a necessary factor to the establishment of basal thermotolerance. 2) On the other hand, the HSF1 (Heat Shock Factor 1, a master regulator) was induced to similar levels in control and mutants, indicating mutants are not defective in initial transcriptional HS response. 3) In response to acclimation treatment, an HSP 23.5 (Heat Shock Protein) was highly induced in control and also in mutants, suggesting that the HS signaling is not impaired in the Rotamase CRISPR-mutants lines. Together, these data demonstrate wheat Rotamase positively regulates thermotolerance, likely by modulation of the basal HS responses.

Keywords: Wheat, Heat Stress, Thermotolerance, Rotamase, CRISPR-Cas9

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Area: Biochemistry and molecular biology

Delving into Calafate's (*Berberis buxifolia*) cell wall: a Patagonian gem.

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The calafate (*Berberis buxifolia*) is a shrub that produces small, dark blue fruits in the Patagonia region of South America. Calafate fruits are used in local cuisine to make jams, sweets, liqueurs, and desserts due to their sweet-tart flavor. They are rich in antioxidants, especially anthocyanins, and also contain vitamin C and fiber. Calafate hold cultural significance in Patagonia and are considered a regional symbol. Given their cultural importance and nutritional richness, we decided to analyze the calafate's cell wall, which is closely related to its fiber content.

The analysis of the total monosaccharides composing the cell wall showed a high content of galacturonic acid, as well as neutral sugars like arabinose, galactose, glucose, mannose, and xylose. The study of pectins revealed a significant presence of homogalacturonans and highly branched rhamnogalacturonan-I (RG-I). The use of antibodies revealed intense labeling for JIM5, consistent with the low degree of methylesterification measured (4.27%). The antibodies also confirmed the presence of xylogalacturonan, arabinogalactan proteins, as well as xyloglucan.

The analysis of isolated RG-I through HPLC and antibodies for different specific epitopes confirmed a high proportion of branching, especially those composed of arabinose and galactose, such as arabinogalactan-I and -II, arabinan, and linear galactan, respectively. Finally, the analysis of the hemicellulose fraction showed the presence of xylan in its components, while the signal for xyloglucans was very weak and only observed at high concentrations. The results observed in this study mark the beginning of the investigation into calafate's cell wall, aiming to explore potential applications for this fruit.

Keywords: Calafate, Cell wall, Pectin, Hemicellulose.

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Area: Biochemistry and molecular biology

The glutamate receptor-like channels (GLRs) at Antarctic moss, *Sanionia uncinata*, and its contribution to ROS balance during abiotic stress.

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The Antarctic continent is characterized by extreme conditions for life in general. A variety of environmental stressors make this environment challenging, including temperature, drought conditions, salinity, and high UV radiation, all having a negative impact on plant life cycle and productivity. In this unique ecosystem, only two species of vascular plants managed to adapt and grow, while mosses, with 109 species, dominate. Among those bryophytes, *Sanionia uncinata* is a remarkable specie, characterized by a remarkable adaptability and tolerance to different types of extreme conditions.

In the signaling pathway leading to plant adaptation to abiotic stresses, a group of ion channels, so called Glutamate Receptor Like (GLR), have been identified that play an important role, through the second messenger calcium (Ca2+) and electric signaling. In vascular plants, understanding mechanisms of action of those GLRs is complicated, due to the large number of family members. On the other hand, moss is a much simpler system, with only 2 GLR expressed in *Sanionia*. We performed analysis of the structural and phylogenetic characteristics of *GLR1.X1* from the Antarctic moss *S. uncinata*, as well as evaluation of the gene expression profile during an assay where a set of plants was subjected to different temperatures (5, 17, 21 and 28°C). Our experiment showed that *SuGLR1.X1* mRNA (as well as *POX, CAT, SOD* and *APX*) transcripts levels were significantly regulated (10-fold), mainly at temperatures investigated of 5°C and 28°C.

Interestingly, in vascular plants, GLRs channels were recently shown to be regulated by ROS, which concentration is triggered by high temperature, but the physiological meaning of this regulation is still unknown. We propose using *Sanionia* as an experimental system to study GLR involvement in abiotic stress response. Such study may participate in understanding the unique adaptability of the Antarctic moss adapted to survive in extreme conditions.

Keywords: Abiotic stress, Moss, *Sanionia uncinata*, Glutamate receptor-Like GLR, Antartic, ROS, Calcium

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Area: Biochemistry and molecular biology

Identification of alternative splicing events under saline stress conditions in tomato (*Solanum lycopersicum var.* MicroTom).

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Alternative splicing (AS) is an essential mechanism in eukaryotic organisms that contributes to post-transcriptional gene expression regulation and genome diversification. In plants, it plays a significant role in responding to various abiotic stressors, including salt stress. Salt stress, in turn, poses a limiting condition for plant growth and development, potentially leading to other abiotic stressors such as osmotic stress and oxidative stress. This limitation results in decreased crop yield, lower fruit quality, and significant economic losses. Environments with high salt content have adverse impacts, and given the current situation of climate change, more cultivable areas are becoming saline. To date, there is a lack of information regarding specific genes undergoing alternative splicing during salt stress events, and the different isoforms of transcripts influencing root architecture modulation in response to salinity stress are not well understood. In this study, phenotypic changes in the radicle of 8-day-old-post-germination Micro Tom tomatoes were analyzed under the influence of an AS inhibitor combined with a 200 mM NaCl stress condition over a one-week exposure period. It was observed that, during treatment with the GEX01A inhibitor (herboxidiene) at 0.2 mM in conjunction with salt stress, there was a length reduction of the main root and a decrease in the density of lateral roots. This suggests a strong association between AS events and modulation in response to salt stress, serving as a tool for plant survival. Furthermore, an in-depth analysis of AS regulation in response to salt stress was conducted using publicly available transcriptomic data to assess which functional gene categories are enriched.

Keywords: Soil salinity, Salt stress, Alternative splicing, Abiotic stress, Root architecture

Financing: Funding was kindly provided by Fondecyt Regular N° 1221832

Area: Biochemistry and molecular biology

Effect of quinoa byproducts on root cell division and expression of growth and salt stress related genes in *Arabidopsis thaliana*.

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Chenopodium quinoa is a halophyte species that produces seeds for human consumption. This crop is expanding worldwide due to its both exceptional seed quality and crop yield in adverse agroecosystems. Quinoa from the salt flats (Chilean highlands) is characterized by saponins, which impart a bitter taste to the grain. Its seeds are consumed after removing saponins through scarification. This byproduct (QSP; Quinoa Seed Powder), due to the presence of saponins, may have an elicitor activity for immune response and resistance to saline stress. We investigated if QSP added to a half-strength MS medium has a growth promoter effect by evaluating growth parameters and transcript accumulation of growth- and salinity resistance-related genes in *Arabidopsis thaliana* (*At*). *Arabidopsis* (Col-0) seeds were germinated on 0.5 X MS medium in a growth chamber at 25°C with a 16/8-hour light/dark cycle. The effect of QSP in *At* reporter lines was analyzed by transferring seedlings to a liquid culture medium with a concentration of 0.05 mg mL^{-1} of QSP. As a preliminary result, increased fluorescence was observed in the seedlings treated with QSP. The BA3-GUS reporter line was subjected to GUS staining, and the root meristem was analyzed under a stereoscope. Additionally, the root meristems of the TCN:GFP line were analyzed using a confocal microscope. *Arabidopsis* seeds germinated in vitro on half-strength MS medium were transferred, after five days, to a medium added with QSP (0.05 mg mL^{-1}) and NaCl (50 mM). After 14 days, the roots and leaves were collected. The cDNA from both tissues was used for gene expression analysis (*CYCD3, EFR, NTR2.1, NPF6.3, P5CS, BADH, ADC, HKT1, and EF1a*) through qPCR.

Keywords: *Arabidopsis*, Quinoa, Salinity, Growth, Apical meristem

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Area: Biochemistry and molecular biology

FchNAC2 and *FchSHP* participates in a coordinated way in the ripening of *Fragaria chiloensis* fruit.

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During ripening each fruit develops a series of attributes, including color, aroma and texture. *Fragaria chiloensis* is a native fruit that softens fastly and intensively. Several cell wall enzymes participate in cell wall disassembly associated to fruit softening, such as *FchXTH1*, *FchRGL, FchEXP2, FchPL* and *FchPG*. During the final stages of *F. chiloensis* development, a substantial increase in the expression level of two transcription factors (TF) was observed: *FchSHP* (SHATTERPROOF) and *FchNAC2*. This suggests their active participation in ripening, and therefore, the role of FchSHP and FchNAC2 in the ripening of *F. chiloensis* fruit was investigated. First of all, both FchSHP and FchNAC2 proteins are localized in the nucleus as indicated by confocal microscopy analysis of GFP-fused proteins, supporting their role in the modulation of gene expression. On the other hand, the physical interaction between FchSHP and FchNAC2 was demonstrated by yeast two hybrid assays, suggesting a possible direct collaboration in ripening regulation. To delve into the regulatory mechanisms, luciferase dual assays were performed. It was discovered that FchSHP transactivates the promoters of cell wall disassembly genes such as *FchXTH1*, *FchRGL, FchEXP2* and *FchPG*, meanwhile FchNAC2 transactivates those of *FchXTH1*, *FchPG* and *FchEXP2*. The data confirms that FchSHP and FchNAC2 modulate the expression of cell wall genes involved in fruit softening. These findings provide a deeper understanding of the regulation at transcriptional level during the ripening of *F. chiloensis* fruit and underscore the importance of the TFs FchSHP and FchNAC2 in this process.

