



X PLANT BIOLOGY MEETING

1ST TO 4TH OF DECEMBER
DREAMS HOTEL VALDIVIA, CHILE.

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Dreams Hotel, Valdivia, Chile
1st to 4th of December

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WELCOME LETTER

Chilean Society of Plant Biologists

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Dear Plant Biology Community

Welcome to Valdivia, Welcome to the First Meeting of the Chilean Society of Plant Biologists!

We are extremely happy to let you know of the existence of the Chilean Society of Plant Biologists. This new society represents the germination and growth achieved by enthusiastic researchers throughout the country of a seed planted almost ten years ago.

In 2005, a small group of Chilean plant biologists had the vision and felt the need to connect and share ideas to advance the field of plant molecular biology. The immediate goal then was to generate an instance for scientific discussions and exchange of ideas, primarily targeting young scientists in different disciplines related to plant molecular biology in Chile. This idea was the seed for creating the Chilean Network of Plant Biologists, which has organized an annual scientific conference every year since 2006.

The first conference hosted by Universidad Andrés Bello included 70 participants, including students, group leaders and recognized international speakers (inset). Since then, every year different institutions held the conference (see Past Meetings) with increased participation and interest.

Today, we are pleased to welcome more than 200 participants in Valdivia, to celebrate the first meeting sponsored by the Chilean Society of Plant Biologists. We believe this new organization, will help in our continuing efforts to discuss research work carried out in Chilean laboratories, participate in scientific discussions (oral and posters presentations) and interact with leading scientists worldwide.



Primera Circular

I Reunión de Biología Vegetal

Fecha 30 y 31 de octubre de 2006

Lugar: Campos Casaca de la Cordes, Universidad Andrés Bello, Las Cordes, Santiago

Comité Organizador:

Loreto Holmgren (lholmg@bio.puc.cl)
Ariel Orellana (arellana@unab.cl)
Claudio Pastenes (cpastene@uchile.cl)
Hugo Peña-Cortés (hugo.pena@mm.cl)
Herman Silva (hsilva@unab.cl)
Gustavo Zúñiga (gzuniga@usach.cl)

Invitados Extranjeros:

Philip Benfey (Universidad de Duke, Estados Unidos)
Gerd Burgans (Universidad de Tübingen, Alemania)
Elizabeth Lord (Universidad de California, Riverside, Estados Unidos)
Natalia Radtke (Universidad de California, Riverside, Estados Unidos)

The Organizing Committee and volunteers across the country have contributed to put together an outstanding program, featuring six international speakers (one opening lecture, 5 plenary lectures, 20 invited speakers, as well as 13 national speakers for concurrent sessions), covering almost all disciplines of plant biology research. Nearly 220 abstracts have been submitted to the Meeting. We are also very pleased that our conference will include nearly 180 young researchers, M.Sc. or Ph.D. students. With their great work and enthusiasm for plant sciences will undoubtedly be a key factor for the success of this conference and the future leaders pushing the boundaries in plant research in Chile.

Dr. Rodrigo Gutiérrez
President
Chilean Society of Plant Biologists (CSPB)

Past Meetings

I	2006	UNAB, Santiago
II	2007	PUC, Santiago
III	2008	U. de Talca, Talca
IV	2009	CEAZA, La Serena
V	2010	U. de Chile, Olmué
VI	2011	CGNA, Pucón
VII	2012	UNAB, Pucón
VIII	2013	INIA Rayantué, Pucón
IX	2014	INIA / U. de La Serena Intihuasi, La Serena



ORGANIZING COMMITTEE

Dr. Rodrigo A. Gutiérrez, Department of Molecular Genetics and Microbiology, Faculty of Biological Science, Universidad Católica de Chile

Dr. Francisca Blanco, Plant Biotechnology Center, Universidad Andrés Bello

Dr. Raúl Herrera, Institute of Biological Sciences, Universidad de Talca

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Dr. Javier Canales, Institute of Biochemistry and Microbiology, Universidad Austral de Chile

Dr. Andrés Zurita, INIA-Intihuasi, Instituto de Investigaciones Agropecuarias

EXECUTIVE COMMITTEE

Dra. Susana Cabello – Conference Coordinator (scabello@bio.puc.cl)

Karem Tamayo – Administrative and Financial Assistance (kptamayo@uc.cl)

GENERAL INFORMATION

Venue

All Scientific sessions will be held in the Río Valdivia Conference Room located in the first floor (level 1) of Dreams Hotel.

Registration Desk

The registration desk will be open at the following times:

Tuesday, 1 November 12:00 – 16:30 at the Hotel foyer.

Name Badges

Participants are requested to wear name badges all times. Name badges will be used as identification for hotel staff to grant access to conference rooms, lunch and dinner as well as social activities organized by the conference.

Wireless access

Dreams Hotel provides wireless access to all conference attendees.

Mobile Phone

Please ensure that all mobile phones are switched off or in silent mode during all scientific sessions.

Lunch

Lunch will be served for all registered participants at the Doña Inés Restaurant.

Location

Dreams Hotel is located at Carampangue 190, Valdivia, Décima Cuarta Región de Los Ríos, Chile.

PROGRAM AT GLANCE

Tuesday, December 1			Thursday, December 3		
🕒	ACTIVITY	ROOM	🕒	ACTIVITY	ROOM
10:00-14:30	Registration		09:00-10:00	Plenary Lecture III	Río Valdivia
16:30-17:00	Opening welcome	Río Valdivia	10:00-11:30	Symposium V	Río Valdivia
17:00-18:30	Symposium I	Río Valdivia	11:30-12:00	Coffee break	Río Cruces / Hallway
18:30-19:00	Coffee break	Río Cruces / Hallway	12:00-13:30	Symposium VI	Río Valdivia
19:00-20:00	Keynote Opening	Río Valdivia	13:30-15:00	Lunch	Skybar
20:00-22:00	Poster I & Cocktail	Río Cruces	15:00-16:30	Symposium VII	Río Valdivia
Wednesday, December 2			16:30-17:00	Coffee break	Río Cruces / Hallway
🕒	ACTIVITY	ROOM	17:00-19:00	Poster III	Río Cruces
09:00-10:00	Plenary Lecture I	Río Valdivia	19:00-20:00	Plenary Lecture IV	Río Valdivia
10:00-11:30	Symposium II	Río Valdivia	20:00-21:00	National Society of Plant Biologist meeting	Río Valdivia
11:30-12:00	Coffee break	Río Cruces / Hallway	21:00-23:00	Dinner	Río Cruces
12:00-13:30	Symposium III	Río Valdivia	Friday, December 4		
13:30-15:00	Lunch	Skybar	🕒	ACTIVITY	ROOM
15:00-16:30	Symposium IV	Río Valdivia	10:00-11:00	Plenary Lecture V	Río Valdivia
16:30-17:00	Coffee break	Río Cruces / Hallway	11:00-12:30	Symposium VIII	Río Valdivia
17:00-19:00	Poster II	Río Cruces			
19:00-20:00	Plenary Lecture II	Río Valdivia			
20:00-22:00	Dinner	Skybar			

PROGRAM

Tuesday, December 1

🕒	ACTIVITY	ROOM
09:00-14:30	Registration	
16:30-17:00	Opening welcome	Río Valdivia
17:00-18:30	Symposium I: Systems and Synthetic Biology	Río Valdivia
18:30-19:00	Coffee break	Río Cruces / Hallway
19:00-20:00	Keynote Opening: Dr. Natasha Reikhel	Río Valdivia
20:00-22:00	Poster I & Cocktail	Río Cruces

Wednesday, December 2

🕒	ACTIVITY	ROOM
09:00-10:00	Plenary Lecture I: Dr. Helen North	Río Valdivia
10:00-11:30	Symposium II: Cell and Developmental Biology	Río Valdivia
11:30-12:00	Coffee break	Río Cruces / Hallway
12:00-13:30	Symposium III: Abiotic Stress	Río Valdivia
13:30-15:00	Lunch	Skybar
15:00-16:30	Symposium IV: Biotic Stress	Río Valdivia
16:30-17:00	Coffee break	Río Cruces / Hallway
17:00-19:00	Poster II	Río Cruces
19:00-20:00	Plenary Lecture II: Dr. Dan Klessig	Río Valdivia
20:00-22:00	Dinner	Skybar

Thursday, December 3

🕒	ACTIVITY	ROOM
09.00-10.00	Plenary Lecture III: Dr. Zhenbiao Yang	Río Valdivia
10.00-11.30	Symposium V: Metabolism	Río Valdivia
11.30-12.00	Coffee break	Río Cruces / Hallway
12.00-13.30	Symposium VI: Post-harvest Molecular Physiology	Río Valdivia
13.30-15.00	Lunch	Skybar
15.00-16.30	Symposium VII: Ecophysiology of Extreme Environments	Río Valdivia
16.30-17.00	Coffee break	Río Cruces / Hallway
17.00-19.00	Poster II	Río Cruces
19.00-20.00	Plenary Lecture IV: Dr. Ian Ferguson	Río Valdivia
21.00-23.00	Dinner	Río Cruces

Friday, December 4

🕒	ACTIVITY	ROOM
10.00-11.00	Plenary Lecture V: Dr. José Quero-García	Río Valdivia
11.00-12.30	Symposium VIII: Molecular Breeding and Genetic Resources	Río Valdivia
12.30-13.00	Closing ceremony and awards	

LECTURES



A drug-like molecule targets conserved EXO70 to inhibit exocytosis and promote vacuole trafficking

Zhang, C⁶., Brown, M⁶., Van De Van, W⁶., Zhang, Z¹., Wu, B²., Young, M³., Synek, L⁴., Borchardt, D³., Harrison, R³., Pan, S⁶., Luo, N⁶., Huang, Y³., Ghang, Y³., Ung, N⁶., Li, R⁶., Isley, J⁵., Morikis, D³., Song, J¹., Guo, W²., Hooley, R³., Chang, C³., Yang, Z⁶., Zarsky, V⁴., Muday, G⁵., Hicks, G⁶., **Raikhel, N⁶.,** ¹Department of Biochemistry University of California. ²Department of Biology University of Pennsylvania. ³Department of Chemistry University of California. ⁴Institute of Experimental Botany Academy of Sciences. ⁵Department of Biology, Center for Molecular Communication and Signaling, Wake Forest University. ⁶Department of Botany and Plant Sciences, Institute for Integrative Genome Biology, University of California.

The exocyst complex regulates the last steps of exocytosis, which is essential to organisms across kingdoms. In humans, its dysfunction is correlated with several significant diseases such as diabetes and cancer progression. Investigation of the dynamic regulation of the evolutionarily conserved exocyst-related processes using mutants in genetically tractable organisms such as *Arabidopsis thaliana* is limited by the lethality or the severity of phenotypes. We discovered that the small molecule Endosidin2 (ES2) transiently arrests the EXO70 subunit of the exocyst complex, resulting in inhibition of exocytosis and endosomal recycling in both plant and human cells and enhancement of plant vacuolar trafficking. An EXO70 protein with a C-terminal truncation results in dominant ES2 resistance, uncovering possible distinct regulatory roles for the N-terminus and C-terminus of the protein. This study provides not only a valuable tool in studying exocytosis regulation but also offers a potentially new target for drugs aimed at addressing human disease

Funding DOE Grant DE-FG02-02ER15295

Sticking together: polysaccharide interactions in Arabidopsis seed mucilage.

North, H., Crépeau, M-J., Vigouroux, J., Tran, J., Berger, A., Sallé, C., Botran, L., Ralet, M-C., Saclay Plant Sciences, INRA.

Seed mucilage is a polysaccharide hydrogel that is formed on imbibition in certain species. Mucilage polysaccharides are accumulated in the epidermal cells of the seed coat during their development then dehydrate on desiccation. The hydrophilic properties of these polysaccharides causes them to swell dramatically when they are rehydrated, rupturing the outer wall of the cells and allowing them to encapsulate the seed. In Arabidopsis, the mucilage is formed of two distinct layers, an outer water-extractable layer, and an inner adherent layer. Mutants affected in the partitioning of the two layers are proving useful tools for understanding polysaccharide interactions. Notably, the reduced adherent layer in the *cellulose synthase5* mutant has previously demonstrated that cellulose plays a critical role in mucilage adherence^{1,2,3}. The pectin rhamnogalacturonan I (RG-I) is the major constituent of both mucilage layers and the adherence of RG-I is also affected in *mucilage modified5*. Results will be presented that show that this is due to reduced xylan ramifications on RG-I and demonstrate that the high affinity of these branches for cellulose makes a major contribution to the formation of the adherent mucilage layer. Other constituents identified in adherent mucilage and their interactions with RG-I and cellulose will be discussed.

[1] Harpaz-Saad *et al.* (2011) *Plant J* **68**: 941-953; [2] Mendu *et al.* (2011) *Plant Physiol* **157**: 441-453; [3] Sullivan *et al.* (2011) *Plant Physiol* **156**: 1725-1739.

Salicylic acid and its binding proteins in plant and human health

Klessig, D¹., Choi , H¹., Tian, M¹., Manohar, M¹., Moreau, M¹., Park, S¹., Song, F²., Venereau , E⁵., Nagy , P³., Harraz, M⁴., Snyder , S⁴., Schroeder , F¹., Montelione , G²., Bianchi , M⁵., ¹Boyce Thompson Institute for Plant Research Cornell University. ²Department of Molecular Biology and Biochemistry, Center of Advanced Biotechnology and Medicine, The State University of New Jersey. ³Department of Plant Pathology University of Kentucky. ⁴The Solomon H. Snyder Department of Neuroscience Johns Hopkins University School of Medicine. ⁵Division of Genetics and Cell Biology San Raffaele University and Research Institute.

Since our discovery in 1990 that SA regulates plant immunity, we have attempted to determine its mechanisms of action in plant immunity and other biological processes using genetic, molecular, and biochemical approaches. Over two dozen plant SA-binding proteins (SABPs) have been identified primarily through biochemical methods, including three high-throughput screens (see <http://bioinfo.bti.cornell.edu/SA2010/>). SA binding alters the biochemical and/or biological activities of these proteins, generally by inhibiting them. We have extended this work to humans, since the most widely used medicine aspirin (acetyl SA) is rapidly converted to SA after ingestion and SA has most of the same pharmacological activities of aspirin. Two novel targets of SA/aspirin have been identified across the animal and plant kingdoms. Together the two human SABPs are associated with most of the major human diseases, including heart attack, stroke, sepsis, rheumatoid arthritis, inflammation-associated cancers, hepatitis, and neurodegenerative diseases. One of the identified human SABPs is High Mobility Group Box1 (HMGB1). In addition to its nuclear role in condensing DNA and regulating gene expression, extracellular HMGB1 is a damage-associated molecular pattern (DAMP), which activates immune and inflammatory responses. SA suppresses both the chemo-attractant activity of HMGB1 and the increased expression of pro-inflammatory cytokine and COX-2 genes induced by HMGB1. An HMGB1 protein mutated in one of the SA-binding sites identified in the HMG-box domain by NMR analyses retained its chemo-attractant activity, but lost binding of and inhibition by SA, thereby firmly establishing that SA binding to HMGB1 directly suppresses its pro-inflammatory activities. Natural and synthetic SA derivatives also have been identified with greater potency for inhibition of the pro-inflammatory activities of HMGB1. Interestingly, our parallel study of the plant ortholog AtHMGB3 revealed that it also functions as a DAMP to activate plant immunity. Moreover, it binds SA and mutations in its corresponding HMG box suppress both SA binding and SA inhibition of its immune-inducing activity.

The second novel target in humans is Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH). In addition to its central role in glycolysis, human GAPDH participates in several pathological processes including neuronal cell death associated with Alzheimer's, Parkinson's, and Huntington's diseases. We discovered that SA, like the anti-Parkinson's drug deprenyl, suppresses nuclear translocation of GAPDH, an early step in cell death, as well as cell death induced by the DNA alkylating agent N-methyl-N-nitroso-N¹-nitroguanidine. Two synthetic SA derivatives and two natural compounds from the Chinese medicinal herb *Glycyrrhiza foetida* (licorice), glycyrrhizin and the SA-derivative amorfrutin, were identified which appear to not only more tightly bind GAPDH, but also more effectively suppress nuclear translocation of GAPDH and cell death than SA. In addition to GAPDH's role in neuronal cell death, some animal and plant viruses, such as human Hepatitis A, B, C Viruses and Tomato Bushy Stunt Virus (TBSV), usurp this host protein for their replication. We discovered that SA binding to GAPDH inhibits its interaction with the TBSV minus RNA strand, thereby suppressing viral replication. This finding reveals a novel mechanism of SA action in defense against viral pathogens. In summary, these studies demonstrate that SA can modulate both plant and human health via shared SABPs. Furthermore, the identification of human HMGB1 and GAPDH as pharmacological targets of SA/aspirin provides new insights into the mechanisms of action of one of the world's longest and most used natural and synthetic drug. It may also provide an explanation for the protective effects of low-dose aspirin usage. Moreover, the identification of natural and synthetic SA derivatives with greater potency for inhibition of HMGB1 and GAPDH provides proof-of-concept that new SA-based compounds with high efficacy are attainable.

Auxin acts a self-organizing hierarchical hormone in plants

Yang, Z., Department of Botany and Plant Sciences, Institute of Integrated Genome Biology, University of California.

Auxin regulates plant growth and development at all developmental stages. The TIR1/AFB-based auxin signaling pathway that regulates auxin-induced gene expression has been well established, but this pathway does not account for the action of auxin that is independent of transcriptional regulation. The mechanisms underlying the non-transcriptional auxin responses are poorly characterized, and the relationship between the transcriptional and non-transcriptional auxin signaling pathways is unknown. We have been using Arabidopsis leaf pavement cells as a model system to investigate the mechanisms of auxin action. Pavement cells develop interdigitated lobes and indentation to form the puzzle-piece cell shape. Auxin is required and sufficient for the formation of the pavement cell interdigitation. We recently found that this process is regulated by the cytoplasmic auxin signaling pathways that Auxin-Binding Protein 1 (ABP1), the TMK subfamily receptor-like kinases, and ROP GTPases. These pathways interact with each other in a self-organizing manner to locally coordinate the formation of interdigitated lobes and indentations within and between pavement cells. In addition, the TIR1-based transcriptional auxin-signaling pathway is required for the generation of a global auxin signal that coordinates pavement cell interdigitation throughout the entire leaf epidermis. These findings support the hypothesis that auxin acts as a self-organizing hierarchical signal to regulate the interdigitated pattern of pavement cell morphogenesis in Arabidopsis leaves. This work is supported by US National Institute of Health to ZY (GM081451).



Regulation of fruit quality traits: using minor crops to look beyond temperate fruit models.

Ferguson, I., Plant & Food Research The New Zealand Institute for Plant & Food Research Limited.

Most of our knowledge on the development of fruit quality traits, and postharvest responses impacting on fruit quality, has come from decades of research on temperate crops. However, fruit which traditionally have been called minor crops, occupy an increasingly large place in global fruit production, for both domestic consumption and export. Most of these are tropical and subtropical and almost all have poor postharvest resilience, particularly sensitivity to low temperature. Fruit crops such as loquat, Chinese bayberry, mangosteen, and persimmon provide novel information on molecular regulation of traits such as texture changes, particularly lignification, colour development, both pre- and postharvest, taste and aroma, and postharvest responses to low temperature stress and ethylene. Some of these will be described, both in relation to control of trait development, and impact on storage life and postharvest fruit quality.

Breeding sweet cherry varieties adapted to climate change and producing high-quality fruits.

Quero-García, J., Barreneche, T., Beauvieux, R., Campoy, J. A., Christmann, H., Fouilhaux, L., Joly, J., Le Dantec, L., Richard, L., Vimont, N., Wenden, B., Dirlewanger, E., UMR1332 Biologie du Fruit et Pathologie (BFP), Equipe Adaptation du Cerisier au Changement Climatique (A3C) INRA Centre de Recherche de Bordeaux.

Sweet cherries are highly appreciated fruits for their taste and nutritional value. However, producing high-quality cherries in an economically efficient manner remains an important challenge. In particular, sweet cherry is a highly vulnerable species face to climatic conditions, even more in the context of global warming. Indeed, an increase of autumn and winter temperatures will impair the correct satisfaction of chilling requirements for sweet cherry flowering. Moreover, higher temperatures at spring will provoke an advance in the bloom dates and subsequently an increased risk of frost damage. Sweet cherry fruits can also be highly damaged by rain-induced fruit cracking. It is hypothesized that climate change will entail a higher frequency of storms during spring and summer, which could lead to higher production losses due to cracking. For all these reasons the A3C team aims at deciphering the genetic determinism of agronomic traits underlying the adaptation of sweet cherry to climate change. In parallel, a breeding program is led by A3C team and results from research are applied through marker-assisted selection (MAS) strategies. Quantitative Trait Loci (QTL) detection studies have been conducted over the last eight years on many important sweet cherry agronomic traits by working with three mapping progenies, issued from the crosses 'Regina' x 'Lapins', 'Regina' x 'Garnet' and 'Fercer x X'. Candidate genes (CG) co-localizing with these QTLs have been identified, either through functional or expressional approaches. The validation of the most promising CGs has been initiated by expression studies (qRT-PCR) and association genetics. Finally, modelling approaches are being implemented in order to assist breeders with the construction of ideotypes. An overview of the main A3C team scientific results will be presented, as well as their integration in new sweet cherry breeding strategies.



SYMPOSIUM

Symposium
Systems and Synthetic Biology
Chair: Dr. Rodrigo Gutiérrez

Quantitative phosphoproteome analysis in response to nitrate in Arabidopsis roots.

Vega, A^{1,3}, O'Brien, J.³, Álvarez, J.³, Rivera, E.³, Zhouxin, S.², Briggs, S.², Gutiérrez, R.³, ¹Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica De Chile. ²Division of Biological Sciences University of California, San Diego. ³Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.

Nitrate is one of the most important nitrogen sources for plants. Nitrate acts as a signaling molecule that modulates the expression of a wide range of genes, with an impact in plant physiology, metabolism, growth and development. Although the transcriptional responses induced by nitrate in roots have been comprehensively described, molecular components of nitrate-signaling pathways are yet to be identified. Signaling pathways often involve post-translational modification of their components. Protein phosphorylation stands out as a major modification in signaling pathways, affecting protein function, protein-protein interactions and in general signaling network function. As a first step to identify components involved in nitrate signaling, we performed quantitative time-course analyses of the Arabidopsis root phosphoproteome in response to nitrate by tandem mass spectrometry (MS/MS). The large-scale analysis of proteins by MS/MS combined with liquid chromatography offers the opportunity to concurrently identify proteins and discover differences in protein abundance on a global scale. We identified peptides with change in their phosphorylation level at 5 min after nitrate treatments (fast responses), that include signaling associated proteins as well as plasma membrane proteins related to nitrate and auxin transport. Our data provide new insights into signalling events underlying nitrate responses in plants. * These authors contributed equally to this work

Sponsored by Nucleo Milenio BSSV NC 130030, Fondap CRG 15090007, Howard Hughes Medical Institute, Fondecyt-11110095, Fondecyt-3140374, Fondecyt-1141097.



Defining morphogenetic mechanisms in multicellular systems.

Nuñez, I¹, Matute, T¹, Del Valle, I¹, Rudge, T², **Federici, F.**³, ¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Universidad Católica de Chile. ²Escuela de Ingeniería, Facultad de Ingeniería, Pontificia Universidad Católica. ³Genética Molecular y Microbiología Universidad Católica de Chile.

We use Synthetic Biology methods and biophysical models to develop mechanisms for engineering of patterns and morphologies in multicellular systems. We apply:

- transcriptional-translational design principles with the aim of avoiding DNA context effects
- combinatorial DNA assembly techniques that accelerates the design-build-test prototyping cycle.
- new characterization methods to parameterize gene expression
- novel methods for establishing arbitrary domains of cells and patterns.
- CRISPR/Cas9 to regulate cell growth and communication
- biophysical models that explicitly couple gene expression and mechanical properties of the cell

We combine these approaches with the aim of defining formalisms that make morphogenetic engineering more tractable, scalable and predictable. In the first part of my talk, I will describe the application of these approaches to the engineering of morphology in bacterial colonies and in the second part, I will show advances in the development of plant specific tools that will be essential to transfer these metaphors to plant systems (work in collaboration with Haseloff, Gutierrez, Pollak and Rudge).

Atbzip60 regulates gene expression in a upr independent manner under osmotic stress conditions in *Arabidopsis thaliana*.

Moreno, A., Mitina, I¹., Sandoval-Ibañez, O¹., Parra-Rojas, J¹., Henriquez-Valencia, C¹., Carrasco-Valenzuela, T¹., Meneses, C¹., Orellana, A¹., ¹Centro de Biotecnología Vegetal, FONDAP Centro de Regulación del Genoma, Facultad de Ciencias Biológicas, Universidad Andrés Bello.

AtbZIP60 is a transcription factor associated to the unfolded protein response (UPR). It has been described that AtbZIP60 messenger RNA is processed during UPR activation by IRE1, a transmembrane protein that could sense the accumulation of unfolded proteins in the endoplasmic reticulum. In addition, several abiotic and biotic stress conditions can activate the UPR during plant life cycle. Moreover, in some particular abiotic stress conditions such as salt and osmotic stresses, an important role for AtbZIP60 has been proposed, given the fact that overexpression of this gene led to an enhanced tolerance to these conditions. Nevertheless, the processing of AtbZIP60 does not take place under these abiotic stresses, posing a question regarding the role of AtbZIP60 into the response to these environmental cues. Our work reveals that an undescribed nuclear form of AtbZIP60 could be detected under osmotic stress conditions. The generation of this form does not depend on the processing of AtbZIP60 mRNA by IRE1; instead it depends on the action of RHOMBOID proteases as depicted by the use of a RHOMBOID protease inhibitor and the presence of a putative RHOMBOID protease cleavage site on AtbZIP60 transmembrane domain. Interestingly, we determined by transcriptomics and chromatin immunoprecipitation (ChIP) that the nuclear form derived from the proteolytic processing of AtbZIP60 could regulate the expression of genes different from the classical UPR markers described to be targets of the one encoded on the processed AtbZIP60 mRNA. Our data suggest that regulation of UPR in plants is more complex process than expected.

Sponsored by FONDAP CRG 15070009; Basal PFB-16.

Members of the BTB gene family negatively regulate nitrate uptake and nitrogen use efficiency in *Arabidopsis thaliana* and *Oryza sativa*.

Araus, V¹., Vidal, E²., Puelma, T³., Alamos, S⁴., Gutiérrez, R. A.²., ¹Departamento de Genética Molecular y Microbiología/ Ciencias Vegetales, Ciencias Biológicas/ Agronomía e Ingeniería Forestal, Pontificia Universidad Católica De Chile. ²Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. ³Departamento de Ciencias de la Computación, Ingeniería, Pontificia Universidad Católica de Chile. ⁴Plant & Microbial Biology, Plant & Microbial Biology, University of California, Berkeley.

Development of genetic varieties with improved nitrogen use efficiency (NUE) is essential for sustainable agriculture. However, achieving this goal has proven difficult possibly due to the fact that NUE is a complex trait encompassing multiple physiological and developmental processes. We thought to tackle this problem by taking a systems biology approach to identify candidate target genes. First, we used a supervised machine-learning algorithm to predict a NUE gene network in *Arabidopsis thaliana*. Second, we used network statistics to rank candidate genes and identified BT2, a member of the Bric-a-Brac/Tramtrack/Broad (BTB) gene family, as the most central and connected gene in the NUE network. Third, we experimentally tested BT2 for a role in NUE by reverse genetic strategies. We found NUE decreased in plants overexpressing BT2 gene as compared to wild-type plants under limiting nitrate conditions. No difference was observed for bt2 mutant plants. However, NUE increased as compared to wild-type plants under low nitrate conditions in double mutant plants in bt2 and its closely related homolog bt1, indicating a functional redundancy of BT1 and BT2 for NUE. Expression of the nitrate transporter genes NRT2.1 and NRT2.4 increased in the bt1/bt2 double mutant as compared to wild-type plants, with a concomitant 65% increase in nitrate uptake under low nitrate conditions. Similar to *Arabidopsis*, we found that mutation of the BT1/BT2 ortholog gene in rice OsBT increased NUE by 20% as compared to wild-type rice plants under low nitrogen conditions. Our results demonstrate a conserved mechanism exist in *Arabidopsis* and rice to modulate NUE. BT gene family members (BT1/BT2 in *Arabidopsis* and OsBT in rice) are at the center of a gene network acting as negative regulators of gene expression, nitrate uptake and NUE. These results prompt BT genes are prime targets for future biotechnological strategies to improve NUE in crops

COPEC-UC 2012.R.022, FONDAPE CRG 15090007, Núcleo Milenio en Biología Sintética y Biología de Sistemas Vegetales NC130030, HHMI.

Symposium
Cell and Developmental Biology
Chair: Dr. Raúl Herrera

Moving along cell tracks leads plant development.

Norambuena, L., Biology Department, Faculty of Sciences, University of Chile.

Plants are able of sensing and respond to both short- and longer-term changes in their environment. As part of the adaptation process plants are capable of generating post-embryonic organs such as lateral roots and root hairs. Also special organs are developed for embryo maturation and seeds propagation. The development of these structures strongly relies on the correct location and organization of the components of the cell. Also it depends on protein relocation constructively or upon certain stimuli. This cell dynamics is mainly task of the endomembrane system which is essential for protein and membrane trafficking through the cell tracks. Using chemical biology, we have described an Arabidopsis lateral root formation mechanism induced by endocytic trafficking through late endosome towards the vacuole. This mechanism results to be distinctive from auxin-driven promotion of lateral root formation. Actually the molecular requirements as well as the establishment of a gene transcriptional program are distinctive between the two mechanisms. Furthermore the evidences point that endocytosis and trafficking to the vacuole also plays an important role under nutrient deprivation affecting root branching. On the other hand we have started a fruitful research on fruit development of the Chilean white strawberry *Fragaria chiloensis*. The role of proteins that traffics trough the endomembrane system has been subject of our interest. Due to the lack of cell biology and genetic tools on *F. chiloensis* we have started a characterization of some proteins involved on fruit development and hormone regulation. We are now in the process of unraveling their molecular function. Overall we aim to step forward in the comprehension of cellular mechanisms of plant development and their impact on plant physiology and the response to environmental challenges.