Keywords: Transcription factor, Ripening, Softening, Yeast two hybrid assay, Luciferase dual assay, MADS-box, NAC

Financing: This work was supported by ANID/FONDECYT Project #1210948.

Area: Biochemistry and molecular biology

OGOX1 controls the Oligogalacturonides-triggered defense responses during Aphid infestation.

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Aphids are the dominant group of phloem-feeding insects, represent one of the most important agricultural pests causing extensive crop losses worldwide. During feeding, aphids use their mouthparts called stylets to navigate through intercellular pathways to reach the vascular tissue and feed on phloem sap. Here we investigate the role of Oligogalacturonides (OGs), cell wall-derived fragments, in plant defense against aphid attacks, focusing on their production and subsequent metabolic modifications. In addition to the length of the galacturonic acid chains, the defensive responses triggered by OGs depend on chemical modifications, such as oxidations at their ends. In *Arabidopsis thaliana*, OGOX1 negatively controls the OG-triggered defense responses through the oxidation of the reducing end of OGs to galactaric acid. Thereby, we are exploring if the OGs generated during stylets probing can promote the resistance against aphids, and how OGOX1 maintains the balance between stress and developmental responses. Our preliminary results show that ogox1 expression levels are down regulated during early stages of *Myzus persicae* infestation, and then upregulated in later stages. This data suggests that OGs are released from cell walls during aphid infestation and then oxidated. To understand whether the decrease in oxidized OG species has an impact on resistance against aphids, ogox1 knockout plants have been developed using CRISPR/Cas9. We have identified that ogox1KO plants have lower *ogox1* expression levels and total OGOX activity than wild type plants. The ongoing work aims to delineate the spectrum of OGs produced during aphid infestation and unravel the molecular mechanisms governing OG-mediated defense responses. Our research may provide insights into novel plant defense strategies, enhancing crop resilience to aphid predation.

Keywords: Plant-Aphid interaction, Cell wall, Oligogalacturonides, Defence, *Arabidopsis thaliana*, *Myzus persicae*

Financing: ANID-FONDECYT regular 1210320, Programa Iniciativa Científica Milenio-ICN17_022, NCN2021_010, ANID-PIA/BASAL FB0002 and ANID/Doctorado Nacional 2022/21221245.

Area: Plant physiology

Biochemical characterization of the direct defense response in mandarin leaves *W. murcott* (*Citrus reticulata* L.) infested by *Panonychus citri*.

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In the context of climate change, the increase of CO_2, long-term drought, high temperatures and low relative humidity promote the development of parasitic herbivores. One of them is the spider mite (*Panonychus citri*), a plague that exclusively attacks citric, feeding on the cellular contents of the leaves and affecting plant metabolism. After the damage caused by the spider mite, direct defenses are induced. The production of secondary metabolites and biomolecules that accumulate because of synthesizing primary metabolites, such as sugars, triggers the defense mechanisms against this plague. On the other hand, using tolerant and/or resistant rootstocks has allowed the development of citriculture under biotic and abiotic stress conditions. In addition, rootstocks can affect the primary and secondary metabolism of commercial cultivars (-scion).

In this study, the levels of polyphenols, total sugars, and photosynthetic pigments were determined in spider mite-infested leaves (20 mites per leaf) from mandarin plants "*W. Murcott*" grafted on four rootstocks: "Macrophylla" (MA), "Carrizo" (CA), "C35" and "Citrumelo" (CI). The identification and quantification were performed by UV-visible spectrophotometry. The extracts were prepared from lyophilized leaves using different solvents for each metabolite analyzed. In mandarin leaves infested with mites compared to uninfected leaves (control), the polyphenols total content increased from 84% to 97%. The total sugar content decreased between 22% and 46%, and chlorophylls lowered from 14% to 58% depending on the rootstock. The CI, CA and MA rootstocks with the mite treatment showed a significantly high variation of the analyzed metabolites concerning their controls. The combination *W. Murcott* / C35 responded differently. The chlorophyll α content increased while polyphenols and total sugar contents were not significantly affected concerning control. These preliminary results show the C35 rootstock enhances resistance against phytophagous mite infection.

Keywords: Mandarin, Red spider mite, Polyphenol, Sugar, Chlorophyll, Resistance, Infection

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Area: Plant physiology

Low temperature-induced root hair growth: revealing the genetic basis using genome-wide association.

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Root hairs (RH) have become an excellent model system for studying cell size regulation, as they can elongate several hundred times their original sizes. The cell growth rate is determined by both intrinsic cell factors and signals from the external environment. RH represents a significant proportion of the root surface, crucial for nutrient and water absorption. Although the mechanisms of expansion of RH cells are known, the roots are in direct contact with the soil, making them highly susceptible to environmental stress, which can affect the entire plant. Therefore, understanding how these mechanisms change in extreme environmental conditions is very relevant. To date, part of the modulation of the cell elongation process by environmental factors, such as temperature, is known, but there are many variants that can alter the specific response. Previous findings from our group indicate that low temperatures (10°C) can trigger exacerbated RH growth compared to cell expansion at room temperature. To explore the molecular basis of this strong growth response, we performed genome-wide association studies (GWAS) on approximately 200 natural accessions of *Arabidopsis thaliana*. The analysis yielded several SNPs that could be relevant; after filtering the data, we identified a gene encoding a protein of the alpha/beta hydrolase superfamily, which is poorly described. We are currently investigating this gene and its mutants, as it also shows differential expression in the root, which we consider promising within the task of unveiling the mechanism and regulation of HR growth under low temperature conditions.

Keywords: Root hairs, GWAS, *Arabidopsis thaliana*, Low temperature

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Area: Plant physiology

The impact of silicon and arbuscular mycorrhizal fungi on plant growth, water status and photosynthetic traits in wheat plants cultivated under water deficit.

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Water deficit stress significantly impacts wheat production globally, highlighting the needed to explore new strategies to cope with this constraint. Although current evidence suggest that a sustainable alternative could involve the application of silicon (Si) and arbuscular mycorrhizal fungi (AMF) to improve the performance of plants growing under water limitation, the potential synergistic action between Si and AMF symbiosis under low water condition remains poorly understood. This study is aimed to investigate the interactive impact of Si and AMF on plant growth, water status, and photosynthetic traits of wheat grown under water deficit. A pot experiment using wheat (*Triticum aestivum*) *cv*. Kiron grown under two irrigation regimes [90% of water holding capacity (WHC) and 40% WHC], two Si doses [0 or 1000 mg Si kg - 1 soil], and two AMF levels [-AMF, +AMF; using *Claroideoglomus claroideum* as inoculant] was conducted. Plants were harvested at tillering stage, and plant biomass, relative water content (RWC), leaf water potential (Ψ_w), CO_2 assimilation (*A*), stomatic conductance (g_s), transpiration rate (*E*) and PSII photochemical efficiency (F_v/F_m) were evaluated. Despite water deficit decreased RWC and Ψ_w, a significant increase in this parameter was observed when +Si and Si+AMF were supplied to low-watered plants. Silicon application also improved the Ψ_w of plants grown at 40% WHC, with a further increase being observed in plants treated with Si in combination with AMF. Similarly, Si+AMF induced an improvement of A under low water availability. In addition, in plants exposed to water limitation, g_s and F_v/F_m increased by Si and AMF supplied alone or in combination. A positive synergistic impact of Si+AMF on *E* and shoot dry matter production was also found in plants subjected to water scarcity.

Keywords: Silicon, Arbuscular mycorrhizal fungi, *Triticum aestivum*, Water deficit stress

Financing: FONDECYT Regular Projects N°1201257 and N°1220190, Vicerrectoría de Investigación y Postgrado de la Universidad de La Frontera Postdoctoral Project VRIP PD 01 2023, and FONDECYT Postdoctoral Projects N°3220092 and N°3210588.

Area: Plant physiology

Glutamate receptor-like proteins involvement in physiological response to salt stress in tomato.

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Plant growth is significantly impeded by the high salinity levels in the soil, impacting negatively in the development, growth, and yield of crops. Plants respond to salinity by redistributing root mass between main and lateral roots, adjusting root system architecture (RSA). However, the genetic mechanisms underlying this process in crop plants remain largely unknown. Glutamate Receptor-like (GLR) channels are multimeric, ligand-gated transmembrane receptors in plants, functioning as key ionotropic channels that integrate multiple hormone signaling pathways in various physiological processes and stress responses. While GLRs have been reported to play a role in salt stress, no study has investigated the mechanism of their involvement in RSA adjustment. To understand the developmental and physiological role of GLR in the salt stress response, we evaluated the physiological response to salt stress in an i*n vitro* and hydroponic approach using pharmacological treatments with GLR agonist (Glu, Gly) and antagonist (AP-5) molecules, alone or combined with NaCl treatments, using tomato as a crop model. Our focus was on physiological parameters (Fv/Fm, PI, chlorophyll content), morphological parameters (root architecture, FW, DW), and molecular parameters (gene expression). Additionally, we determined the elemental composition with X-ray Fluorescence to assess differences in ion homeostasis at the end of each treatment. Finally, we analyzed the gene expression of salt stress-related genes at different time points to evaluate the role of GLR in the transcriptional regulation of gene expression. We will present the results of the set of experiments and discuss the contribution of GLR to the tomato's response to salt stress.