FONDECYT 1120289, Anillo ACT-1110 and Conicyt Graduate Students Fellowship



Genetic and chemical approaches for the global identification of targets of the histone acetyltransferase GCN5 in *Arabidopsis thaliana*.

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GENERAL CONTROL NON-REPRESSIBLE 5 (GCN5) appears to be an important histone acetyltransferase required for gene expression involved in many development pathways in plants and animals. Mutations in *Arabidopsis thaliana* GCN5 (AtGCN5) show various pleiotropic defects as a consequence of affecting the activity meristem activity. Although AtGCN5 plays an essential role in chromatin modification and transcriptional regulation, its mode of action is still not understood. Proteins involved in chromatin remodelling control the development of plants and animals through directly regulating the expression of specific developmental transcription factors. In this work, we have identified a set of potential direct target genes of AtGCN5 through a combination of chromatin immunoprecipitation/DNA sequencing (ChIP-Seq) and genome-wide transcriptional profiling using RNA-seq. This analysis revealed that AtGCN5 control directly the expression of genes involved in metabolic process, nutrient transport and transcription. Among these targets, we identified 7 transcription factors belonging to different families. Almost all of them have not been characterized in plants. In order to validate the targets of AtGCN5, qRT-PCR and reporter lines of several transcription factors has been monitored in mutants allele. In addition, we are using the absence or presence of the broad-spectrum Histone Deacetylase inhibitor and the GCN5-specific Histone Acetyltransferase inhibitor, that efficiently target aminoacids within the catalytic active site of the mammalian GCN5 enzyme. Functional analysis will reveal the role of these transcriptions factors in plant development and their genetic interaction with AtGCN5 in *Arabidopsis thaliana*.

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Study of the role of UDP-glcA transporters in the biosynthesis of non-cellulosic polysaccharides.

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The cell wall is a complex extracellular matrix mainly composed of polysaccharides. Non-cellulosic polysaccharides are synthesized in the Golgi apparatus by glycosyltransferases (GTs), which use nucleotide sugars as donors to glycosylate the nascent polysaccharides chains acceptors, which are exported to the extracellular space. UDP-glucuronic acid (UDP-glcA) is a key molecule involved in this process since it serves as precursor for the synthesis of several polysaccharides of cell wall, contributing to 50% of the biomass present in the extracellular matrix. Nevertheless exists a topological problem, as UDP-glcA is synthesized by UDP-glucose dehydrogenase (UGD) in the cytosol. This molecule needs to get into the Golgi apparatus lumen where the catalytic site of the epimerases and GTs are located. It has been shown that in this process Nucleotide Sugars Transporters (NSTs) are needed. To date, no UDP-glcA transporter has been identified so their role in non-cellulosic polysaccharides remains unclear. In this work we identified a family of UDP-glcA transporters located in Golgi apparatus. To determine their contribution to non-polysaccharides synthesis we studied 4 of 5 members of the UUATs family (UUAT1, UUAT3, UUAT4 and UUAT5) and determined their expression pattern using transcriptional fusions of promoters to GUS reporter gene. We confirmed UUATs are Golgi localized proteins and we analysed their contribution to non-cellulosic polysaccharides biosynthesis, analysing their cell wall composition on plants presenting altered expression levels of UUATs genes by High Performance Anion Exchange Chromatography (HPAEC) and we analysed xylan structure and composition by Polysaccharides Analysis by Carbohydrates Electroforesis (PACE).

Sponsored by Conicyt Fellowship, Fondap-CRG 15090007, Fondecyt N° 1151335 , Basal PB-16



Characterization of 4 pollen-specific Receptor-like kinases coding genes from *Arabidopsis thaliana*.

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Pollen grains are the male gametophyte of plants and thus are essential for plant reproduction and productivity. However, despite their biological and agronomical importance, little is known about the molecular mechanisms that regulate its development and function. In *Arabidopsis*, three cells compose mature pollen grains: a large vegetative cell and two small sperm cells engulfed in the cytoplasm of the vegetative cell. During fertilization, the vegetative cell must germinate and produce a pollen tube, a growing tip structure that directionally transports the sperm cells to the ovule to produce the double fertilization event. Currently, little is known about signal transduction pathways and molecular components involved in these processes. Using microarray data we have previously identified 4 genes encoding kinases proteins (PS-RLCK1-4, for POLLEN-SPECIFIC RECEPTOR-LIKE CYTOPLASMIC KINASE) that are expressed exclusively in last stages of pollen development, germination and tube elongation. To analyze the physiological relevance of these genes, we have generated transgenic plants expressing specific amiRNAs under the control of a pollen-specific promoter (LAT52) and we have analyzed pollen development and tube elongation in insertional mutant and transgenic plants expressing amiRNAs. Tube guidance was affected in *ps-rlck2* mutant and transgenic plants, suggesting a role for this protein in male-female interaction, specifically in guidance of the pollen tube into the micropyle. On the other hand, pollen tubes from *ps-rlck4* mutant and amiRNA silenced plants lacks of a callose plug suggesting a possible role in the positioning or synthesis of the callose plug. Also, we have determined the sub-cellular localization of each kinase protein using 35S:PSK:GFP constructions in agroinfiltration experiments in tobacco leaves and in pollen tube using endogenous and LAT52 specific promoter. Taken these together, we present 4 pollen specific receptor-like cytoplasmic kinases required for pollen development and tube elongation in *Arabidopsis*.

Funded by Fondecyt 1120766 and UNAB DI-74-12/R

**Symposium
Abiotic Stress**

Chair: Dr. Francisca Blanco

Abiotic stress tolerance in *D. carota*: crossregulation between carotenoids and abscisic acid.

Simpson, K., Acevedo, O., Fuentes, P., **Stange, C.**, Departamento de Biología, Facultad de Ciencias, Universidad De Chile.

In plants, carotenoids are pigments synthesized in plastids that participate in photosynthesis and protect cells against photo-oxidation given by its antioxidant feature. Carotenoids are precursors of phytohormones such as abscisic acid (ABA), involved in abiotic stress tolerance, among others. A key point in carotenoid biosynthesis regulation is the production of phytoene from geranylgeranyl pyrophosphate, reaction catalyzed by the enzyme phytoene synthase (PSY), the first committed step in the pathway. *Daucus carota* (carrot) accumulates large amounts of carotenoids, preferably α - and β -carotene, in the storage root. In carrot, two PSY paralogs have been described, of which DcPSY2 codifies for a functional plastidial enzyme that is mostly expressed in the mature storage root. In carrot only DcPSY2 is induced after ABA treatment. Moreover, transgenic DcPSY2 tobacco plants present a boost in carotenoids and a higher survival rate and ABA levels when cultivated in salt stress conditions. A 850pb of DcPSY2 promoter was obtained by genome walking. The in silico analysis indicates that DcPSY2 promoter possesses ABA-responsive elements (ABREs), suggesting the presence of an ABA responsive complex. DcPSY2 promoter characterization indicates that it responds to ABA treatment. Using a yeast one-hybrid assay we found 3 sequences belonging to the carrot AREB/ABF transcription factor family that bind to DcPSY2 promoter and activate the transcription of reporter genes. Finally, the expression analysis of these genes in response to ABA treatment, showed a significant induction of AREB3 in roots that correlates with DcPSY2 expression. Taken the results together we propose that ABA modulates the expression of DcPSY2 in carrot through AREB/ABF transcription factors leading to an increment in carotenoids and a better salt stress tolerance.

Fondecyt 1130245, Beca CONICYT 22130956



Salt stress response triggers activation of the jasmonate signaling pathway leading to inhibition of cell elongation in *Arabidopsis* primary root

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Salinity is a severe abiotic stress that affects irrigated croplands. Jasmonate (JA) is an essential hormone involved in plant defense against herbivory and in responses to abiotic stress. However, the relationship between the salt stress response and the JA pathway in *Arabidopsis* is not well understood at a molecular and cellular level. In this work we investigated the activation of JA signaling by NaCl and its effect on primary root growth. We found that JA-responsive *JAZ* genes were upregulated by salt stress in a *COI1*-dependent manner in the roots. Using a JA-Ile sensor we showed that activation of JA signaling by salt stress occurs in the meristematic zone and stele of the differentiation zone. This activation was dependent on JAR1 and proteasome function. We also found that the elongation zone (EZ) and its cortical cells were significantly longer in JA-related mutants (*AOS*, *COI1*, *JAZ3* and *MYC2/3/4* genes) compared with wild-type plants under salt stress, revealing the participation of the canonical JA signaling pathway. Noteworthy, osmotic stress, a component of salt stress, inhibited cell elongation in the EZ in a *COI1*-dependent manner. We propose that salt stress triggers JA-Ile accumulation in the roots leading to activation of the JA signaling pathway followed by transient inhibition of cell elongation in the EZ. Therefore, we demonstrate a crosstalk mechanism between the salt stress response and the JA pathway that regulates root growth in *Arabidopsis*.

CONICYT, FONDECYT/Regular [1120086]

Exogenous GABA application transiently improves the tolerance to root hypoxia on a sensitive genotype of prunus rootstock.

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In general terms, *Prunus* species are considered as moderately sensitive plants to root hypoxia. Previously, from a set of commercial *Prunus* rootstocks we identified to the plum-based rootstock 'Mariana 2624' and the cherry-based rootstock 'Mazzard F12/1' as the most tolerant- and sensitive-genotype to root hypoxia by waterlogging, respectively. Then, a study about the root transcriptome responsive to hypoxic condition revealed a transcript with high homology to a root-specific Glutamate decarboxylase of *Arabidopsis*, AtGAD1, among the set of early differentially expressed genes. A homology search on peach genome detected four putative GAD sequences. They exhibited a very high identity in their deduced amino acid sequences except in the C-terminal region. GAD isoforms showed organ specific expressions and different transcriptional responses to root hypoxia stress. GAD is the enzyme responsible for the biosynthesis of GABA, an amino acid that reaches high levels in response to stresses. We assessed the effect of exogenous GABA application over molecular and physiological parameters in both genotypes. The root hypoxia tolerant-genotype was unaffected by GABA supplementation. However, exogenous GABA triggered transient but substantive changes in 'Mazzard F12/1' plants under waterlogging, mainly represented by a modulation of the mRNAs abundance of some GAD isoforms, an improvement of photosynthetic parameters, smaller loss of chlorophyll content, lower levels of H₂O₂ and increased levels of endogenous GABA, providing a transient amelioration of the response of sensitive-genotype plants to hypoxia.

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The master coactivator npr1 is involved in the salt tolerance response by its interaction with BZIP17 and the transcriptional regulation of salinity stress response genes in *Arabidopsis thaliana*.

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In response to salt stress, bZIP17, a membrane-associated transcription factor that reside in the endoplasmic reticulum (ER) is activated by intramembrane proteolysis. The N-terminal bZIP component (bZIP17ΔC) is translocated to the nucleus, where it activates the expression of salt stress response genes. bZIP17 is homologous to tobacco transcription factor TGA1b, which interact with the transcriptional coactivator NPR1. NPR1 is the master regulator of salicylic acid (SA) signaling pathways during the establishment of plant defense response against biotrophic pathogens. In this work we show that low concentrations of SA promote the maintenance of membrane fluidity and seed germination in Col-0 plants treated with NaCl 100 mM. In contrast, the protective effect of SA under salt stress conditions is absent in bZIP17 and NPR1 mutants. Moreover by Yeast two hybrids and Bimolecular Fluorescence Complementation assay, we found that NPR1 interacts with bZIP17ΔC and additionally NPR1 is translocated to the nucleus in response to NaCl treatments. This was observed in roots and shoots. Furthermore, using qRT-PCR we found that induction levels of salt stress response genes in both mutants decreased after salt treatment suggesting that NPR1 and bZIP17 are required for the transcriptional regulation of these genes during salt stress. Finally, we performed ChIP assays to test the binding of NPR1 through bZIP17 to the promoter sequence of salt induced genes. Taken together our results demonstrate that SA signaling pathway is contributing to the salinity stress response through the interaction between NPR1 and bZIP17, which modulate the expression of salt stress response genes to increase the probability that plants adapt to live in unfavorable conditions such as salinity.

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Whole plant water potential and loss of hydraulic conductivity does not depend on stomatal control upon water stress: evidence from three grapevine varieties.

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Stomata have persisted as a critical element to avoid dehydration, as evidenced by their presence already in earlier terrestrial plants. Therefore, a great deal of attention has been paid to stomata considering that their functioning regulates the $\text{CO}_2/\text{H}_2\text{O}$ exchange in a dry environment. An important consequence of drought on the physiology of plants is the xylem cavitation and hydraulic conductivity loss; then, stomatal control would prevent such effect, alleviating the increasing tension within the xylem conduits upon water scarcity. Here, we report on three contrasting grapevine varieties responses to water shortages in a commercial vineyard: Cabernet Sauvignon (CS), Carmenere (C) and Syrah (S). The plants were irrigated from veraison onwards, supplying a range from 60 to 180 mm of water. CS and C had a higher correlation between whole plant conductivity and assimilation vs. stomatal conductance reductions compared to S, suggesting that the CS and C strategy against dehydration relay on stomatal responses, contrary to S. The latter variety had a narrower stomatal conductance range compared to CS and C. Correlation analyses reveals that S reached lower leaf water potential values but associated with higher stem water potentials than CS and C. Interestingly, the loss of hydraulic conductivity at harvest in S was lower than CS and C. Still, the three varieties maintained the same whole plant water potential. We conclude that even though S was less effective in stomatal control, was capable of achieving lower xylem tension than CS and C. The physiological convenience and threats of the observed responses to water constrains will be discussed.

Sponsored by Project Funded By Fondecyt 1140880

Symposium
Biotic Stress

Chair: Dr. Loreto Holuigue

Wrky7, -11 and -17 transcription factors, are repressors of unfolded protein response genes during the basal defense against bacterial pathogens in *Arabidopsis thaliana*.

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Plants have evolved sophisticated mechanisms that allow them to avoid pathogens. The recognition of pathogens triggers a signaling cascade, leading to the biosynthesis of salicylic acid (SA), reactive oxygen species (ROS) and callose. Furthermore, the defense response involves the transcriptional activation of pathogenesis related proteins (PRs). The accumulation of PRs generates reticulum stress triggering UPR (Unfolded Protein Response). However, constitutive activation of UPR may have harmful effects on the plant. In this context, WRKY7, 11 and 17 transcription factors play a key role in the regulation of the defense response since mutant plants lacking these factors are more resistant to pathogenic bacteria. Here we describe that double mutant plants (*wrky7/wrky11* and *wrky11/wrky17*) are more effective in establishing a defense response against bacterial infection (*Pst* DC3000) but its response to fungal (*B. cinerea*) and aphid (*M. persicae*) infestation is not altered. Additionally, double *wrky* mutant plants showed a greater amount of callose deposits and ROS in response to Flg22 treatment. Furthermore, our results indicate that after Flg22 or SA treatment the double mutant plants showed an increase in transcripts coding for UPR genes and *CalS12*, suggesting that WRKY7, -11 and -17 act as transcriptional repressors. Our results allow us to postulate a fine-tuning model of basal defense response regulation in *Arabidopsis*, including the negative control of gene expression associated with UPR and other key-defense response genes like *CalS12* that together control the physiological response of plant-pathogen interactions.