Keywords: GLR, Root system architecture, Salt stress, Tomato, Fv/Fm, AP-5

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Area: Plant physiology

Germination and antioxidant activity in purple basil.

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Purple basil (*Ocimum basilicum var. purpuracens*) is a species known for its high content of phenolic compounds, which possess high nutritional value and antioxidant properties, compared to its counterpart, common basil (*Ocimum basilicum*). Seed germination tends to be a delicate process, and in purple basil, it remains a crucial process. To enhance seed germination and phytochemicals in purple basil, formulations into hydrogels of *Bacillus pumilus* were developed.

Treatments based on the use of *Bacillus pumilus* on physiological aspects and phenolic compound synthesis in *Ocimum basilicum var. purpuracens*, such as alginate microcapsules and encapsulated *B. pumilus* microcapsules within an alginate matrix, were compared to a liquid formulation of *B. pumilus* and water as a control, with four repetitions for each treatment and a completely random distribution.

Significant differences were observed among the treatments due to the influence of hydrogels and bacteria as biostimulants. The application of the three treatments, while delaying post-sowing germination, improved aerial biomass (g) and height (cm) of the treatments 20 days after sowing compared to the control. Furthermore, antioxidant properties were higher in the hydrogel treatments with *B. pumilus*, resulting in a nutritional enrichment of basil. These findings could have significant implications for the future development of biostimulants, focusing on healthy and sustainable food.

Keywords: Biostimulants, Antioxidants, Phenolic compounds, Alginate.

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Area: Plant physiology

Black Rice: bioactive compounds and effect of water deficit on grain chemical composition.

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Black rice stands out for its high content of anthocyanins (flavonoid compounds with antioxidant activity) in its pericarp and a wide variety of other bioactive compounds. Furthermore, comparative studies between pigmented and non-pigmented rice have reported a higher tolerance of pigmented rice to water deficit. Three rice genotypes, two black advanced lines (Quila 279101 and Quila 292008), and one white rice cultivar (Zafiro-INIA) were grown under flooding (F) and alternate wetting and drying (AWD) irrigation at two locations, San Carlos (SC) and Parral (PA). Total polyphenol content (TPC), antioxidant capacity (ORAC and DPPH), color parameters (L*, a*, b*, c*, and h*), and phenolic profile were evaluated in polished grain extracts. The TPC, ORAC, and DPPH were 3.8-, 3.6-, and 8.7-fold higher in Quila 279101 regarding Zafiro-INIA, respectively. High and positive correlations were obtained between ORAC, DPPH, TPC, and color parameters. Among color parameters, L* showed the highest correlation with TPC, ORAC, and DPPH ranging from -0.705 to -0.805. The total mean concentration of compounds in the phenolic profile was 0.76 mg g^{-1}. Quila 292008 showed the highest phenolic concentration regardless of location and water regime with 1.24 mg g^{-1}, which was 4.3 times higher than that of Quila 279101. Cyanidin 3-*O*-glucoside (C3G) and quercetin (QCT) were not detected in white rice grains. Polished black grains showed similar phenolic concentrations under flooded and AWD conditions, with 0.72 and 0.81 mg g^{-1}, respectively. PCA analysis explained 75.5% of the variance of the data, contributing quercetin derivate (AGNd*), vanillic acid derivate (Vad*), ORAC, QCT, L*, TPC, DPPH, vanillic acid (VA), and h* to 50% of the variability. The evaluation of the bioactive compounds of black rice under water deficit conditions allowed determining their potential in the production of functional foods, as well as the effect of the environment on grain composition.

Keywords: Secondary metabolites, Stress, Grain quality

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Area: Plant physiology

Effect of drought and heat stress on yield and protein content of *Phaseolus vulgaris* (L.) Chile landrace.

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Common bean (*Phaseolus vulgaris L.*) is the most important direct-consumption leguminous crop in the world. Due to its high protein and mineral content, it is an essential dietary component, especially in Central and South America. Common bean cultivation faces a series of abiotic limitations for its production, among which drought (DS) and heat stress (HS) can result in flower and pod abortion and can cause the dissociation of reserve proteins (globulin and phaseolin) in the seed. Since it is predicted that these stress factors will continue to increase due to climate change, consequently, we expect yield losses and a marked decrease in the dietary protein content. In this study, we aimed to evaluate the effects of drought (ETc 50%) and heat stress (35°C/6h), both independently and combined, on the accumulation of reserve proteins, using contrasting genotypes of *P. vulgaris* of Chile landrace (varieties Palo and Mantequilla). For this purpose, a greenhouse experiment was conducted with both varieties under optimal conditions, DS, HS, and DS+HS; we analyzed the storage protein content along with productivity parameters. The stressed and control plants were harvested in the R9 stage, and the different parameters associated with yield were obtained (number of pods, seeds, seeds per pod, individual seed weight, and total weight). Likewise, a total protein analysis was carried out on the seeds. Briefly, both varieties were negatively affected by heat stress, with the Palo variety being the most severely affected in terms of yield and having a loss of up to 10.3% in the amount of protein in the seed. In contrast, the Mantequilla variety was less affected in yield but had a 12.6% protein loss. Overall, so far these results show the effects of these stresses and emphasize the need to continue studying the diverse and robust response of the Chile landrace.

Keywords: Common bean, Protein content, Drought stress, Heat stress

Financing: Centro de Estudios en Alimentos Procesados, Fortalecimiento Científico de los Centros Regionales ANID, Proyecto R20F0001.Dirección de Postgrado, Facultad de Agronomía y Sistemas Naturales, Pontificia Universidad Católica de Chile.

Area: Plant physiology

Differential nodulation capacity on two different Chilean *Phaseolus* landraces

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Global food security is compromised by climate change, environmental deterioration and growing population. In this complex scenario legumes can play a crucial role in restoring soil health by converting inert atmospheric nitrogen (N_2) into plant-available form and become an important source of human dietary protein and other nutrients. Legume plants roots are often associated with bacteria of the family *Rhizobiaceae* and generate highly specialized structures –nitrogen-fixing named nodules. The legume/rhizobia symbiosis is a complex controlled process that involve rhizobia infection, nodule development, regulation of nodule number per root and nodule senescence. Additionally, to a common response required for infection and nodule organogenesis, there is evidence that the compatibility between *Phaseolus vulgaris* and rhizobia is determined at species-specific level, being the plant genotype, the major determinant of the symbiotic interaction. Therefore, we proposed that it is a differential nodulation capacity, measured as number and nodule area, on different Chilean *Phaseolus* landraces and also that the irrigation with nitrate and without it also provoke differences on nodulation capacity. For instance, “Cimarron” and “Zorzal” Chilean *Phaseolus* landraces were cultured under greenhouse with those irrigated conditions. Roots from plants in flowering stage were carefully washed and the nodules were extracted. The nodules were counted and photographed to determinate nodule area. These preliminary results show that “Zorzal” tends to form more and bigger nodules than “Cimarron”, and the irrigation with nitrate (12 mM) does not inhibit nodulation but tends to form smaller nodules in both landraces. Additionally, the nodule bacteria were isolated and identified by colony PCR.

Keywords: Nodule, *Phaseolus*, Symbiosis, *Rhizobium*

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Area: Plant physiology

Involvement of Glutamate Receptor-Like GLR in salt stress tolerance in the Antarctic moss *Sanionia uncinata*.

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In the Antarctic continent and particularly in ice-free areas, plant organisms that reach to colonize these environments must overcome multiple extreme abiotic factors such as cold, drought, radiation and salinity, being the latter one of the most important. One of the plant species with the greatest coverage and that stands out for its high tolerance to salinity is the moss *Sanionia uncinata*. Although this moss has a high tolerance to abiotic stress, the molecular mechanisms that confer this characteristic are not yet known. Therefore, our study aims to understand the participation of signaling pathways in the stress response and particularly how calcium would be related to this tolerance in this moss. In this way, we focus on glutamate receptors (GLR) whose activation allows the movement of calcium ions, achieving the activation of signal transduction pathways that contribute to the tolerance mechanisms of vascular plants, although their function has not yet been fully elucidated.

Our evidence show that when plants are treated for 30 days with a high concentration of NaCl (350 mM) or in association with AP5, a pharmacological inhibitor of GLR channels, there is a significant difference between the parameters analyzed. Furthermore, plants treated only with NaCl showed a reduced chlorophyll concentration as well as a lower number of gametophores. In addition, elemental composition analysis (Micro-XRF) showed a higher accumulation of [Na+], [Cl-] and lower [K+]. The NaCl+AP5 treatment showed a higher concentration of chlorophyll and a high number of gametophores. In terms of elemental composition, a higher concentration of K+ and lower accumulation of Na+ and Cl- were observed.

Based on our results, we will discuss the participation of glutamate receptors GLR in the Antarctic moss *Sanionia uncinata* in relation to abiotic stress and particularly when it is under a salt stress condition.

Keywords: salt stress, Glutamate receptor Like-GLR, AP5, *Sanionia uncinata*, Antartic, moss, chlorophyll

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Area: Plant physiology

Evaluation of perception and response mechanisms to UV-B radiation in the sub-antarctic moss *Polytrichum strictum*.