Transcriptome analysis reveals regulatory networks underlying differential susceptibility to *Botrytis cinerea* in response to nitrogen availability in *Solanum lycopersicum*.

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Nitrogen (N) is one of the main limiting nutrients for plant growth and crop yield. Despite its role as a nutrient, nitrate can also act as a signaling molecule that modulates gene expression of a wide range of plant processes. It is well documented that changes in nitrate availability, the main N source found in agricultural soils, influences a myriad of developmental programs and processes including plant defense responses. Plant N nutritional status influences their ability to respond effectively when challenged by different pathogens. However, the molecular mechanisms involved in N-modulation of plant susceptibility to pathogens are poorly characterized. In this work, we show that *Solanum lycopersicum* defense response to the necrotrophic fungus *Botrytis cinerea* is affected by plant N availability, with higher susceptibility in nitrate-limiting conditions. Genome-wide expression responses of tomato against infection by the fungus under contrasting nitrate conditions reveals that plant primary metabolism is affected by the fungal infection regardless of N regimes. This result suggests that differential susceptibility to pathogen attack under contrasting N conditions is not explained by a metabolic alteration. We used a systems biology approach to identify the transcriptional regulatory network implicated in plant response to the fungus infection under contrasting nitrate conditions. Interestingly, hub genes in this network are known key transcription factors involved in ethylene (ET) and jasmonic acid (JA). Our results provide insights into potential crosstalk mechanisms between necrotrophic defense response and N status in plants.

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Kiwifruit canker disease, deciphering the molecular basis of a worldwide problem.

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Kiwifruit (*Actinidia* spp.) is an important crop for the economy of several countries. In terms of production and exportation this industry is led by Italy, New Zealand and Chile. Currently, the kiwifruit is suffering from a disease produced by the attack of the hemibiotrophic bacteria *Pseudomonas syringae* pv *actinidiae* (Psa), which severely affects kiwifruit production worldwide. Psa is able to infect plant tissues, multiply and move systemically through the leaves and shoot vasculature until the whole plants is infected, producing its death. Several factors, such as the virulence of the Psa strains, the genetic background of the plants, the growth conditions, among others, might contribute to the spread of this disease. To have a better understanding of the Kiwifruit bacterial canker disease in Chile, we are evaluating several physiological and molecular parameters that will help us to define on one hand the response of the kiwifruit cultivars to Psa and secondly the characteristics of the Psa Chilean strains. We have identified, amplified and sequenced putative defense and housekeeping genes in 6 different kiwifruit varieties, in order to quantify the expression of the defense genes after Psa infection. Concerning the characterization of Chilean Psa strains, we have site-directed mutagenize 2 independent Chilean isolates in putative effector genes conserved in *Pseudomonas* spp., in order to evaluate their contribution to the development of the disease.

This work is founded by FONDECYT 1141029

Identification and characterization of isochorismate synthase 1-interactor proteins in *Arabidopsis thaliana*

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Salicylic acid (SA) is a phytohormone widely known for its role in the regulation of plant defense responses. SA accumulates in response to a variety of biotic and abiotic stress conditions, such as pathogen infections and UV-C light exposure. In *Arabidopsis thaliana* the main source of SA under these conditions is a chloroplastic pathway in which isochorismate synthase 1 (ICS1) is, so far, the only enzyme known to be involved. However, it is known that in some bacteria ICS1 forms a complex with other enzymes involved in SA biosynthesis, therefore, we hypothesized that ICS1 could work in a similar way in plants. To test this possibility, we performed immunoprecipitation of ICS1 and used mass spectrometry to identify co-purifying ICS1-interacting proteins (IIPs). In this analysis, we found a large number of peptides from a protein family that was previously described as mitochondria-localized. Here we report that IIPs also localize in chloroplasts, similar to ICS-1. We evaluate a mutant line lacking the major IIP isoform under UV-C treatments and *Pseudomonas syringae* AvrRpm1 (Pst AvrRpm1) infections. In both conditions the levels of SA as well as the levels of PR1 protein (coded by a SA-regulated gene) are reduced in mutant compared to WT plants. This IIP mutant plant also shows higher susceptibility to Pst AvrRpm1 infection. This evidence suggests that IIP could be involved in the regulation of both the accumulation and the response mediated by SA in *Arabidopsis thaliana*.

Sponsored by FONDECYT (1141202) And Millenium Nucleus Center For Plant Systems And Synthetic Biology (NC130030)

Characterization of the gene *FcCYS1* encoding a putative cystatin proteinase inhibitor in *Fragaria chiloensis*.

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Plants are constantly affected by insects, necrotrophic and biotrophic pathogens, leading to hormone-induced activation of defense mechanisms. In this sense, salicylic acid (SA) activates the response mechanism to biotrophic pathogens, whilst jasmonic acid (JA) and methyl jasmonate (MeJA) activate the response to necrotrophic pathogens and insects. These hormones induce expression of genes encoding Pathogenesis Related proteins (PR). *Fragaria chiloensis* is less sensitive than the commercial strawberry *Fragaria x ananassa* to the infection caused by the fungus *Botrytis cinerea*. Our research has identified a type of PR gene (*FcCYS1*) that encodes for a cystatin protein whose molecular function is the inhibition of proteases. The amino acid sequence of *FcCYS1* has high identity to other *Fragaria* CYS sequences, with two noticeable protease domains. Molecular modelling of *FcCYS1* reveals a conserved 3D structure, and folding similar to other plant cystatins, with a characteristic helix-sheet fold and the conserved residues for biological function. Additionally, *FcCYS1* is expressed in different vegetative as well as reproductive organs of *Fragaria chiloensis*. This suggests that *FcCYS1* could be involved in the inhibition of endogenous proteases in different physiological processes. *FcCYS1* responds transcriptionally to the application of SA and MeJA, suggesting that *FcCYS1* could be involved in the response against pathogen attack. Furthermore, *FcCYS1* is differentially expressed in achenes at different stages of fruits development, suggesting an additional role in the prevention of degrading storage proteins that nourish the embryo during seed germination.

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Symposium Metabolism

Chair: Dr. Javier Canales

Sugar alcohol metabolism in plants.

Handford, M., Donoso, A., Olivos, K., Rojas, B., Aceituno, U., Bracamonte, M., Espinoza, A., Figueroa, A., Zapata, S., Norambuena, L., Centro de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

In Plantaginaceae and Rosaceae species, sugar alcohols like sorbitol are synthesised in source organs, phloem-translocated and then degraded in sink organs. They allow more efficient use of carbon, act as compatible solutes in abiotic stress and facilitate boron mobilisation. The main enzyme required for sorbitol synthesis is aldose-6-P reductase (A6PR), which reduces Glc-6-P to sorbitol-6-P, which is then converted to sorbitol. In sorbitol degradation, sorbitol dehydrogenase (SDH) is key, oxidising the sugar alcohol to fructose. Curiously, A6PR- and SDH-like enzyme activity is found in non-Plantagineaceae and non-Rosaceae species that accumulate sucrose, and in *Arabidopsis* (a sucrose-translocating Brassicaceae), we have identified several proteins with the structural features and substantial amino acid identity with known plant A6PRs and SDHs; we call these AtA6PR1, AtA6PR2 and AtSDH. These three genes are differentially-expressed in different *Arabidopsis* organs. Using GFP-tagged versions and specific antisera, we determined that these enzymes are cytosolically localised, consistent with bioinformatic predictions. In the presence of NAD⁺, recombinant AtSDH exhibits greatest oxidative activity with sorbitol, ribitol and xylitol as substrates. Furthermore, Molecular Dynamics simulations of AtSDH ternary complexes with these sugar alcohols indicate that differences in interaction with structural water molecules correlate well with the observed enzymatic parameters. Under normal growth conditions, *atsdh*- mutants develop as wild-type. However, under short-day conditions, the mutants are more resistant to dehydration stress, with a reduced loss of leaf water content when watering is withheld, and a greater survival rate on re-watering. Additionally, potential *ata6pr2*- mutants have been genotyped, and taken together our evidence indicates that limitations in the metabolism of sugar alcohols alter the growth of *Arabidopsis* and its response to different abiotic stresses. Finally, we are using the molecular and biochemical tools employed in this research to study metabolic aspects of the ripening process in a truly Chilean species: the white strawberry.

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Phosphorus resorption efficiency during leaf senescence in *Chilean Proteaceae* is modulated by acid phosphatase activity depending on phosphorus availability in the soil.

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The majority of species in the family Proteaceae thrive on ancient, phosphorus (P) impoverished soils in Australia (SWAP) and South Africa. Almost all species develop specialized, short-lived, cluster (proteoid) roots that mediate highly efficient P acquisition. Moreover, species typically display highly efficient photosynthetic P-use efficiency (PPUE) and P remobilization efficiency (PRE) from senescing tissues. In Chile there are six species of Proteaceae distributed across a range of climatic and edaphic conditions where young volcanic soils contain very high total but low available P. Compared with SWAP, the Chilean species similarly has cluster roots to acquire soil P with high efficiency, lower PPUE and PRE. Acid phosphatases (APases) play a pivotal role in scavenging P from organic P substrates during leaf senescence, but there is no data on the regulation of APase activity in senescing leaves of Chilean Proteaceae, and whether or not leaf P remobilization efficiency is related to P availability in the soil. The aims of the present study were to i) measure PRE and PPUE in leaves of several Proteaceae species and ii) determine leaf APase activities and leaf P remobilization efficiency in Proteaceae species from two sites where soils have relatively high concentrations (Anticura, site 1) or relatively low concentrations of available P (Cochamó, site 2). For *Embothrium coccineum* (notro) and *Lomatia ferruginea* (fuinque) leaf PRE was on average 2.8 times greater in plants from site 2 compared with that for plants from site 1. Furthermore, APase specific activity was significantly greater in leaves in clarified extracts of senescing leaves of plants from site 2 compared with the same species growing in soils with high P availability. Leaf APase specific activity is upregulated to a greater extent in Chilean Proteaceae species growing in soils of lower P availability. This likely facilitates the leaf P remobilization.

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Nitrate exerts a quick local effect on nodule activity and a mid-term systemic impact comparable to that of ammonium.

Cabeza, R., Ingeniería y Suelos, Ciencias Agronómicas, Universidad De Chile.

Nitrogen (N_2) fixation is a complex biological process tightly regulated by the higher plant. It is known that N_2 fixation in legumes can be impaired by the addition of external N sources. Recently, our group described the physiological and molecular changes in active nodules of *Medicago truncatula* after the addition of nitrate. Nitrate impacts negatively the nodule activity (measured as apparent nitrogenase activity [ANA]) after a couple of hours of application. Then, nodule activity decreases continuously. At the same time, a change of the transcriptome is observed, mainly related to a down-regulation of key genes encoding for nodule-specific cysteine-rich peptides (NCRs), leghemoglobins and nicotianamine synthase. NCRs are responsible for bacterium differentiation into bacteroid which is the state that triggers the expression of genes for nitrogenase building. We hypothesized that because the N_2 fixed in nodules is rapidly reduced to ammonia, the effect of N sources should be different. Nitrate and the direct products of its reduction are known to have various impacts on plant tissue and gene expression. As it is also the product of N_2 fixation, ammonium as an external source of N might not affect nodule activity directly and its effect on the N_2 fixation might more be a consequence of the N status of leaves, i.e. a systemic effect due to N satiety. In order to test our hypothesis, we measured continuously the ANA and analyzed the transcriptome of active nodules of *M. truncatula* after 4 and 28 h after ammonium and nitrate application. These results will be presented.



Integration of photoperiod and cytokinin metabolism through FLOWERING LOCUS T during dormancy transition in grapevine (*Vitis vinifera* L.) buds.

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In the vine (*Vitis vinifera* L) as in other woody perennial plants, preliminary evidences suggest that FLOWERING LOCUS T controls growth cessation and endodormancy (ED) by regulating the expression of the cell cycle genes (CCG) mediated by the APETALA1 and AINTEGUMENTA transcription factors. In order to elucidate whether VvFT effectively regulates the expression of CCG in vines, constructs containing VvFT, VvFT-GFP and GFP, under the control of the 35SCaMV promoter were transiently overexpressed in grape somatic embryos (SE) and the effectiveness of the transformation was assessed by fluorescence microscopy. The expression of VvAP1, VvAIL2 and CCG was analyzed in the genetically transformed grape SE, results showed that despite the high levels of VvFT expression in the transformed embryos (approx. 2000 folds), no significant changes in the expression of CCG was observed, however, the expression of VvAP1 increased significantly. Because, in *Arabidopsis* it has been reported that AtAP1 regulates the expression of cytokinin related genes, the expression of two cytokinins biosynthetic genes (VvIPT1, VvLOG1) and one cytokinin degradation (VvCKX3) gene was analyzed. Results showed that in SE overexpressing VvFT, the expression of VvIPT1 and VvLOG1 transcripts increased relative to control embryos. Moreover, the expression of VvFT, VvAP1, VvIPT1 and VvLOG1 transcripts was downregulated either by the transition of buds to ED and by the exposure of vines to short day (SD)-photoperiod, suggesting that VvFT could control the input and output of grapevine buds from ED by regulating the cytokinin levels.

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Symposium
Post-harvest Molecular Physiology
Chair: Dr. Alejandra Moya

Pectin Structure in Citrus Fruit.

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Pectin, a key component of plant cell walls, is a widely used, after extraction, as gelling agent in food industry. The structure of pectin is based on a backbone of polygalacturonic acid, which is called homogalacturonan (HG). Another major building block of pectin is rhamnogalacturonan I (RGI), which consists in alternating sequences of l-rhamnosyl and d-galacturonosyl residues ramified with side chains of arabinans, arabinogalactans and galactans. The length of both HG and RGI domains, the fine structure of RGI, and the HG/RGI ratio are likely to influence pectin functionality both *in vitro* and *in planta*.

Dry citrus peel (orange, grapefruit, lime and lemon) was subjected to nitric or oxalic acid extractions. We showed that both the type of acid and pH value are important parameters influencing pectin features. Pectin samples with different sub-domain structure and diverse macromolecular characteristics were obtained depending on extraction conditions and citrus sources. Notably, oxalic acid allowed efficient extraction of very well-preserved pectin macromolecules exhibiting particularly high molecular weight and intrinsic viscosity values. Variation in (i) pectin overall composition, (ii) macromolecular characteristics, (iii) HG/RGI ratio, (iv) HG length, and (v) RGI fine structure depending on extraction conditions and citrus sources will be presented and discussed.



Texture of berry grape, new insights and questions.

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One of the most important quality parameters of table grapes corresponds to berry texture that strongly affect the consumer preferences. Because of this, growers/exporter invest energy to maintain the organoleptic characteristics of fruit during long periods during postharvest storage. One approach is to understand the changes that occur on berries related with loss of firmness. For this reason, our laboratory carried out a comparative study of table grape varieties with contrasting firmness. Results reveled that firmer variety (NN107) obtained significantly higher levels of uronic acids and calcium in cell wall material of its berries compared to Thompson Seedless variety. Additionally, PACE experiments suggest differences in the structures of pectin, which together with immunohistochemistry results and PG and PME enzyme activities suggesting greater integrity and postharvest maintenance of cell wall pectin in the firmer variety. Transcriptome analysis by RNASeq of contrasting varieties showed differential expression of several genes involved in the metabolism of pectins and hemicellulose. In addition, in-vitro culture calcium experiments showed significant effect in berry firmness associated to pectin structure. Taking together our results provide new insights in the biology of grape berry texture.

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Understanding apple (*Malus domestica* borkh.) Sunscald development postharvest through metabolite profiling.

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Sunscald (=delayed sunburn) is a physiological disorder on apples that appears after cold storage as a consequence of photooxidative and heat stress pre-harvest and low temperature postharvest. Research has shown that severity and time of appearance is directly correlated with sun-damage (=sunburn) level of the fruit prior storage. Currently, there is no control for sunscald, therefore, metabolic profiling during its expression may lead to molecular markers to predict its appearance postharvest. Granny Smith apples were harvested with different sunburn levels (treatments) and stored in regular atmosphere (RA) for 120 days. Every 15 days peel samples were removed from apples of each treatment and flashed frozen with liquid N₂. Tissue (350 mg) was extracted with methanol (80%) and separated using C-18 columns and propanol to elute. Dried samples were reconstituted in 200uL methanol. Metabolites were analyzed using UHPLC-MS techniques. Data processing for non-targeted profiling was carried out using Mzmine v.2 and MetaboLyzer for statistical analyses. Non-targeted semi-polar metabolite profiles were statistically different between sun injury levels and sunscalded and non-sunscalded tissue. Fruit with sun injury had a higher number of metabolites associated with the fatty acid and linolenic acid metabolisms, ABC transporters, carbohydrate metabolism, and terpenoid backbones biosynthesis, among others. Specific flavonoids were well correlated with sunscald appearance. Among 18 targeted flavonoids kaempferol, kaempferol 1-3-glucosidase, rutin, quercetin, apigenin glucosidase, quercitrin, ferulic acid, luteolin 3-glucosidase were significantly higher in sunscalded versus non-sunscalded tissue.



Fruit development and functional properties of arrayan (*Luma apiculata*), a native berry of Chile.

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In recent years, there has been a growing interest in sustainably produced food that can provide beneficial effects to human health. As a result, many native fruits have been studied for their potential as a source of antioxidants and functional food. The arrayan berry (*Luma apiculata*) is a native fruit from South America that belongs to the Myrtaceae family. To elucidate and characterize the developmental process of this edible fruit, quality and physiological parameters, along with antioxidant capacity, were evaluated during four clearly defined developmental stages of the fruit. In addition, the potential health benefits of ripe fruit -vascular protection and antimicrobial activity- were evaluated. Fruit firmness slowly decreases during fruit development, whereas the solid soluble content/titratable acidity ratio (SSC/TA) increases significantly in the final stages of development. The measurement of low respiration rates and low ethylene production during growth and ripening suggested that the arrayan berry should be classified as a non-climacteric fruit. In addition, ripe fruit extracts show presence of flavonol and anthocynins, high ORAC value, vascular protection under high glucose conditions and inhibition of both gram positive and gram negative foodborne bacteria. The results obtained show that these endemic berry fruits have a promising potential as functional food.

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Symposium
Ecophysiology of Extreme Environments
Chair: Dr. Herman Silva

Physiological traits associated with drought tolerance in wheat

Del Pozo, A¹., Yañez, Alejandra¹., Tapia, Gerardo²., Castillo, Dalma²., Matus, Iván²., ¹Producción Agrícola, Facultad de Ciencias Agrarias, Universidad de Talca. ²Quilamapu INIA. Sponsored by Fondecyt 1150353

Plant phenotyping using physiological traits, as a complement to agronomic traits, can help in identifying key features that accelerate breeding for yield potential and performance under drought. In this work we examine the genotypic variability of leaf chlorophyll, stem water-soluble carbohydrate content at anthesis (WSCa) and maturity (WSCm) and carbon isotope discrimination ($\Delta^{13}\text{C}$), and their relationship with grain yield (GY) and other agronomical traits. The study was performed on a large collection of 384 wheat genotypes from Chile, Uruguay and CIMMYT, grown under contrasting water regimes in a Mediterranean environment: water stress (WS, rainfed), mild water stress (MWS, deficit irrigation) and full irrigation (FI). The average GY of two growing seasons was 2.4, 4.8 and 8.9 Mg ha⁻¹ under WS, MWS and FI, respectively. Chlorophyll content at anthesis was positively correlated with GY and the agronomical components. WSCa as well as the apparent WSC remobilisation, calculated as WSCa-WSCm, were positively correlated with kernel per spike (KS) and thousand kernel weight (TKW), but negatively correlated with numbers of spikes per m². The relationship between $\Delta^{13}\text{C}$ and GY was positive under FI and MWS but negative under WS. The positive correlation of $\Delta^{13}\text{C}$ and GY suggests that apart from severe water stress conditions the genotypes exhibiting higher water use are the most productive.



How to survive in the Blooming Atacama Desert? Lessons from *Cistanthe longiscapa*.

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The flowering desert is an ephemeral phenomenon that occurs when rainfalls are unusually high in the Atacama Desert, like in the “El Niño” years. Hundreds of species, mostly endemics, bloom, being *Cistanthe longiscapa* (Montiaceae) the dominant. This plant outstands by its great capacity to survive several months after it emerges and colonize diverse environment extensively. Our aim is to characterize the ecophysiological response of *Cistanthe longiscapa* in coastal and inland populations in order to understand the mechanisms that underlie their drought resistance strategy in different environments. We focus on the ecological and physiological mechanism, like leaf mass:area ratio (LMA), plant height, plant size, population density, photosynthetic rate (A_{max}), chlorophyll fluorescence, Isotopic composition, day/night Δ leaf acidity. Our results show that plants from coastal populations exhibit traits that favor water conservancy, like high LMA, smaller leaves, low A_{max} , and a trend to CAM photosynthesis. In contrast inland populations exhibit higher plant size, low LMA, high A_{max} and tends to C3-CAM intermediacy. We also observe reversal of the photosynthetic CAM traits in inland plants following mesic conditions of acclimation of the plants. These results suggest that *C. longiscapa* can make use of its great capacity of change their photosynthetic via (CAM to C3) to colonize broad environment and survive long periods of time during drought conditions, explaining in part their dominance pattern during the marvelous phenomenon of the flowering desert.

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Can ecophysiology really help us increasing crop performance under drought stress? Experience with arid zone fruit tree production systems in northern Chile.

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Plant ecophysiology integrates physiology and ecology, which is the ideal level of analysis for proposing technical solutions for farmers because they mainly work at the individual and/or community scale. Moreover, ecophysiology has a long history of studying the effect of abiotic stress on plant performance at different levels of organization: from tissues to plant communities; and has, therefore, become a powerful tool for predicting and mitigating the effect of such stress on agricultural production systems. Nonetheless, for ecophysiological studies to effectively produce solutions for the productive sector, their results and methodologies need to be properly up-scaled and simplified in order to meet farmers ability to use them and economic requirements. As with other disciplines, ecophysiology has often been 'driven by tools': favoring information generated with sophisticated and expensive equipment (eg. gas exchange) while neglecting variables which are assessed with simple tools (eg. growth). This has created an imbalance which hampers the possibility of using the published information at the farm level. We here present our experience working with the effect of mild and severe drought stress on the ecophysiology and the productive outcome of fruit trees under the extreme environmental condition of the Chilean arid zones. The integration of results at different levels of organization, in the aim of generating technological solutions, are discussed from the viewpoint of the requirements of the growers of different fruit tree species. We include examples of success and failure and attempt to identify the reasons for these contrasting outcomes. The importance of identifying the relevant agricultural problems we need to tackle, and the variables we need to assess for doing so, are pointed out. We also show that the traits which determine the success of fruit tree production under drought stress are highly variety and species-dependant and are not necessarily acquired with sophisticated equipment.

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Symposium
Molecular Breeding and Genetic Resources
Chair: Dr. Basilio Carrasco

Identification of candidate genes involved with fruit quality in peach [*Prunus persica* (L.) Batsch] using QTL analysis and deep sequencing.

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Peach fruit quality traits such as texture, flavor and juiciness are important for consumer acceptance. Maturity date (MD) also plays a role in the fruit-ripening process and is an important factor for marketing fresh fruit. On the other hand, cold storage produces a physiological disorder known as chilling injury where the most important symptom is a lack of juice in the flesh or mealiness (M). In this study we analyzed an F2 population obtained from a self-pollination of 'Venus' nectarine. Besides we analyzed an F1 population obtained from the cross between 'O'Henry' and 'NH-053'. Using the first population we built a linkage map with 1,830 SNPs, 7 SSRs and two slow-ripening (SR) morphological markers, spanning 389.2 cM distributed over eight linkage groups (LGs). The SR trait was mapped to LG4 and we compared the whole genome sequences of a SR individual and 'Venus' and identified a deletion of 26.6 kb containing ppa008301m co-localized with the SR trait. Three Quantitative Trait Loci (QTL) for MD were detected; they all co-localize on LG4 between 31.0 and 42.0 cM. Four co-localizing QTLs on LG4 between 33.3 and 40.3 cM were detected for M, explaining 34% of the phenotypic variation. We identified five and nine candidate genes for MD and M from the QTL regions, respectively. Our results suggest that the transcription factors ANAC072 and ppa010982m are CGs for both traits. LG4 contains a cluster for genetic factors that possibly regulate M and MD, but functional validation is necessary to unravel the complexity of genetic control responsible for fruit traits. Using the second population, we constructed a genetic map by pseudo-testcross strategy and we expect performed a QTL analysis to identify candidate genes for fruit quality traits.

This project was supported by Fondecyt 11121396, Genoma 4 G13i10005 and CORFO 09PMG-7240.

Differential expression of phytoene synthase (psy) during durum wheat (*Triticum turgidum* L. var. durum) grain filling affects semolina yellowness distinctly.

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Phytoene synthase (Psy) is an essential synthesis gene influencing semolina (milled, coarse durum wheat product used as raw material for pasta production) yellowness (SY) while catalase (*Cat3-A1*) is a catabolic enzyme that causes color loss. The main objective of this study was to evaluate the expression of *Psy-A1*, *Psy-B1* and *Cat3-A1* as candidate genes for SY, using a population of 94 durum wheat genotypes differing in SY. Real Time-qPCR experiments of a subset of 12 genotypes showed greater expression of *Psy-A1*, which specifically peaked at 35 days post-anthesis (DPA). At this time, *Psy-A1* was 21-fold higher expressed in the high-yellowness relative to the low-yellowness genotypes. *Psy-B1* and *Cat3-A1* peaked at 21 DPA, suggesting different timing of expression of these genes during grain filling. The contribution of *Psy-B1* was relatively higher in the genotypes showing low SY than those with high SY. *Cat3-A1* did not exhibit clear differences in transcript accumulation throughout grain filling. We conclude that *Psy-A1* has a major role in determining SY, particularly in genotypes with high levels for this trait.

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The genetic architecture of fruit quality in table grape.

Mejía, N., Ocarez, N., Núñez, R., Morales, I., González, M., Hinrichsen, P., Defilippi, B., Mejoramiento y Biotecnología INIA CRI La Platina.

Grapevine (*Vitis vinifera* L.) is one of the first crops domesticated worldwide and Chile is the first fresh table grape exporter thanks to the excellent crop management focused in the production of high quality and the availability of successful varieties created by foreign breeding programs. Nowadays these programs sell plants to specific clubs or through production quota. To retain the global standing INIA has developed a breeding program that has to produce and evaluate at least 10,000 individuals per season. To improve breeding efficiency we developed a genomic strategy through QTL analysis based in a F1 progeny ($n > 1,000$), high-throughput SNP genotyping and deep phenotyping of quality-related traits: seedlessness, berry size, firmness, aroma, color, ripening date, texture and cluster size. We built a high-density map with 3,909 SNPs that reduced distance between markers down to 116 kb. QTL analysis revealed the complex architecture of quality related traits: 5, 6, 11, 8, 6, 4 and 3 QTLs were identified for cluster size, berry size, texture, firmness, ripening date, seedlessness and color respectively. Most of the QTLs detected have small effects over phenotypic variance; however, the use of a combination of SNPs for each trait captures most of this variance, enabling the design of haplotype-assisted selection. Identification of associated SNPs markers and the development and validation of intragenic markers will create the opportunity to improve the bases and efficiency of breeding and additionally provide novel genes involved in quality traits that generate unique studies along this large population.

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Biotechnology tools to assist the domestication of *Gaultheria pumila*, a native Chilean Ericaceae: Preliminary results.

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Gaultheria pumila (Chaura) is a small native plant that grows in the Andes Mountains in Chile and Argentina. No previous studies regarding the Chilean Ericaceae family have been conducted before; therefore, this project will be a pioneer attempt to identify the suitability of native small fruit from this family as an agricultural product. The expected results of our main project is the identification of candidate genotypes for selection based on their potential productivity. Despite the species, as well as other representative from the genus, like *Gaultheria mucronata*, could have a large commercial potential as a berry small fruit, it is necessary to assess its genetic diversity. It would permit the identification of the most interesting individuals and populations to establish a domestication program. Molecular markers can play a very important role in the evaluation and management of the genetic diversity for native species with agronomic potential since they can give information about the genetic composition of the populations, the degree of diversity and heterozygosis. Furthermore, a complete description of fruit morphometric characteristics and their variations among the different ecological conditions where the species inhabit will also be studied. Propagation methods assisted by biotechnological approaches will be available at the end of the project helping to establish an *in vitro* germplasm bank, but also to accelerate the propagation of interesting accessions selected due their commercial potentials. A germplasm bank with 30 accessions under two different ecological conditions (the Maule Valley and the Andes Mountains) is currently under construction. This report will inform about the preliminary results obtained after two years of research with this species, informing about the isolation of SSR with highly informative and the establishment of *in vitro* multiplication protocols. Also, chemical composition of chaura fruits will be informed.

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Poster I

Systems and synthetic biology

1.- Genome-wide mapping of regulatory DNA in response to nitrate in Arabidopsis root

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Our understanding of plant gene regulation is constrained by our limited knowledge of plant cis-regulatory DNA and its dynamics in response to environmental cues. We used DNaseI hypersensitivity-seq (DNase-seq) to map regulatory DNA sequences in Arabidopsis genome in response to nitrate, a potent signal that control global gene expression, shaping plant growth and development. Integrating transcriptomics, chromatin immunoprecipitation with RNA Pol II antibodies coupled to sequencing (ChIP-seq) and DNase-seq data we uncover transcriptional regulatory landscape dynamics, disclosing hundreds of nitrate sensitive elements and enabling mapping of key TF regulatory circuits underlying this fundamental response. This information is helping to uncover the transcriptional mechanism controlling nitrate response and will facilitate developing strategies to improve N-use efficiency and enhance plant production.