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Polytrichum strictum is a cosmopolitan moss with a high tolerance to adverse climatic conditions. Each spring, the thinning of the ozone layer occurs over Antarctica, reaching the sub-Antarctic ecoregion and increasing up to 85% of UV-B radiation in early December. UV-B radiation acts as a critical environmental factor that determines plant fitness, modulating morphological and physiological processes by activating gene expression and metabolism changes. A genomic analysis by NGS was performed to study the putative genes of the UV-B signaling pathway of *P. strictum* moss collected from Isla Navarino, which is regularly affected by the seasonal ozone hole phenomenon. Our objective was to identify the putative homologs of genes encoding UVR8, COP1 and RUP1/2, 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) whose accumulation has been proposed as crucial for UV-B tolerance in mosses. Our results showed 22,744 predicted genes in the *P. strictum* genome, of which almost 75% were assigned by BlastP to a biological function using GO terms. A comparative genomic analysis of orthologous genes was performed using ten moss genomes, looking for those associated with the phenylpropanoid and UVR8 signaling pathways. These genes are being evaluated at the transcriptional level in *P. strictum* grown in an in vitro system under a UV-B radiation treatment condition and a control condition. Our research seeks to understand the defensive molecular response of mosses to UV-B radiation stimulus and its level of similarity/conservation with the mechanism observed in higher plants.

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

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Area: Plant physiology

JETCUTTER technology for the formulation of agricultural hydrogels and their effects on water deficit tolerance.

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In agriculture, hydrogels are a good tool to mitigate water stress in plants, as well as for the development of biostimulants. The morphology and composition of the hydrogels influence the stability and water retention, so the type of methodology used for the formation of the spheres will determine their properties. The main problems with the techniques used in hydrogel bead processing are low homogeneity, very large sizes and limitations in yield rates, restricting their application on a larger scale. JetCutter technology is a new bead forming technique with the ability to generate small, uniform beads with a high production rate, achieving adequate yields. Therefore, in this research, the optimal operating parameters of the JetCutter to improve alginate-based hydrogels were studied and established. Different polymer concentrations were studied in search of high yields and decreasing losses in the process. In addition, the response of basil plants to hydrogel encapsulated with the bacterium *Bacillus pumillus* was studied. With the established parameters, high yields were obtained in the production of spheres, high homogeneity in shape and size, small diameters of 2 mm and water retention capacity of the spheres greater than 55%, as well as an encapsulation efficiency of 80%. Basil plants treated with hydrogel formulations containing *B. pumillus* showed greater tolerance to water stress.

Keywords: Alginate, Water stress, Encapsulation, Biostimulant

Acknowledgments: Fondecyt Regular 1201950 / FONDEF IDEA ID21I10050, ANID

Area: Plant physiology

Root gene expression of a new salt-tolerant hybrid rootstock (*Solanum lycopersicum* x *S. chilense*) grafted with plants of "Old Limachino Tomato" cultivated under saline conditions.

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The expression of genes associated with salt tolerance in the plant at the root level was analysed in the new rootstock, JUPAFORT1, during the vegetative phase in grafted plants with the well known Chilean tomato landrace "Old Limachino Tomato" (OLT), grown under greenhouse and saline conditions. This rootstock was developed from an interspecific cross between the cultivated tomato (*S. lycopersicum* cv Poncho Negro) and its wild relative, the halophyte (*S. chilense*). The experiment included grafted (with JUPAFORT1 rootstock), self-grafted and ungrafted OLT plants grown at three different salinity treatments: 0 (control), 80 and 160 mmol L^{-1} NaCl. The salt treatment was applied by irrigation with a nutrient solution every three days for 21 days. The expression of genes [1] Na^+ transporter HKT1.1 (*HKT1.1*), [2] Na^+ transporter HKT1.2 (*HKT1. 2*), [3] potassium channel SKOR-like (*SKOR*), [4] potassium channel (*LKT1*), [5] plasmalemma Na^+/H^+ antiporter (*SOS1*), [6] calcineurin B-like interacting protein kinase (*SOS2*) and [7] calcium sensor calcineurin B-like (*SOS3*) was determined by qPCR. In addition, their transcriptome profiles were compared by RNAseq analysis. The results on gene expression in Na^+ and K^+ transporting mechanisms in roots and their transcriptomes suggest that JUPAFORT1 confers good salt tolerance to OLT plants, which involves transcriptional control associated with their physiological salt tolerance.

Keywords: Rootstock, Salinity, Gene expression, Tomato, Wild tomato, Tolerance, Landrace

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Area: Plant physiology

Effect of a rhizobacterium isolated from Osorno grasslands on the growth of plants of *Lolium perenne* and *Trifolium repens* exposed to drought stress.

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The grasslands of southern Chile are the most abundant source of feed for the dairy and beef cattle industry. In recent years, due to climate change, grasslands have been exposed to high temperatures and periods of drought that have an impact on plant yields and consequently on milk and meat production. In order to look for alternatives to reduce the impact of heat and drought on grasslands, we isolated rhizobacteria from grassland plants that appear to be more tolerant to these stresses. Of these isolates, one called 'C17' was able to produce indole acetic acid derivatives and improve phosphate solubilisation. To test whether this isolate can improve the tolerance of *Lolium perenne* and *Trifolium repens* plants to drought stress, we inoculated it into the germinating seeds of these plants. Thereafter, after 1 month of growth, the plants were exposed to drought for one week and morphological and physiological parameters were measured before treatment, after treatment and one week after re-watering. From these analyses, it could be observed that the isolate 'C17' has a positive effect on the growth of *Lolium perenne* plants, such as longer roots and an increase in fresh and dry weight, whereas no effects were observed in *Trifolium repens* plants. In addition, the internal structure of the root tissue shows differences between inoculated plants compared to non-inoculated plants. Thus, these results suggest that isolate 'C17' is able to induce changes in *Lolium perenne* plants that may enhance their ability to tolerate drought stress. Based on these results, the host specificity of this isolate will be discussed.

Keywords: *Lolium perenne*, *Trifolium repens*, *Rhizobacterium*, grasslands

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Area: Plant physiology

Pressure-volume curves reveal the mechanics behind root resistance to drought stress in *Opuntia ficus-indica*.

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Succulent plants have developed several mechanisms to overcome long periods of drought. Yet, many questions regarding plant water relations at root tissue level remain unanswered. Here, we used pressure-volume curves with two levels of dry-bench (i.e., 2 and 5 min) to evaluate root tissue traits of *Opuntia ficus-indica* under well-watered, severe drought stress, and recovery conditions. Also, fluorescence light microscopy was used to understand structural modifications under the mentioned conditions. Our data revealed that *Opuntia ficus-indica* fine roots presented, after severe drought stress (i.e., recovery), a higher osmotic potential than at full hydration (π_o) during the 5-minute dry-bench level. Plants exposed to 5 minutes of dry-bench conditions exhibited a lesser decrease in relative water content (RWC_{tlp}), and no statistically significant differences were observed across stress levels. This observation aligned with the values of capacitance (C_{tlp}), as fine roots showed lower compared to the 2-minutes dry-bench level. Additionally, fine roots subjected to 5 minutes of dry-bench conditions displayed a higher turgor loss point (π_{tlp}) across stress levels. Based on these findings, it is advisable to subject *Opuntia ficus-indica* to longer durations of drought stress to accurately assess the effects of drought on the biophysical behavior of fine roots.

Keywords: Biophysical behavior, *Opuntia ficus-indica*, Pressure-volumen curves, Fine roots, Drought

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Area: Plant physiology

Deciphering root resilience of grapevine native to the Atacama desert of Chile.

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Drought causes root cell damage modifying fine root hydraulic performance. Yet, physiological mechanisms underlying root cell damage during drought remains incomplete. Here, we performed fine root hydraulic conductivity using a root pressure probe (RPP). Besides, electrolyte leakage and fluorescent light microscopy were implemented to quantified and characterized the impacts of drought stress over fine roots. Ultimately, a naturalized grapevine rootstock of the Atacama Desert (R-65) versus a well-known drought susceptible one (101-14MGT) were used as model plants. R-65 displayed a less pronounced decline in RPP during severe drought associated with no cortical lacunae formation. Electrolyte leakage (%) measurements supports this hypothesis due to released cell material when are injured. On the other hand, 101-14Mgt experienced a sharper decline in root hydraulic conductivity during severe drought stress. Structurally, root cortical lacunae formation during severe drought stress could be related to the reduction of hydraulic properties. Suggesting an intimate connection between the hydraulic elastic behavior of fine root with cortical tissue mechanics, allowing naturalized grapevines of the Atacama Desert in northern Chile to tolerate drought.

Keywords: Atacama desert, Hydraulic conductivity, Cortical lacune, Drought, Physiological mechanisms, Naturalized grapevines

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Area: Plant physiology

Development of enhanced tolerant lines of *Solanum lycopersicum* (Tomato) to inorganic phosphate deficiency combined with high salinity stress.