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2.- *In vitro* germination of *Calceolaria corymbosa* (Calceolariaceae), native vulnerable species of Chile.

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The genus *Calceolaria* L. consists of about 250 species distributed from Mexico to southern Argentina. It has great economic importance for their ornamental interest. *Calceolaria corymbosa* Ruiz & Pav. is one of the 86 species in Chile, that is listed as being vulnerable native species. In this context, the purpose of this study was to establish a protocol for *in vitro* propagation of *C. corymbosa* from seed. Disinfection consisted of a solution of 70% ethanol followed by washing with 30% sodium hypochlorite. The seeds were established in MS medium with ¼ macronutrients, and held for 14 days in darkness, to further evaluate the effect of temperature and light on germination. The results shows that germination began after 35 days, presenting the 50th day, germination rates of 100% in treatment maintained in darkness and 22 + 1°C. In proliferation stage, different concentrations of cytokinins and constant concentration of auxin was used, being the optimal 1.0 mg L⁻¹ BAP and 0.1 mg L⁻¹ IBA. These preliminary studies, can demonstrate the feasibility of using *in vitro* culture techniques for the propagation of *C. corymbosa*, in order to encourage *ex situ* conservation of this species, listed as vulnerable.

3.- Spatio-temporal gene regulatory networks in response to nitrate in Arabidopsis roots

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Genome-wide transcriptional analyses have provided an impressive catalog of N-responsive genes participating in a wide range of processes. Despite this solid groundwork, the molecular mechanisms involved in regulating and coordinating N-responses at the organism, organ or cellular level are largely unknown. In addition, the majority of these genome-wide studies were performed at defined time points in whole plants or organs impairing our understanding of cell-specific regulatory gene networks and how they interact to coordinate organ responses over time. In this research, we mapped and characterized dynamic N-regulatory networks acting within cell types in Arabidopsis roots. We combined cell-sorting, transcriptomics analysis and integrative network bioinformatics to identify cell type specific regulatory gene networks controlling root responses to nitrate over time. We have been able to identify genes with specific regulation patterns at cell-type level over timewith tissue specific gene clusters showing significant enrichment for different gene ontology terms. Analysis of predicted gene regulatory networks revealed that network complexity and specificity varies in cell types and over time. Regulatory networks are specific at earlier time points but common to all cell types at later time points. These findings suggest a fine tune control over gene responses to N at both cell-type and temporal levels.

Núcleo Milenio BSSV NC130030, FONDECYT 1141097, FONDAP CRG 1509007, HHMI.

4.- Quantitative phosphoproteome analysis in response to nitrate in Arabidopsis roots

Vega, A^{1,2}., O' Brien, J. A²., **Fredes, I².,** Álvarez, J. M²., Rivera, E²., Gutiérrez, R. A²., ¹Ciencias Vegetales, Agronomía e Ingeniería Forestal, Pontificia Universidad Católica De Chile. ²Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.

Nitrate is one of the major N sources for higher plants in agricultural soils. Nitrate activate a signaling pathway that lately modulate the expression of a wide range of genes with impact in growth, metabolism and plant development. Although the transcriptional responses induced by nitrate have been widely studied, our work is focused on the elucidation of new protein components involved in nitrate signaling. Most signaling pathways components regulate protein activity, localization or interaction through phosphorylation. Hence, we analyzed the impact of nitrate in the global dynamics of phosphorylation in Arabidopsis roots through quantitative time-course analysis by mass spectrometry (MS/MS) combined with liquid chromatography. From this global approach, we identified proteins with changes in their phosphorylation status after 5 and 20 minutes of nitrate treatment (fast and late responses, respectively). This analysis shows phosphorylation changes in a wide range of components including signaling associated proteins, kinases and importantly, key regulators of gene expression. Most changes triggered by nitrate occur in one of the periods of time and possibly reflect the reversibility of phosphorylation in response to a stimulus. Our data provide new insights into signaling events underlying nitrate responses in plants.

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5.- Nrt1.1 and plcare involved in to increase of ip_3 and $[Ca^{2+}]_{cyt}$ causing induction in the expression of nitrate-responsive genes in *Arabidopsis thaliana* roots.

Ibarra, C¹., Riveras, E¹., Alvarez, JM¹., Vega, A^{1,2}., Gutiérrez, R¹., ¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. ²Ciencias Vegetales, Facultad de Agronomía e Ingeniería forestal, Pontificia Universidad Católica De Chile.

Nitrogen (N) is an essential macronutrient for plants growth and development. Besides its nutritional role, N can also acts as signal that regulates the expression of hundred of genes, modulating plant metabolism, physiology, growth and developmental processes. However, the molecular components involved in N sensing and signaling remain still poorly characterized. With this aim, we show that the nitrate transceptor NRT1.1/AtNPF6.3 sense nitrate availability, inducing an inositol 1, 4, 5- trisphosphate (IP_3) and cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) increase in the cytoplasm. These signals cause an induction in the expression of nitrate-responsive genes in roots of *Arabidopsis* in response to nitrate treatment. This effect was abolished using U732122, a pharmacological inhibitor of phospholipase C (PLC), but not for the nonfunctional analog U73343. In addition, the expression nitrate-responsive genes were severely affected in a mutant of this nitrate transceptor or in pre-treatments with inhibitor of PLCs or Ca^{2+} channel blockers. Our finding suggest a model where nitrate is sense by NRT1.1 and a phospholipase C activity mediates the increase of Ca^{2+} required for changes in expression of canonical nitrate-responsive genes. In *Arabidopsis*, it had been described 9 PLCs genes, which are transcriptionally induced by various different environmental stimuli. Experiments are underway to understand the role of these *AtPLCs* genes in response a nitrate treatment in *Arabidopsis* roots.

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6.- Linking transcriptome states and biological processes using information attached to gene pairs.

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It is widely accepted that organismal changes during development or in response to environmental cues are mediated by changes in global gene expression. Conversely, albeit less conventional, transcriptomes reflect the state of the system under specific conditions. Transcriptome states are typically bounded by the architecture of gene networks, however, these boundaries in transcriptome states have not been formalized neither structures systematically explored for any biological system. Throughout this work we sought to model restrictions that bind transcriptomes of an organism and from these boundaries learn transcriptome structures and gene function. We uncovered inherent restrictions in gene expression at the genome level as well as clues for mechanisms that are essential for an organism to change during development or in response to perturbations in experimental conditions using a novel entropy framework and publicly available transcriptome data for *Arabidopsis thaliana* and *Saccharomyces cerevisiae*. We found that low entropy gene networks allows for functional gene network inference, interestingly, using this method, we found gene pair relationships that were not attained by methods such as correlation analysis or mutual information, and were experimentally validated. Our distinct conceptual framework to understand transcriptomes provides new insights to old data and highlights candidates that mediate adaptation to environmental perturbation for further studies.

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Cell and Developmental Biology

7.- De novo transcriptome analysis of *Daucus carota* modified root under dark or light grown conditions

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Carotenoids are lipophilic pigments synthesized by all photosynthetic and some non-photosynthetic organisms. In plants, these compounds are involved in photosynthesis, photo-oxidative damage protection and phytohormone synthesis. *Daucus carota* (carrot) is one of the few plant species able to synthesize and accumulate carotenoids in the modified root. The orange varieties are the most worldwide consumed, because of its high levels in carotenoids, mostly β - and α -carotene. Four weeks old carrot roots are thin and pale. At eight weeks of dark culture they begin to swell and accumulate carotenoids. Previously, we determined that light inhibits secondary root growth and carotenoid accumulation suggesting the existence of mechanisms that regulate both processes. In order to understand the role of light and darkness in the modulation of genes that regulate carotenoid synthesis and carrot root development, we performed RNA-Seq analysis from eight weeks old dark and light grown carrot roots. Using this highthroughput sequencing methodology, we obtained a transcriptome model with 63,124 contigs by *de novo* assembly, from which 18,488 are differentially expressed (DEG) between the two experimental conditions. About 0.4% of them belong to genes involved in light perception, 0.7% in hormone response, and about 1% might correspond to transcription factors. Interestingly genes such as *Phytochrome-Rapidly Regulated 1 (Par 1)*, *Alfin-like* and *Orange (Or)* are preferably expressed in dark roots. Enrichment analysis of GO terms with DEGs genes, validation of the transcriptome model and DEG analysis through qPCR will be also presented to verify whether these genes are involved in the light-machinery that regulates the synthesis of carotenoids and carrot modified root development.

Sponsored by CONICYT, FONDECYT/Regular N° 1130245

8.- Zinc-finger transcription factors possibly involved in pollen development in *Vitis vinifera*

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Vitis vinifera cv. Carménère is one of the most important grapevine cultivars for the Chilean wine industry. This variety shows high tendency to parthenocarpic fruit development (PFD), i.e. a reproductive disorder originated by defective ovule fertilization due to a failure in pollen tube growth, which leads to development of low quality seedless grapes. A straight correlation was observed between the extent of the PFD and the occurrence of morphologically abnormal pollen grains which are unable to germinate and do not develop the pollen tube. Reasons underlying this phenomenon in grapevine are not well known but uncoupling of the pollen development regulation could be inferred. In *Petunia x hybrida* and *Arabidopsis thaliana* this process is modulated by several anther and pistil-specific zinc-finger transcription factors (*PhZPT* family and *AtDAZ1/AtDAZ2*). Silencing of the respective genes leads to alteration in pollen morphology and pollen abortion. Little is known about the functional role in anther and pollen development of these proteins in grapevine. Through in silico analysis we found 72 zinc-finger proteins in the grapevine reference proteome, constituting the 0.2% of the putative proteins in this plant. Most of them contain typical transcription factor domains as the nuclear localization signal (NLS), DNA binding domain and an EAR repressor domain. Six sequences homologous to *PhZPT* genes and one to *AtDAZ* genes have been identified in grapevine (*VvZPT*-like and *VvDAZ*-like genes). Four of them show highest transcription level during flower anthesis, suggesting candidates probably involved in pollen development. Further studies are required for the characterization of these genes and their products in *Vitis vinifera* and to determine their role in pollen development.

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9.- Study of auxin accumulation in pollen and its role on stamen maturation in *Arabidopsis thaliana*

Gutierrez, L., Leon , G., Centro de Biotecnología Vegetal, Ciencias Biológicas, Universidad Andrés Bello.

In *Arabidopsis* the first phase of stamen development involves morphogenesis, histospecification and pollen mother cells generation. Later, during the late phase of stamen development, microspores go through a division process in the anther, producing mature pollen grains, stamen filaments elongate and the anther dehiscence occurs to release pollen grains at anthesis. The phytohormone auxin plays a key role in coordinating many aspects of plant development, including flower development and maturation. The anther, pollen and pollen related tissues accumulate auxin in large quantities. Pollen maturation is a critical step in plant development for successful breeding and seed formation. Thus far, the role of auxin in this process remains poorly understood. To assess the contribution of auxin accumulated in pollen during the different stages of its development, and to determine if this hormone is important to the late stamen developmental processes, we generated transgenic plants expressing the auxin inactivator gene *iaaL* in a pollen specific manner and in different times of pollen development using tissue-specific promoters. These promoters were carefully selected from bibliography and their specificity was confirmed using the *uidA* reporter gene and GUS staining. The transgenic plants, expressing the auxin inactivator gene *iaaL* were generated in the DR5:GUS background for the analysis of the distribution of the hormone in the anther. Phenotypical analyses of the three main processes of stamen maturation (stamen elongation, pollen maturation and anther dehiscence) will be performed in the transgenic plants.

Fondecyt 1120766

10.- Time-course of growing grain dynamics of wheat (*Triticum aestivum* L.) allows to hypothesize plastic and elastic growth in cultivars contrasting in grain weight potential

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Grain weight (GW) is a key trait affecting grain yield, but the mechanisms controlling are still poorly understood. Studies hypothesized that: “pericarp imposes an upper limit to GW Potential (GWP) and grain growth could be conditioned by yours structures”. Our hypothesis indicates that the grain wheat has two growths, a plastic growth that determines the final grain size, followed by an elastic growth. The objective of this research was evaluated different component of dynamic grain growth, of two contrasting cultivars in GWP. Two spring wheat of different GWP (Bacanora and Kambara), were sown in plot of 370 pl m⁻² and 44 pl m⁻², under field conditions and split-split plot design with three replications. The experiment was carried out in the E.E.A.A. of the Universidad Austral de Chile (2012-13). From anthesis, 4 grains from the first 4 grain position (G1 to G4) of the central spikelets of 4 spikes were collected every 3-4 days. Grain weights and dimensions were recorded or calculate. At harvest, the same measurements were carried out in 20 grains from 10 spikes. Dry weight was registered after drying at 60°C during 48 hours. The time-course of the measured and calculated traits were fitted by using mono, bi, tri or tetra-linear regression analysis. The datas were analyzed by ANOVA and Tukey's test ($p < 0.05$). As previous studies, GW had differences between cultivars ($p < 0.05$). The highest values of dry and fresh weights and length, height, width and volume were recorded in Kambara. Replace di-lineal adjustment by other tri-lineal, showed a plastic growth to about 300 °Cd, followed by a plastic growth to physiological maturity (Bac G2 = 6.64 and 2.52 um °Cd⁻¹). This behavior is repeated in both genotypes and GP.

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11.- Auxin and jasmonic acid-mediated coordination of late stamen development in *Arabidopsis thaliana*.

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In plants like *Arabidopsis*, the floral meristem produces four successive whorls tissue, starting with the outermost whorl of sepals, progressing with the petals, a third whorl of stamens and finally the carpels. Specifically, the anther development can be classified in two stages: (1) the formation of the stamen primordia and (2) the late stamen development. The latter, consists in three phases: the anther dehiscence, pollen maturation and filament elongation. Coordination of this three processes contribute to a successful pollination at anthesis. It has been shown that jasmonic acid (JA) is involved in the control of this processes, as mutant defective in JA biosynthesis or perception shows indehiscent anthers, short stamens and are male-sterile. On the other hand, auxin has been shown to regulate early stages of stamen development and recent evidence suggest this hormone is also involved in late developmental stages by regulating jasmonic acid biosynthesis. Previously, our laboratory generates male-sterile plants using the bacterial RNase barnase under the control of pollen-specific promoters. Interestingly, stamen development was also affected showing short stamens and indehiscent anthers, phenotypes that were reverted with exogenous treatments with methyl jasmonate and the auxin IAA. Similarly, jasmonic acid biosynthesis mutant *opr3* have the same phenotypes. *opr3* mutants treated with methyl jasmonate reverts the male sterile, indehiscent and have short stamens phenotypes in this plant, while IAA treatments doesn't, suggesting that auxin acts through jasmonic acid to control the anther development in a pathway that also depends on pollen development. This work was funded by FONDECYT 1120766.

12.- The existence of a lateral root formation-program leading a pericycle cell fate determination promoted by the induction of cellular trafficking towards the vacuole

Morales-Herrera, S., Pérez-Henríquez, P., Rubilar-Hernández, C., Cruz-Amaya, M., Norambuena, L., Plant Molecular Biology Centre, Faculty of Sciences, University of Chile.