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Plants must adapt to stress conditions in their natural environment. Roots, especially root hairs (RHs), play a crucial role in this adaptation by increasing the root surface area, enhancing water and nutrient uptake. RH growth is stimulated by low inorganic phosphate (Pi) conditions, which is an essential element for plant growth and development. However, Pi is a limited and nonrenewable natural resource with low solubility in soils. High salinity is another common abiotic stress present in soils of cultivated lands and modifies the root architecture. Tomato has become an important model for plant genetics, fruit development, and stress tolerance research. To the date evidence of tomato response to low Pi combined with salt is scarce and developing plants with enhanced Pi uptake under salt stress could play a significant role in optimizing crop yield in the future. To achieve this, we aim to identify key genes involved in RH growth regulated under low Pi combined with high salinity conditions in *Arabidopsis thaliana* and extrapolate this genetic data to improve stress tolerance in tomato. For this purpose, we use 16 lines of TRANSPLANTA collection of Arabidopsis lines that express transcription factors (TFs) under the control of a ß-estradiol-inducible promoter, under this multi-stress (Pi+Salt). The lines of TFs selected were detected by single cell RNA-seq information and shows a specific expression in RH cells. The TFs lines with major tolerances to these stresses will be further test in tomato seedling to determine RHs response to these conditions. Additionally, using X-ray microfluorescence spectrometry we determined the accumulation of P, Na and Cl in leaves to generate information related to tomato resistance and processes of Pi and Salt. All these strategies approaches have the final goal to use the Arabidopsis homologous genes in tomato and generate super adaptable plants to these combined conditions.

Keywords: Phosphate, High salinity, *Solanum lycopersicum*, *Arabidopsis thaliana*, Abiotic stress

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Area: Plant physiology

Effects of deficit irrigation on the physiological and agronomic performance of durum wheat lines (*Triticum turgidum* L. *var.* durum).

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Food production faces a great challenge by 2050, in which the world population is projected to reach 10 billion people, with increasing demand for basic staple crops such as wheat that will have its productivity increased by 60% (De Clercq et al., 2018). Events such as the increase in temperature and reduction in rainfall have been accentuated over time due to climate change, increasingly impacting the stability of agricultural production systems, therefore, it will be necessary to accelerate the crop breeding programs for the development of more drought-tolerant varieties. In the present study, 6 drought contrasting lines of durum wheat subjected to deficit irrigation (TD) of 40% container capacity (CP) were evaluated from the full stage of stem elongation until harvest, from which physiological and agronomic parameters were measured to select those more stable genotypes in the two water scenarios (TD versus optimal irrigation (TI), 100% CP). Our results show that measurements of foliar water potential and chlorophyll stand out as physiological parameters of greater consistency. The most important results indicate that genotype 4 (G4) and genotype 2 (G2) were identified as drought-tolerant genotypes, in contrast to genotype 3 (G3), which associated with the genotype most clearly affected by water stress based on the measured parameters. In conclusion, the combination of appropriate and consistent measurement tools enabled to identify more tolerant genotypes, speeding up and benefiting the work of the durum wheat breeding programs.

Keywords: Durum wheat, Drought tolerance, Water potential, Chlorophyll content, Sustainable production, Climate change

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Acknowledgments: Fondecyt Regular n. 1210092

Area: Plant physiology

Arabidopsis LIFEGUARD gene function has a negative role in plant defense response against *Pseudomonas syringae pv.* tomato infection.

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The induction of regulated cell death (RCD) is vital for the plant response against biotrophic or early hemibiotrophic pathogens. Plant response to pathogenic infection increases protein demand, causing endoplasmic reticulum (ER) stress and triggering the unfolded protein response (UPR). Under maintained ER stress, cell death is activated. In plants, UPR has been associated with the defense response against the hemibiotrophic pathogen *Pseudomonas syringae*. The LIFEGUARD (LFG) family protein is described as regulator of the UPR response. Arabidopsis genome codes for five LFG genes (*LFG1-5*), and *LFG1* to *LFG3* show a redundant role in inhibiting cell death triggered by UPR under chemically induced ER stress. Considering the participation of LFGs in the process of the UPR-associated cell death, and the involvement of the RCD in the defense response, we are interested in elucidating the role of the *LFG* genes in plant defense response. To reach this aim, we used as models the plant *A. thaliana* and *Pseudomonas Syringae tomato* (*Pst*) as pathogen. We performed *Pst* infiltration assays on WT plants, and by using RT-qPCR, we found that two out of five *LFG* genes were induced at 24 hours post-inoculation, suggesting the participation of these genes in the plant-pathogen interaction. We also generated and characterized plants overexpressing *LFG1* and *LFG3*. To evaluate the contribution of these genes to the defensive process, we performed *Pst* infiltration assays to evaluate the plant phenotype. We found that the bacterial title is statistically significantly higher in the overexpressor lines compared to WT plants, indicating an increased susceptibility to the *Pst* infection. Additionally, we are characterizing *LGF2* and *LFG5*-insertional mutant lines to study their contribution to the plant response to the *Pst* infection. Our results suggest that *LFG* genes might modulate defense response, showing new evidence that links their function over UPR response with plant immunity.

Keywords: Plant immune response, *Arabidopsis thaliana*, *Pseudomonas syringae*, LIFEGUARD

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Area: Plant physiology

Changes in White Lupine physiology and soil microbial activity induced by microplastics.

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Intensive agriculture relies on plastic mulches, nets, tapes, ropes and sacks made of low-density polyethylene (LDPE), polyamide (PA), and polypropylene (PP) to improve productivity and profitability. However, the degradation of these plastics results in microplastics (MPs; < 5mm), which can damage plants and the environment. White lupine is a fabacea used in agroecosystems to improve soil due to its ability to fix nitrogen. Thus, the objective of this study was to evaluate the effects of 3 types of MPs (PE, PA, PP) on soil microbiological activity. For this, glass pots containing 700 g of soil, each with a 2 % w/w (70 g) addition of LDPE, PA, PP mechanically fragmented control MPs, and control were utilized. Two white lupine plants were grown in each pot, totaling 40 plants across the experiment. After 110 days of exposure, we assessed chlorophyll content, soil basal respiration, plant height, biomass dry weight, microbial biomass carbon, enzymes activities (urease and phosphatase), fluorescein diacetate (FDA) activity, as well as the number of nodules and root clusters. This experiment revealed that the exposure of white lupin to MPs yielded multifaceted effects on both the plants and the soil ecosystem. While plant height, biomass and soil respiration remained largely unaffected. Alterations in chlorophyll levels, microbial biomass carbon, enzyme and FDA activity were observed. These results indicate potential impacts on plant health and soil microbial activity, underscoring the intricate interactions between microplastic pollution and terrestrial ecosystems.

Keywords: Microplastics, Soil, Microbiological activity, Lupin physiology

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Area: Plant physiology

Conservative and 'risk-taking' bottle gourd genotypes: evaluation of water uptake capacity, leaf gas exchange of Watermelon grafted onto different *Lagenaria siceraria* landraces under water deficit.

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Climate change has induced the strongest droughts in the last 10 years, causing major alterations in the climate with negative impact on most of the world's crops, including watermelon. We aimed to study the dynamics of water uptake and gas exchange in leaves of watermelon plants grafted onto *Lagenaria siceraria*landraces to identify promising genotypes with different tolerance to water deficit. Four *L. siceraria* landraces were used as rootstock: 'Illapel' and 'Osorno' (from Chile), 'BG-48' (from South Africa) and 'Philippines' (from Philippines), and one commercial genotype: 'Pelops'. Commercial watermelon variety 'Delta' (*Citrullus lanatus*) was grafted onto the rootstocks. The rootstock/scion combinations were grown under two water availability conditions: well-watered (WW) and progressive water deficit (WD). We evaluate the fraction of transpirable water soil (FTSW), photosynthesis (A_n), stomatal conductance (g_s) and water use efficiency (WUE_i). We found variability among landraces in the capacity of water uptake, from conservatives (landraces from humidity environments) to 'risk-taking' (landraces from arid or semiarid environment). Gas exchange analysis (A_n, g_s, WUE_i) reveled that under WD the landraces reduced progressive the g_s, but affecting differentially the A_n, inducing variability in the WUE_i from short to long term response to water deficit. These physiological traits analyzed may be used in studies for the selection of *L. sicerari* landraces (rootstock) tolerant to water deficit.

Keywords: Climate change, Drought, Landraces, Rootstock, Watermelon, Tolerance

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Area: Plant physiology

In vitro establishment of *Vasconcellea pubescens* locality of Lipimávida.

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Vasconcellea pubescens is a species that is cultivated from the Coquimbo Region to the Ñuble Region, mainly propagated by seed. Production has been decreasing over time due to various technological factors, such as the limited availability of planting material of high genetic quality. Therefore, *in vitro* culture techniques emerge as an essential alternative for multiplication, either to generate a large number of plants to improve new cultivars. The present study aimed to develop a establishment protocol for *in vitro* culture of *Vasconcellea pubescens* from seeds and mature embryos. The effect of explant management and different growth regulators on germination rate was evaluated. Regarding explant management, the embryo with endosperm treatment obtained 86% germination, differing statistically from the embryo without endosperm treatment, which achieved 25%. With respect to the effect of the growth regulators for embryo without endosperm, there were no significant differences between treatments. For the culture of embryos with endosperm, the treatment of MS medium free of growth regulators obtained the highest values in the variables germination and root presence, 70% and 40%, respectively. It can be concluded that embryo culture with endosperm is a favorable technique for *in vitro* germination of *Vasconcellea pubescens*.

Keywords: *Vasconcellea pubescens*, Growth regulators, Germination, Embryo, Endosperm, Micropropagation.

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Area: Plant physiology

Dissection of the balance between ethylene and lipoic acid synthesis mediated by SAM consumption.

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Plants face adverse conditions such as drought that increases their oxidative state. Plants thus produce antioxidant compounds such as lipoic acid (LA). In a reaction catalysed by lipoyl synthase, LA requires the precursor S-adenosylmethionine (SAM) for its synthesis, a molecule that is also required for the synthesis of ethylene, a phytohormone of senescence and fruit ripening in climacteric plants, processes also associated with an increase in ROS. Therefore, we propose that there is an antagonistic regulation between the synthesis of AL and ethylene due to competition for SAM.

Therefore, we evaluated the effects of altering the ethylene and AL synthesis pathways with respect to senescence and fruit ripening in *Solanum lycopersicum* (tomato), by determining the effects of the exogenous application of AL, and of 1 -MCP, an inhibitor of the action of ethylene, and also by using plants that overexpress lipoyl synthase in fruits.

Our results show that the application of AL reduces the chlorosis of the oldest leaves of the plants (as a parameter of leaf senescence), but that this positive effect disappears if AL is applied over prolonged lengths of time. Additionally, extended application of AL does not alter fruit ethylene production. Likewise, the application of 1-MCP delays fruit ripening but does not have a significant effect on ethylene production when comparing the same fruit ripening states.

Studies using other means of altering ethylene and AL synthesis are necessary to confirm the existence of a balance between ethylene and AL mediated through SAM consumption.

Keywords: Lipoic acid, ethylene, 1-MCP, S-adenosylmethionine, *Solanum lycopersicum*

Financing: Fondecyt 1231417

Area: Plant physiology

Dilucidating the RSL4-mediated regulatory gene networks driving root hair growth at low temperature conditions in *Arabidopsis thaliana*.

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Root hairs (RH) serve as excellent models for investigating the regulation of cell size, given their ability to rapidly elongate to hundreds of times their original size. The control of their growth involves a complex interplay between intrinsic and environmental signals, with the transcription factor ROOT HAIR DEFECTIVE 6-LIKE 4 (RSL4) playing a pivotal role in early gene regulation associated with RH growth. Previously, we observed that in Arabidopsis thaliana plants subjected to low-temperature conditions (10°C), RHs elongate to approximately twice their original size, while overall plant growth is hindered. Nutrient bioavailability in soil is directly affected by temperature, which suggests a possible link to this exacerbated growth. However, the precise molecular mechanisms governing the sensing and downstream signaling pathways remain elusive. In this study, we sought to uncover and characterize the transcriptional networks that RSL4 governs under cold conditions using a combination of transcriptomic and gene networking methodologies. Through a comprehensive analysis that integrated RNA-seq and DAP-seq techniques, we were able to pinpoint early responsive gene networks associated with RSL4 in a low-temperature context and identified three critical nodes downstream of RSL4 in the response to cold: the homeodomain leucine zipper group I 13 (HB13), the trihelix transcription factor GT-2-LIKE1 (GTL1), and a previously uncharacterized MYB-like transcription factor (AT2G01060). Furthermore, we delved into the transcriptional dynamics of these regulatory nodes, along with other genes, by examining transcript accumulation via RT-qPCR in both 10 °C and control conditions. This was done using a series of knockout and overexpressor lines of RSL4, HB13, GTL1, and MYB-like. Collectively, our findings serve to expound the fine control that these transcriptional regulatory mechanics have on a previously unknown RH phenotype.

Keywords: Root Hairs, Cold, RSL4

Financing: Supported by ANID-FONDECYT Regular (Grant 1200010) and ANID-Programa Iniciativa Científica Milenio (Grants ICN17_022 and NCN2021-010).

Area: Plant physiology

Silicon modulates the cell wall structural compounds in oat plants grown under different nitrogen doses.

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Nitrogen (N) is an essential element for the formation of structural tissues in plants. Thus, N deficiency or excess produces alterations in the plant, weakening its structure. Recent evidence has suggested that silicon (Si) could increase cell wall structural compounds of such as lignin and cellulose improving structural strengthening in plants grown under stressful conditions. This study aimed to evaluate the Si effect on the cell wall structural compounds in oat plants grown under different N rates. A hydroponic experiment was conducted by using two oat cultivars (N-tolerant and N-sensitive) treated with three N doses (0.5, 5 or 10 mM N) with two Si doses (0 or 2 mM) for 21 days. At harvest, Si, N, cellulose, hemicellulose and lignin concentration were assayed. The accumulation pattern of cellulose and lignin in shoots were visualized by LSCM. Nitrogen concentration was increased in oats cultivars to higher-rate N application. Nevertheless, when higher N doses were applied, both cultivars decreased the Si concentration in shoots. In plants grown without Si, cellulose concentration was reduced by increasing N doses in the N-tolerant cultivar, whereas the N-sensitive cultivar did not shown differences. However, Si improved cellulose concentration in the N-sensitive cultivar irrespective of N dose. Changes in hemicellulose concentration were also induced by Si in cultivars grown under different N rates. In addition, lignin concentration was reduced by Si application in the N-tolerant oat grown at all N doses, whereas Si reduced lignin content only at the highest N dose in the N-sensitive cultivar. A differential lignin and cellulose accumulation pattern was also observed as a consequence of N and/or Si application. Thus, Si increased lignin and cellulose accumulation in the N-sensitive cultivar at 5 mM N, but it decreased the accumulation of these cell wall structural compounds at either N deficiency or N excess.

Keywords: Silicon, Nitrogen, Oat, Cellulose, Hemicellulose, Lignin

Financing: FONDECYT Postdoctoral Project N° 3220092, FONDECYT Postdoctoral Project N° 3220092, FONDECYT Regular Project N°1190585 and N°1201257, and Vicerrectoría de Investigación y Postgrado de la Universidad de La Frontera- Postdoctoral Project VRIP PD 01 2023.

Area: Plant physiology

Auxins play an important role in root hair elongation under conditions of low phosphate and high salinity in *Arabidopsis thaliana*.

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Plants are sessile organisms, meaning they can move but not relocate. For this reason, they have developed mechanisms to adapt when faced with changes in their environment, such as alterations in soil composition due to variations in pH levels, water deficits, and increased salt concentrations. These changes result in reduced nutrient availability, affecting the proper development of plants. One adaptation strategy employed in these situations is the remodeling of the root system architecture. Root system architecture, comprising primary roots, secondary roots, and root hairs, offers a valuable model for studying cell elongation, as these structures originate from a single epidermal cell called the trichoblast. The elongation of root hairs is profoundly influenced by external cues, such as high salinity, which retards their growth, and nutrient deficiencies, particularly inorganic phosphate (Pi), which stimulates their development. The phytohormone auxin (IAA) plays a pivotal role in the root system's response to phosphate deficiency, primarily through transcription factors like ARFs. Nonetheless, there remains a knowledge gap regarding how plants respond to simultaneous phosphate deficiency and high salinity. In this study, we propose that Auxins play a pivotal role in root hair elongation under low phosphate and high salinity conditions in *Arabidopsis thaliana*. RNAseq analysis showed that Auxin pathway is affected in the dual stress conditions. Mutant analysis suggests that Auxin transport and signal transduction pathway could be a critical factor influencing root hair elongation under the specified conditions.

Keywords: *Arabidopsis thaliana*, Auxin, Low Phosphate, High Salinity, RNAseq, Root Hair

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Area: Plant physiology

The bioherbicidal effect of *Chenopodium quinoa* (Willd.) saponins-enriched by-products on *Portulaca spp.*: an experimental approach.

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Weeds can adapt to many growth conditions. In the field, they invade crops, which means competition for light, water, and nutrients between the crops and the weeds, risking crop production [1]. Many herbicides, due to their composition and synthetic origin, are harmful to both weeds and crops. In addition, weeds can develop resistance to pesticides. To reduce the impact of chemical herbicides on the environment, it is urgent to explore the use of phytometabolites as biopesticide formulations or as part of them [2]. These compounds from plant metabolism present a possible tool for effective and sustainable weed management. Quinoa (*Chenopodium quinoa* Willd.) is a species of agronomic interest that grows in the Andean region of South America. A by-product enriched with saponins is produced from the post-harvest process of quinoa; these secondary metabolites contained in the husk may have potential as biopesticides and growth promoters depending on their origin and concentration [4]. *Portulaca oleracea* (*fam.* Portulacaceae) is a worldwide distributed succulent species that can adopt weedy growth habits. The latter trait suggests *Portulaca* as a model to study the effect of herbicides. The objective of this research is to evaluate the bioherbicidal effect of quinoa by-product saponins-enriched by determining the suitable dose response to produce growth inhibition in *Portulaca*.

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[2] Marques, M., *et al.,* (2023). *Plants,* 12, 663 doi:10.3390/plants12030663.

[3] Ruiz, KB., *et al.,* (2017). *Ind. Crops Prod.*, 104, 156-163. doi: 10.1016/j.indcrop.2017.04.007.

Keywords: weed, saponin, bioherbicide, quinoa, growth, inhibition.