Lateral roots (LR) are organs that develop post-embryonically from root pericycle cell layer. A LR founder cell (LRFC) will start cell divisions for initiate LR primordium. Certain pericycle cells acquire the fate to be LRFC in a process called priming. This process includes pre-initiation events such as activation of the transcription factor GATA23 and accumulation of a membrane associated-kinase regulator MAKR4. By means of the synthetic compound Sortin2, we have shown that induction of trafficking towards the vacuole promotes LR formation. Indeed Sortin2 promotes the differentiation of pericycle cells to be a LRFC in a trafficking towards the vacuole depending manner. The goal of ourwork is locating the acceleration of trafficking towards the vacuole by Sortin2 as a pre-initiation event of LR formation. We have found that Sortin2 induces both pre-initiation events, GATA23 activation and MAKR4 accumulation in pericycle cells. Sortin2 trafficking towards the vacuole induction is detected first than the induction of the pre-initiation events, temporally consistent with been trafficking toward the vacuole the leading event. Continuing our understanding of how this process takes place we used the advantages of Arabidopsis genetics. Mutants on protein trafficking and in LR formation were analyzed in terms Sortin2 sensitivity. We found a relationship between resistance of the LR formation and the induction of trafficking towards the vacuole by Sortin2. In some of these mutants, Sortin2 is able to promote the activation of GATA23 and MAKR4, however this pre-initiation events activation is at lower levels when compared to wildtype plants. Overall, our results indicate a cellular response that affect the endogenous LR-program where the induction of trafficking towards the vacuole promotes a change of cell fate in pericycle cells to LRFC, allowing remodeling of the root architecture in *Arabidopsis thaliana*.

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13.- Ectopic expression of *VviAGL11* from *Vitis vinifera* in tomato alters seed and flower development.

Morales, I., Ocarez, N., Mejía, N., Mejoramiento y Biotecnología INIA CRI La Platina.

Table grape is the most important exported crop within the national fruit industry, which makes it a priority candidate for the study of the molecular mechanisms underlying quality traits of agronomic interest such as flavor, color, seedlessness, etc. Within this frame, *Vitis vinifera* *AGAMOUS-Like 11* (*VviAGL11*) is a transcription factor that belongs to the MADS-box MIKC^c family of genes and has been proposed as the best candidate gene responsible for the genetic control of seedlessness in grapevine. Besides its role in seedlessness, it appears to affect berry size, firmness and harvest date, however, it is not clear if *VviAGL11* affects these traits directly or through the pleiotropic effect mediated by hormones produced by the seed.

With the purpose to further characterize the role of *VviAGL11* in seed development, we created tomato (cv. Micro-Tom) transgenic stable lines that express *VviAGL11* under control of CaMV 35S promoter. Transgenic lines produced alterations in flower morphology through its development, such as curled petals, thicker sepals, overdeveloped carpels, among others. These alterations are associated with high expression levels of *VviAGL11* in early stages of flower development. Seed morphology was also affected in transgenic lines: alterations in seed size and shape were detected when compared to the wild-type plants. Together, expression analysis of *VviAGL11* in different stages of flower development, and the phenotypic characterization of seeds, allowed us to conclude that *VviAGL11* influences seed size and development, as well as flower organ development in the studied conditions, validating its predicted role in the process of seed development.

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14.- Propagation of seeds by *in vitro* culture of native south american plant *Physalis peruviana* L.

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Physalis peruviana is a perennial shrub plant from the Solanaceae family, native of the South American Andes which is characterized for having a fruit covered by a calice which is marketed by its flavor and antioxidant properties. As allogamous plants shows great phenotypic variability in the population, the ideal is to obtain varieties of a particular habit of growth, high productivity and uniform quality, characteristics that can be achieved through asexual micropropagation. The objective of this study was to create a method for propagating *in vitro* genetic homogeneity and increase the number of individuals. Seeds were collected from fruits of three plants of the Biobío region to which three types of disinfection treatment were applied. The culture medium used was MS¼ macronutrients. For multiplication of shoots, five treatments of growth regulators were used. The quantitative variables evaluated were contamination, germination, number of lateral buds and shoot length. The best treatment of seed disinfection was using 50% commercial bleach plus 70% ethanol, which achieved 91.7% germination. The number of buds developed by explants, show no significant differences between treatments. However 0.5 mg L⁻¹ of AIA plus 1 mg L⁻¹ BAP, showed greater shoot length and better development. After 30 days, the plants generated spontaneously roots and were transferred to a substrate of perlite, peat and vermiculite (1:1:1) for acclimatization, obtaining a 100% survival of viable plants. It was possible to establish a methodology for *in vitro* propagation of plants of *Physalis peruviana*.

15.- Epigenetic and genetic markers for embryogenic capacity of *Pinus radiata* d.don somatic cultures

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Somatic embryogenesis is a vegetative method for producing embryos from somatic tissue. This process is well-known in the genus *Pinus* and is it currently being used for commercial purposes in the *Pinus radiata* forestry industry in some countries, such as Chile. Despite the importance of this process, the molecular bases of somatic embryogenesis in *P. radiata* are barely known. Nevertheless, a group of genes related to early embryogenesis in conifers such as WOX2 and BBM among others, have been described as molecular markers, and in a few cases, their epigenetic regulation has been reported. During the earlier steps of somatic embryogenesis, the proliferation of embryogenic masses which are unable to produce proper embryos (embryogenic capacity) frequently occurs. Externally, these masses are indistinguishable from cultures with high embryogenic capacity and they represent an important leak of resources. The aim of this work was to develop a molecular tool to discriminate between cultures with high and low embryogenic capacity. In order to identify epigenetic differences global DNA methylation by ELISA assay was performed. To detect differential expression of conifer embryogenesis-related genes, relative and absolute qRT-PCR was performed. Both global DNA methylation and embryogenesis related gene profile are presented as a valuable tool to assess the embryogenic capacity.

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16.- Suppression of MADS-box *AGL11* gene produces seedlessness in fleshy fruits.

Ocañez, N., Mejía, N., Mejoramiento y Biotecnología INIA CRI La Platina.

AGAMOUS like-11 (AGL11) is a MADS-box gene that controls ovule identity in model species. *VviAGL11* has been proposed as the main candidate gene responsible for seedlessness in grapevine because ovules develop into seeds after fertilization. In this work we demonstrate that *AGL11* has a direct role in the determination of the seedless phenotype in grapevine and tomato. In grapevine, broad expression analysis revealed very low expression levels of the seedless allele compared to the seeded allele at the pea-size berry stage, when seeds begin their development. Heterozygous genotypes have lower transcript accumulation than expected considering the diploid nature of grapevine, thereby revealing that the dominant phenotype previously described for seedlessness is based on its expression level. In Sultanina Monococco, a seeded somatic variant of Sultanina (Thompson Seedless), structural differences were identified in the regulatory region of *VviAGL11*. These differences affect transcript accumulation levels and explain the phenotypic differences between the two varieties. Functional experiments in tomato, based on stable genetic transformation, demonstrated that suppression of *SlyAGL11* produces seedless fruits and that the degree of seed development depends on transcript accumulation levels. Additionally, genes putatively controlled by *SlyAGL11* and *VviAGL11* and involved in seed coat development, *SlyδVPE1* and *SlyδVPE2* in tomato and *VviδVPE* in grapevine, are expressed at lower levels in silenced tomato lines and in seedless grapevine genotypes. In conclusion, this work provides evidence that the MADS-box gene *AGL11* plays determinant role in the establishment of the seedless phenotype of fleshy fruits, providing a valuable tool for further analysis of seed and fruit development.

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17.- The splicing RNP Variant STEP1 regulates the composition of the pollen tube cel wall in *Arabidopsis thaliana*.

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Germination of pollen grains and the correct formation of a pollen tube is a crucial step in plant reproduction. Although many of these processes have been characterised, the underlying molecular mechanisms have not been elucidated yet. The RNP STEP1 is a putative ortholog to the Liliun Germline Restrictive Silencing Factor in *Arabidopsis thaliana*, proposed as pollen-specific gene repressor in vegetative tissues. Here we report the diverse defects in *step1* mutants regarding the structure of mature pollen grains and the rheological proprieties and structure of the pollen tubes, showing pectin burst at the tip, emerging of multiple pollen tubes and also the fragmentation of the pollen tube wall, producing wall bubbles that display circular cytoplasmic streaming, a striking phenotype not previously reported. Our analysis also suggest a reduction in the cellulose and callose deposition on the wall of pollen tubes, which may explain in part the abnormal phenotypes observed.

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18.- Evaluation of the participation of auxin accumulated on tapetum in the process of late STAMEN development in *Arabidopsis Thaliana*

Peña, J., Leon, G., Centro de Biotecnología Vegetal, Ciencias Biológicas, Universidad Andrés Bello.

In flowering plants, proper development of the male reproductive organs, stamens, is required for successful sexual reproduction. The stamens are formed by a filament and an anther. The anther contains the reproductive and non-reproductive tissues that contribute supporting gametogenesis. The anther tissue is organized in four concentric cell layers with special tasks in stamen development. The most inner anther cell layer is the tapetum, which works as a somatic helper that surrounds the microsporocytes and produces nutrients, structural components, and enzymes necessary to build up the outer layer of the pollen wall. In *Arabidopsis*, stamen development is divided in early and late development. The early phase involves formation and morphogenesis and the late phase that involves three highly coordinated processes, anther dehiscence, pollen maturation, and filament elongation. Alteration on any of these processes generally results in masculine sterility. Additionally, it is known that auxin plays a determinant role on both phases of stamen development. Recent studies suggest the presence of auxin transporters in the tapetum, and also that active auxin accumulates in this tissue. Plants deficient in auxin synthesis, transport or perception on stamen are also usually masculine sterile. On this work we aim to determine the importance of auxin accumulated in the tapetum for the late development of the stamen. For this purpose we inactivated auxin locally generating transgenic lines of *Arabidopsis thaliana* expressing the bacterial gene *iaaL* –which codes for an auxin conjugating enzyme– under the control of tapetum specific promoters. These lines will be analyzed to assess possible alterations on the stamen development process.

Fondecyt 1120766

19.- A distinctive from auxin transcriptional scenario for setting up lateral root formation in *Arabidopsis thaliana*

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Lateral roots (LR) are formed postembryonically, shaping the root system architecture, in a process accompanied by hormone auxin transport and accumulation. Our laboratory has described Sortin2 as a LR formation inducer, which does not activate an auxin transcriptional response as auxin does. Moreover, Sortin2 action mechanism is independent of the polarized auxin transport. Therefore, in this work we investigated whether Sortin2 could induce a particular and distinct transcriptional profile, different from canonical auxin signaling, resulting in LR-formation. We detected that Sortin2 induces LR formation by boosting up the LR initiation in the absence of transcriptional activity of different auxin transcriptional reporters. Interestingly, Sortin2 and exogenous auxin stimuli have distinctive and common molecular requirements for LR induction activity. Consistently with that, a transcriptome profile of Sortin2 and auxin-stimulated primary roots, revealed that transcriptional response to Sortin2, have both a common and excluded from auxin response gene set. Interestingly, those genes modified exclusively in response to Sortin2, enrich completely different biological processes and cis-regulatory elements on their promoter region, than those genes exclusively responding upon auxin. Based on the transcriptional regulatory networks built among the Sortin2 response genes we decided to further evaluate genes with annotated function as either transcriptional factor or co-activator for their putative participation in the LR formation in response to Sortin2. Insertional mutants on those genes showed decreased LR formation rate and diminished Sortin2-induced LR response. Taken together these results revealed specific molecular players, different from the canonical auxin signaling, mediating a transcriptional scenario of LR formation in response to Sortin2. The role of these molecular players on endogenous or Sortin2-induce LR formation should be further evaluated.

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20.- Comparative analyses between *Prunus persica* and *Arabidopsis thaliana* reveals a novel set of conserved cytokinin-responsive genes

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Cytokinin activates an ancient hormonal pathway that existed prior to the development of well-defined vasculature, but has diversified during land plant evolution. This phytohormone plays a very important role in plant growth and development; including cell division, meristem maintenance, shoot initiation and growth, vascular development, nutrient uptake, chloroplast differentiation, light perception, leaf senescence and fruit ripening. Recently, we have reported the conservation of the cytokinin signaling and homeostasis pathways between the *Arabidopsis thaliana* and *Prunus persica*. In order to better understand cytokinin signaling during peach fruit development, we performed transcriptomic analyses of peaches treated exogenously with trans-zeatin at different stages of fruits development. Comparative analyses of cytokinin-responsive genes in these peach fruits with published *Arabidopsis* meta-analyses, reveals a novel set of cytokinin-responsive genes conserved between these species.

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21.- Apoplastic calcium uptake and phosphoinositide kinases as key regulators for configuring a novel molecular signaling of lateral root initiation on *Arabidopsis thaliana*.

Rubilar, C., Norambuena, L., Plant Molecular Biology Centre, Biology Department, Faculty of Sciences, University of Chile.

Lateral roots (LR) are organs which increase contact area with the soil to absorb water and nutrients. The formation of LR can be sequentially summarized in an initiation step where LR primordia are developed and the emergence step where these primordia emerge from the primary root. LR formation is stimulated by a signaling pathway dependent of the auxin nuclear complex receptor SCF^{TIR1/AFB₅}. In *Arabidopsis thaliana*, we had shown that endocytic trafficking is a positive regulator of a different pathway which is SCF^{TIR1/AFB₅}-independent by means of the biomodulator Sortin2. Therefore, we have been characterizing this novel mechanism to find new regulators in LR formation. We have observed that phosphatidylinositol 4-kinase (PI4K) activity is an important component of Sortin2-induced LR formation. Particularly, the PI4KIIIb protein family is essential to promote Sortin2-induced LR initiation. Consistently, Sortin2 induces a change in the phosphatidylinositol 4-phosphate (PI4P) levels in different cellular compartments. The PI4P increases transiently at the plasma membrane while it rises at intracellular compartments suggesting that the PI4K activity plays a key role on the LR formation induced by Sortin2. On the other hand, we have observed that apoplastic Ca⁺² uptake is required for Sortin2-induced LR initiation. The evidence suggests that cytoplasmic Ca⁺² increment is important to promote LR initiation. Genetic evidence suggests the apoplastic Ca⁺² uptake as a downstream event from PI4KIIIb family function on Sortin2-induced LR initiation. Overall, these molecular events configure a signaling pathway which participates on the LR initiation promoted by a distinctive SCF^{TIR1/AFB₅}-independent mechanism.