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Area: New and emerging areas of knowledge: Molecular Breeding, Phenomics and new breeding technologies; Teaching, Educational and social aspects in Plant Biology

Generation of CRISPR/Cas9-based gene editors to inactivate susceptibility genes to Potato virus Y (PVY) in Potato (*Solanum tuberosum*) *cv.* Patagonia

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Solanum tuberosum is one of the most important crops for human consumption. The local variety Patagonia-INIA significantly supplies the local market, with optimal use for oven cooking. Patagonia stands out for its resistance to oomycetes but is susceptible to Potato virus Y (PVY), which utilizes the molecular translation machinery to carry out its infection processes and is the main virus affecting the species. To multiply, PVY hijacks and utilizes initiation of translation factors (eIFs), so mutations in eIFs could generate resistant individuals; unfortunately, natural mutations in eIF genes have not been described in potato. The emergence of editing (GE) tools such as CRISPR/Cas9 makes feasible to obtain PVY-resistant potato genotypes by this technique. In this work we aimed the development of GE in Patagonia due to its relevance. Previously, we characterized the complete set of eIF4E candidate genes (named *eIF4E1*, *eIF4E2*, *eIF(iso)4E*, and *nCBP*); from these and considering very recent findings, GE by double cut of the eIF4E1 isoform was prioritized. Guide RNA (gRNA) couples for eIF4E1 were designed and cloned into pVH-X vectors; a home-made technology based on T-DNA modified plasmids containing geminivirus genome modules and expression cassettes for Cas9, GFP, hygromycin resistance, and multiple gRNAs. The functional evaluation of these vectors and gRNA couples was carried out by transient leaf agroinfiltrations using the EHA105 strain. Infections were monitored by GFP expression, and 7 days post-infection (the higher reporter expression), explants were selected for target gene analysis. Among the evaluated vectors, we identified one construct able to induce a clear double cut in the Patagonia eIF4E1 gene. This identified gene editor construct allowed for long-term experimentation from which completely regenerated have been already obtained. Propagation and molecular analyses of these materials are currently under progress towards the identification of eventually PVY-resistant Patagonia individuals.

Keywords: Double gRNA cut, Geminivirus-based editors, *A. tumefaciens*-transformation, eIFs.

Financing: Fontagro – Procisur grant "Edición génica para mejoramiento genético en especies vegetales y animales" and Núcleo INIA grant # 502955-70 (Humberto Prieto).

Area: New and emerging areas of knowledge: Molecular Breeding, Phenomics and new breeding technologies; Teaching, Educational and social aspects in Plant Biology

Species-specific effects of plant growth promoting bacteria on *Solanum lycopersicum* under phosphorus scarcity

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Phosphorus (P) is an essential element for plant growth and survival. P naturally occurs in organic and inorganic forms, although largely insoluble, making it inaccessible to plant roots. Plant Growth Promoting Bacteria (PGPB) are pivotal in regulating various plant traits, including nutrient acquisition and overall health. The role of specific PGPR in plants facing P scarcity is still an underexplored domain. Here we studied the effects of three PGPB (*Pseudomonas putida* KT2440, *Azospirilum brasilense* SP7, and *Paraburkholderia phytofirmans* PsJN) in *Solanum lycopersicum* cv Moneymaker grown under low P conditions.

First, the plant growth was analyzed under different P conditions (pots with perlite/vermiculite and H_2PO_4 as the sole source of P delivered by irrigation). The optimal growth was detected at 1mM and those concentrations equal to or lower than 0.25mM restricted the aerial and root growth. Then, the effects of the three strains were studied by comparing plants inoculated at sowing versus non-inoculated plants. In contrast to our findings in *Arabidopsis*, these strains did not increase plant growth under normal or low P conditions, indicating possible competition for P between the plant and the associated bacteria. Furthermore, the anthocyanin production, a response typically associated with abiotic stress in plant vegetative tissues was increased in the inoculated plants. The study underscores the species-specific nature of plant-beneficial bacteria interactions and highlights the importance of exploring diverse plant-bacteria-environment combinations to advance more efficient and sustainable agriculture.

Keywords: Phosphorus, Abiotic stress, *Solanum lycopersicum*, Plant Growth Promoting Bacteria, Nutrient acquisition

Financing: ANID - Millennium Science Initiative Program - Millennium Nucleus for the Development of Super Adaptable Plants (MN-SAP).

Area: New and emerging areas of knowledge: Molecular Breeding, Phenomics and new breeding technologies; Teaching, Educational and social aspects in Plant Biology

From image to phenotype: an efficient methodology pipeline for measuring fruit size, shape and color using computer vision

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Fruit phenotyping is a mandatory task in breeding programs aiming to improve fruit quality, and it is also required for the genetic and genomic studies that assist them. This is time-consuming and intensive labor, mainly when thousands of fruits must be evaluated for many traits. The high-throughput phenotyping of some of these traits can be accomplished using computer-vision approaches, enabling accurate measurements from whole fruits in less time. We present a pipeline developed to measure fruit size, shape, and color, using 675 plum fruits in 157 images (1 to 5 per image). The methodology relies on ImageJ, a free image software commonly used in the scientific community. All images are calibrated using a color chart reference, and a trained machine-learning algorithm segments all fruits contained in the images. For size and shape, measurements are performed for area, perimeter, width, height, maximum and minimum diameter, contained ellipse, roundness, and circularity. For color, pixel measures are obtained for mean, standard deviation, median, skewness and kurtosis in the RGB and CIELab color models. A macro allows automatizing the steps, and a bash script groups all measured data into a single table. Correlation and PCA results indicate that this methodology can precisely phenotype these traits and be useful for genomic studies using many individuals, such as genome-wide association studies. Although not tested here, the same programmed pipeline could be used with fruits of any species and other organs such as leaves or flowers.

Keywords: Phenomics, Image analysis, Fruit phenotyping

Financing: FONDECYT N°1231614 and IDeA ID22I10266

Area: New and emerging areas of knowledge: Molecular Breeding, Phenomics and new breeding technologies; Teaching, Educational and social aspects in Plant Biology

Harnessing *in vitro* methods for Hemp clonal propagation and improvement.

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Cannabis sativa, a dioecious plant, has garnered substantial attention in the pharmaceutical industry due to its synthesis of cannabinoid compounds in its flowers. The primary cannabinoids of interest are cannabidiol (CBD), known for its non-psychoactive properties, and Delta-9-tetrahydrocannabinol (THC), recognized for its psychoactive effects. Notably, the hemp market, characterized by low-THC cannabis plants, has witnessed remarkable growth, thanks to favourable regulatory changes in various countries. Nevertheless, the industry faces a significant challenge related to the limited stability and genetic uniformity of seeds available in the market. In this context, *in vitro* micropropagation emerges as a powerful tool to address issues related to homogeneity and stability. It enables the rapid production of a large number of clonal plants with minimal space requirements. However, micropropagation alone does not facilitate the development of new plant varieties. It is in this specific context that *in vitro* mutagenesis comes into play as a strategy for creating novel varieties under micropropagation conditions. This study focuses on standardizing and optimizing (i) *in vitro* culture media and conditions for clonal micropropagation in various hemp varieties and (ii) a random chemical mutagenesis protocol using Ethyl Methane Sulfonate (EMS) on *in vitro* hemp explants. Consequently, this research establishes the groundwork for *in vitro* clonal propagation of multiple hemp varieties and the potential generation of novel varieties within this species.

Keywords: *Cannabis sativa*, *In vitro* Culture, Chemical Mutagenesis, Micropropagation, EMS, Clonal propagation, Hemp

Financing: CORFO project 22CVID-206655

Area: New and emerging areas of knowledge: Molecular Breeding, Phenomics and new breeding technologies; Teaching, Educational and social aspects in Plant Biology

Use of Oxford Nanopore DNA sequencing and methylation calling to compare early and late varieties in *Prunus avium* (sweet cherry).

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Sweet cherry fruits of different cultivars mature at different times, with early and late varieties being harvested from the end of November to the end of January in Chile. The ripening process is controlled by several genes that regulate the softening and lignification of pericarp layers, as well as the accumulation of sugars, acids, pigments, and release of volatiles compounds. Understanding the underlying processes of fruit ripening is a key factor for crop improvement. Hormones, transcription factors, and epigenetic changes play a significant role in tightly regulating the ripening process. With the increasing importance of epigenetic modifications on fruit phenotypes, high-throughput sequencing technologies can facilitate the routine screening of crop epigenomes, thereby expediting breeding through the analysis of epigenome structure and conventional genetic variation. Epigenetic-based approaches have the potential to greatly impact fruit quality traits, specifically for traits that have experienced reduced allelic variation due to domestication or intensive breeding. Recent advancements in the study of plant DNA methylation variation present a new opportunity for translating epigenetic research into breeding applications. Although current methods for analyzing the methylome have provided insight into the epigenetic influence on fruit traits, they have proven insufficient in distinguishing between early and late sweet cherry varieties. To address this point, we propose the utilization of Oxford Nanopore DNA sequencing on immature fruits at 24 hours after full bloom to detect Differential Methylation Regions (DMRs) between early and late sweet cherry varieties. Our results shown the presence of at least 35 distinct DMRs that span more than 1000 base pairs each one, clearly showing a differentiating between the varieties. This study confirms the significant role of DNA methylation in sweet cherry fruit phenology. We have identified several DMRs that can serve as potential methylation markers to predict ripening time in a breeding program.

Keywords: Epigenetic breeding, Methylation, Epialleles, Oxford Nanopore Sequencing

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Area: New and emerging areas of knowledge: Molecular Breeding, Phenomics and new breeding technologies; Teaching, Educational and social aspects in Plant Biology

W. murcott (*C.reticulata* x *C.sinensis*) protoplasts isolation, transformation and regeneration as a model to overcome for fruit crops editing challenges.

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The use of new breeding techniques (NBTs) in agriculture offers a promising approach to achieve specific breeding objectives, by allowing precise targeted modification, obtaining faster results and preserving desired traits. In order to apply these techniques, single cell to complete plant regeneration techniques must be handled, unfortunately many important fruit species are classified as recalcitrant (low regeneration efficiency, difficult somatic embryogenesis etc) to in vitro culture, which has delayed the use of NBTs in their breeding programs. Citrus fruits are the second most produced fruits worldwide, being tangerines de second most important commercially of them after oranges. *W. murcott* hybrid tangerine is one of the most important citrus produced in the world. In vitro culture regeneration techniques have been widely used in this genus and species making it a good model to apply NBTs as editing on them. We aimed to use W.Murcott protoplasts to edit different target genes as to demonstrate the scope of these techniques, meanwhile we are working to develop protocols of regeneration to support editing techniques in other fruit trees species. Protocolos to obtain friable embryogenic callus, protoplast isolation, transient transformation with plasmids and/or proteins, regeneration and plant culture have been developed at Meristem.

Our fruit crop editing strategies allow us to establish an efficient transitory integrative transformation system. Each construct module must be analysed as for functionality and efficiency, our protoplast platform allows a quick consistent architectural validation of our constructs in a fruit tree crop consolidating it as a good strategy to obtain edited materials.

Keywords: Protoplasts, New breeding tecnologies, Plant editing, Citrus

Financing: Meristem spa

Area: New and emerging areas of knowledge: Molecular Breeding, Phenomics and new breeding technologies; Teaching, Educational and social aspects in Plant Biology

Plant experimentation unit: Efficiency and Quality in your research

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The Plant Experimentation Center of the UC Faculty of Biological Sciences has a modern infrastructure (200m^2) designed to support national research in the plant field.

This center features growth rooms, full-control walk-in environmental chambers, in vitro growth chambers, and a state-of-the-art molecular biology laboratory, enabling the provision of services such as propagation, agro-transformation, genotyping, phenotypic analysis, plant ecology studies, and species conservation, among others.

Current research objectives in plants and climate change are focused on understanding and mitigating the effects of this phenomenon on agriculture and natural ecosystems. This entails studies on plant adaptation to changing climatic conditions, necessitating the long-term measurement of environmental parameters in controlled conditions. The unique full-control walk-in chambers, the first of their kind in Chile, are designed to replicate a wide range of environmental conditions, including dawn and dusk, as well as special situations like heatwaves.

The availability of this facility will provide local students and researchers with the opportunity to conduct cutting-edge science on home turf.

Keywords: Climate change, Transformation, Native plant growth, CO_2 control

Financing: Facultad Ciencias Biologicas - PUCProyecto CONICYT - FONDEQUIP EQM190120.

Area: New and emerging areas of knowledge: Molecular Breeding, Phenomics and new breeding technologies; Teaching, Educational and social aspects in Plant Biology

Genome-Wide Association studies in peach traits related to fruit phenology and quality

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In breeding programs, the identification of favorable gene alleles in available accessions is crucial before using gene-based selection. This knowledge allows the design of crossing schemes with higher probabilities of obtaining desirable selections and performing marker-assisted selection. Genome-wide association studies (GWAS) can identify genomic variants associated with specific traits in germplasm collections. This study aimed to identify genomic variants associated with three traits (HT: Harvest Time; FW: Fruit Weight; and SSC: Soluble Solid Content) in cultivars and advanced selections included in the germplasm collection of the *Universidad de Chile* stone fruit breeding program. For this aim, we performed a series of genome-wide association analyses using phenotyping data of the mentioned traits for 96 accessions of *Prunus persica* (L.) Batsch. Genotypic data of these accessions was generated by 2D-RADseq, identifying 10.131 reliable SNP variants. The phenotypic matrices were generated using BLUE values for each accession, minimizing the intra-genotype phenotypic variation. The results were validated over the two seasons separately and combining both seasons. We identified the following SNPs significantly associated to the traits: for HT, the markers Pp04_10.644.155 and Pp01_39.728.536; for SSC, SNPs Pp05_16.641.136 and Pp06_20.912.828; and for FW, markers Pp01_27.423.616 and Pp03_20.780.938. Considering that future phenotyping seasons and additional materials will be included in this study, these results are promising. They also provide practical applications for fruit breeding programs and provide evidence for unveiling the genetic architecture of the studied traits in the context of the available cultivars and selections in our germplasm collection.

Keywords: Peach, *Prunus persica*, Genome-wide association studies, SNP, Fruit quality

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Area: New and emerging areas of knowledge: Molecular Breeding, Phenomics and new breeding technologies; Teaching, Educational and social aspects in Plant Biology

Developing haploid inducer lines for genetic stability in Hemp.

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Cannabis sativa (Cannabis) corresponds to a dioecious which has garnered great interest in the pharmaceutical industry, due to the cannabinoid compounds it synthesizes in its flowers. Particularly, the Hemp (non-psychoactive cannabis plants) market has experienced exponential growth due to favourable regulatory changes in various countries. However, one of the major problems facing this industry is the low stability and genetic homogeneity of the seeds available on the market. In this regard, the *in vivo* double haploid (DH) technology has garnered great interest due to its capability to develop plants that are virtually 100% homozygous for all their alleles in only two generations. To accomplish this goal, it is required both a haploid inducer (HI) line (natural or mutant line) and a genome duplication method (spontaneous or by colchicine treatment). In this context, gene editing of *DMP* (*DOMAIN OF UNKNOWN FUNCTION 679 membrane protein*) and *CENH3* (*CENTROMERE-SPECIFIC HISTONE 3*) has sparked great interest, as their mutations produce HI lines able to promote *in vivo* haploid induction. This research established the foundations to develop HI lines in cannabis, through the study of orthologous genes to *ZmDMP* and *AtCENH3*. By *in silico* analysis, 13 and 7 putative loci of similar identity to *AtCENH3* and *ZmDMP* were identified in cannabis, respectively. Through phylogenetic analysis, it was estimated that the LOC115705795 locus corresponds to *AtCENH3* ortholog and the LOC115707143 locus to *ZmDMP* ortholog in cannabis (hereinafter called *CsCENH3* and *CsDMP*, respectively). *CsCENH3* and *CsDMP* were cloned and sequenced in two hemp varieties, and gRNAs were designed for gene editing by CRISPR/Cas9 system (presenting high efficiency and low off-targets *in silico*). Thus, establishing the foundations for the development of HI lines in Hemp.

Keywords: Haploid Induction, Gene editing, *Cannabis sativa*, Hemp, Double Haploid, CRISPR/Cas9

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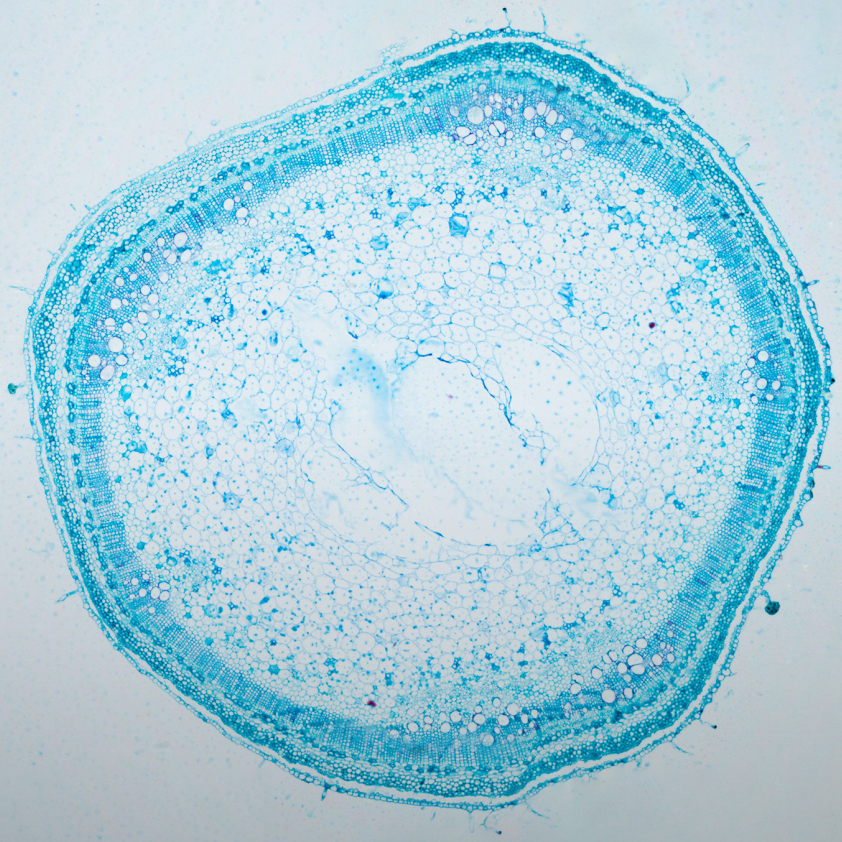
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