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22.- Phenotypic and biochemical characterization of mutants affected in the UDP-Rhamnose /Galactose transporters

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The cell wall is a complex extracellular matrix mostly composed of polysaccharides. In these polysaccharides we can find 3 main domains: homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II). RG-I and RG-II are the main rhamnose-containing polysaccharides present in the cell wall. RG-I is formed by a backbone containing repeats of the disaccharide formed by galacturonic acid (GalA) and rhamnose (Rha) residues, substituted by side chains made of arabinose and galactose. RG-II is the most complex polysaccharides present in the cell wall. It is composed by a backbone of galacturonic acid, which is substituted by complex side chains. These pectin domains are synthesized in the Golgi apparatus by glycosyltransferases (GTs), which use nucleotide sugars as donors to glycosylate nascent polysaccharides chains acceptors. UDP-Rhamnose (UDP-Rha) is a key molecule involved in RG-I and RG-II formation since it serves as precursor for their composition. UDP-Rha is synthesized in the cytosol and needs to be transported into the Golgi apparatus lumen, where the catalytic site of the epimerases and GTs are located. In this process Nucleotide Sugars Transporters (NSTs) are key elements. Recently, the function of the UDP-Rhamnose/Galactose Transporters (URGT1-6) family has been characterized. However no thorough assessment of the lack of the URGTs has been shown in plants. Here we present, a detailed characterization of *urgt* mutant phenotypes (1-6). They show different physiological phenotypes for example, *urgt2* and *urgt4* present a delay in germination whereas *urgt3* show a reduction of the rosette size. These preliminary results suggest the participation of URGTs in an organ specific manner.

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23.- Characterization of a putative bZIP transcription factor in pollen

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Sexual reproduction in plants depends on the production and differentiation of functional gametes. Pollen grains are the male gametophyte and their development encompass a myriad of well-coordinated cellular processes. Currently, we have a limited understanding of the regulatory mechanisms that are involved in gametophyte development. Transcription factors (TFs) are among the most relevant actors during this regulatory network. TFs are usually classified by their structures and DNA binding domains. One of the TFs that can be found abundantly in eukaryotes, are the DNA-binding domains of basic region/leucine zipper (bZIP). This family of TFs have a basic region that binds DNA and a leucine zipper dimerization motif. Genetic and molecular studies of a few of these bZIP factors in *Arabidopsis thaliana* (AtbZIP), show that they regulate diverse biological processes such as pathogen defense, stress signaling, seed maturation and flower development. This AtbZIPs tend to be ubiquitous in the plant, but little is known of pollen-specific bZIP factors. To date, AtbZIP34 is the only bZIP TF that has been characterized as pollen-specific and is involved in pollen wall patterning. In this work we focus on the characterization of a putative, pollen-specific bZIP TF (At1g35490). This protein has been classified as member of the bZIP factor based on sequence similarity of the basic region and the presence of additional conserved motifs. On the other hand, mRNA is detected only in developing pollen grains, with a peak in mature pollen grain, and expression in tobacco leaf suggest a nuclear localization. This preliminar data suggest that At1g35490 is a pollen-specific bZIP transcription factor.

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24.- Expression of genes of TaExpA6, XTH2 and XTH5 during the grain growth in two wheat cultivars contrasting in grain weight

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The need of increasing grain weight (GW) to improve grain yield potential and the efficiency of wheat breeding has been supported by different evaluations carried out in contrasting high yielding environments. However, associated traits and physiological mechanisms determining potential (PGW) and actual grain weight are partially understood. The objective of this study was to identify key molecular and physiological traits/mechanisms controlling the setting of PGW in two spring wheat cultivars contrasting in GW. The study was carried out at field conditions under optimal agronomical management in Estación Experimental Agropecuaria Austral of the Universidad Austral de Chile in Valdivia. A Completed Randomised Block Design with three replicates was used. At the booting stage, control and thinning treatments were set, the last to improve the environmental growing conditions of plants. From anthesis on, the fresh and dry matter weight of grains were recorded every five days as well as grain dimensions in grain position 2 (G2) from central spikelets of spikes. Grain samples were harvested at the same time for evaluating the expression of the TaExpA6, XTH2 and XTH5 genes by qPCR. Data was assessed by ANOVA and regression analyses. Both cultivar and thinning treatments affected ($P < 0.01$) final GW of G2 (from 60.4 to 86 mg, and from 50.5 to 72.5 mg, respectively). Different grain length (GL) was found between cultivars ($P < 0.01$) even though they showed similar timing of length stabilization (20 days after anthesis). ENT2, the higher GW and GL cultivar, showed higher relative expression of TaExpA6 throughout grain filling and GL associated with TaExpA6 expression at A+20. The relative expression of XTH2 in both cultivars was higher between A+20 and A+25. Besides, the XTH5 expression was detected from A+15 to A+35, which could meaning this gene is involved in several processes of grain growth further than the GL dynamic.

Molecular Breeding and Genetic Resources

25.- Genetic variation and population structure in quinoa (*Chenopodium quinoa* Willd.) using microsatellite markers

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Quinoa (*Chenopodium quinoa* Willd.) is an important seed crop domesticated in the Andean region of South America, which is a primary protein source for many of the indigenous inhabitants of this area and recently a worldwide success. Its nutritional properties are outstanding because of perfect balance among lipids, carbohydrates and protein containing all essential aminoacids for human nutrition. The genetic variability of quinoa is fundamental for the adaptation to different ecological environmental constrains (salinity, drought, frost and high radiation). For this reason, Quinoa breeding program are being carried out in Chile and other South American countries. The objective of this work was to characterize the genetic diversity and population structure of Quinoa selected lines from the breeding program at INIA Intihuasi. Twenty-seven microsatellites from public database were genotyped in 96 quinoa selections using capillary electrophoresis. Both genotypic and phenotypic data will be analysed to determine genetic diversity and to assist in traits selection. Phylogenetic dendrogram from UPGMA cluster analysis and two-dimensional PCA analysis using Xlstat will be shown. Similarly, population structure will be determined using Structure v2.3 software. This information is important to characterize the superior lines and to better select parental material for combining superior traits in this breeding program, and to develop new cultivars for different purposes and regions by means of NGS tools.

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26.- Metabolic engineering to develop *M. domestica* and *S. lycopersicum* fruits with high contents of antioxidants and vitamin a.

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Plant carotenoids are attractive pigments synthesized in plastids that play key roles in photosynthesis, photoprotection and phytohormone biosynthesis. Also, carotenoids are necessary in the human diet, acting as vitamin-A precursors and antioxidants. Their consumption is associated with protection against several diseases and aging. Such studies have increased the interest in generating new plant varieties able to produce high levels of carotenoids through genetic engineering. Apples (*Malus domestica*) are one of the most widely consumed and cultivated fruits, mainly because of their high nutritional value, including high fiber and vitamin C contents. In Chile, apples are the second most-exported fresh fruits. Therefore, our aim is to produce apple fruits with improved carotenoid content as a functional food through metabolic engineering in order to increment the antioxidant capacity, pro-vitamin A content and to obtain new varieties with different colors in the flesh. In this work, key genes of the carotenoid pathway from carrot (phytoene synthase (*Psy*) and lycopene cyclase (*Lcyb*)), and carotene desaturase (*crtI*) from *Xanthophylomyces dendrorhous* were cloned under the control of a fruit specific promoter, generating four vectors (CG vectors) with single and multiple gene combinations. Stable transformation of CG vectors in tomato (*Solanum lycopersicum*) and transient expression in *Malus domestica* produces a 2- to 5-fold increment in total carotenoids and β -carotene levels in fruits. A stable apple transformation protocol was optimized, with 0,36% transformed shoot regeneration efficiency (determined by PCR), of which 60% possessed GFP fluorescence, product of a transformation with a control vector. At present, transformed apple shoots with CG vectors are under molecular analysis and *in vitro* rooting.

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27.- Evaluation of antibacterial activity from maqui (*Aristotelia chilensis*) leaves.

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Maqui (*Aristotelia chilensis*) is a native evergreen tree from South America, their height varies among 3 to 4 m, growing from the Coquimbo Region to the Aysén Region, at altitudes up to 2500 meters on sea level. The fruit is a shiny black berry, 3-5 mm diameter, which is used as food and dye. Maqui fruit has analgesic, anti-inflammatory and antioxidant properties. Maqui fruit is characterized by a higher antioxidant activity due to their high anthocyanin and phenol content. The aim of this study is to evaluate the antibacterial activity of maqui leaves extracts from Cipreses River National Reserve and other places of Chile. The leaves extracts were obtained using different solvents. Gram positive and gram negative bacteria were used in this evaluation. Determination of effective 50% reduction concentration (EC_{50}) of maqui leaves extracts against each of the test bacteria used was performed using the microtiter broth dilution assay. The EC_{50} was considered to be the lowest concentration of leaves extracts that reduces the growth by 50% relative to the control.

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28.- Study genetic diversity in orchards of raspberries var. heritage in the region of Maule.

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In this work, were used Inter Sequences Simple Repeat markers to evaluated the genetic diversity in red raspberry cultivars of the variety Heritage in the Region of Maule, Chile. It were analyzed 121, 140 and 149 samples from three sectors denominated North, Central and South, including all the provinces of the Region. The ten primers ISSR used in this study generated 89 polymorphic bands PCR-ISSR. The samples analyzed presented a high grade of molecular variance, with a 21% of variance interpopulations. The Principal Components Analysis (PCA) showed that exist a very low genetic distribution between the sectors north, central and south, give this distribution only by the samples of sector central. This events can are in accordance with the sales of raspberries plant from the nurseries in each province of the Region of Maule or probably for the distances geographic that exist between the different populations.

29.- High-density SNP-based genetic map of Japanese plum (*Prunus salicina* L.)

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Japanese plum (*Prunus salicina* L.) is an important temperate fruit crop that possesses a diploid genome ($2n = 2x = 16$). This species displays great morpho-phenological variability. Many efforts have been made in the past to construct linkage maps in *Rosaceae* species such as peach, cherries, almond, apricot, and Chinese plum, among others. However, Japanese plum has not been studied so far. We applied the Genotyping by Sequencing (GBS) approach to generate a highly saturated map in a biparental segregant population of Japanese plum. This highly saturated genetic linkage map will be extremely important for conducting basic genetic studies and for developing molecular breeding strategies, which can accelerate the breeding cycle of this species. We analyzed 57,536 SNPs in two parental lines (cv. Angeleno x cv. Aurora) plus 152 offspring materials (or lines). The software Tassel 5.0 was used to analyze the filtered Hapmap containing SNPs data. We applied additional filtering to eliminate distortions of segregation, suspected linkages, genotyping errors, missing data, and double recombinants. In addition, Joinmap 4.1 was utilized to build a framework map for each parental genetic map. Finally, regression mapping and Kosambi mapping function were used. The first eight scaffolds were associated with linkage groups identified on peach; the number of observed SNPs ranged between 11,978 SNPs (Scaffold 1) and 5,711 SNPs (Scaffold 5). The Japanese plum genetic map and its relationship with the peach genome is discussed in detail.

Sponsored by Functional Genomics Approach To Understand Cracking Susceptibility In Sweet Cherry: An Integrative View For *Prunus* Species. FONDECYT Nº 1120261

30.- Identification of genes associated with the softening rate in *Prunus persica* (L.) Batsch using QTLs and expression QTL (eQTL)

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Fruit ripening is a highly coordinated and genetically programmed event characterized by physiological and biochemical changes. Softening is a very important aspect of the ripening syndrome and it is one of the most important process that determine quality and postharvest performance. The aim of this work was to identify candidate genes associated with a fruit softening in peach (*Prunus persica*). For this study we evaluated a F2 melting segregating population of 151 individuals (Venus x Venus) at harvest (stage E1) and harvest + shelf-life period (stage E2). We phenotyped the fruit firmness in both stages and we calculated the fruit softening as a percentage of loss of firmness. Twelve contrasting siblings for softening (6 soft and 6 firm) were selected. RNA from five fruits of each selected siblings was isolated and pooled. Twelve libraries were constructed and they were sequenced in two lanes using HiSeq 2000 platform. We obtained an average of 3,891,114,755 bp for each library which were mapped against the peach reference genome with an average of 93.4% mapped reads. The FPKM of this genes were used as a molecular phenotype for the eQTL analysis, finding both *cis* and *trans* eQTL. These eQTL were compared with the conventional QTL in order to find co-localization between them. The genes obtained from this analysis were selected as candidates for the softening rate in *P. persica*.

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31.- Use of oryzalin for polyploidy induction: effect dosage in embryogenic callus survival of *Alstroemeria* spp.

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The genus *Alstroemeria* is native to South America and it is well known by the ornamental characteristics of some of its species. Chile is one of the main center of diversity, offering great opportunities for the breeding new cultivars. Size and color flower are the traits of mayor importance in the breeding of these species. Plant polyploidization is a useful technique to induce an increase in the size of organs such as flowers. Oryzalin, it is antimitotic agent, which has the advantage of being less toxic to humans than colchicine and having a greater affinity with plant cells. In this study we evaluate the effect of oryzalin on callus obtained for interspecific cross (CORUCH 13B08) between two different *Alstroemeria* species, one from Chile (non-aromatic) and one from Brazil (aromatic). A culture media containing Murashige & Skoog (MS) macro and micronutrients, supplemented with 2 mg•L⁻¹ BAP, 1 mg•L⁻¹ 2,4-D and solidified with 7.5 g•L⁻¹ of agar plant TC (Phytotechnology Inc.) was used. Embryogenic callus later was immersed in a solution made with the MS salts, supplemented with 0-5-10 and 20 mg•L⁻¹ oryzalin, for 3 or 6 days in orbital shaker at 100 rpm. Treated embryogenic callus were rinsed with MS salts and planted in petri dishes containing 30 mL of the culture medium previously described. Petri dishes were incubated in a growth chamber at 21±1°C and a 16/8 light/dark photoperiod for 30 days and we evaluated for callus survival. According to the higher percentage of embryogenic callus survival (30-40%), the combinations of 10 mg/L with 3 day and 5 mg/L with 6 days showed the best response. Polyploidization of embryogenic callus obtained by oryzalin treatment effect will be determined by Flow Cytometry.

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32.- Identification of s-genotypes in prunus rootstocks with breeding purposes

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Most *Rosaceae* fruit trees such as *Prunus* species (sweet cherry, peach and almond, among others) exhibit gametophytic self-incompatibility, which prevents self-fertilization, and is genetically controlled by the *S*-locus. This locus contains at least two tightly-linked *S*-determinant genes, a pistil *S-RNase* and a pollen *SFB*. When the *S*-allele of the pollen grain matches one of the *S*-alleles of the style, these ribonucleases inhibit the growth of the pollen tube in the style. Allele-specific and consensus primers have been designed to amplify across each of the two introns of the *S-RNase* gene sequence, to accelerate the determination of *S*-genotypes in *Prunus* cultivars, but there is limited information for *Prunus* rootstocks. The development of new *Prunus* rootstocks is a challenge considering the hybrid nature of most of these materials, flower morphology, genetic distance, among others, and self-incompatibility could be other limitation for obtaining new genotypes. Identification of *Prunus* rootstocks *S*-alleles genotypes may be a useful tool for the *Prunus* rootstocks Breeding Program developing by CEAF (Centro de Estudios Avanzados en Fruticultura), helping with the selection of appropriate pollen donors and for checking the hybrid nature of new materials obtained by the Breeding Program. The objective of this study was to characterize *S*-alleles of the main *Prunus* rootstocks available at a germplasm collection of CEAF, comprising genotypes with different ploidy. As a first approach, the partial nucleotide sequence of *S-RNase* from six diploid *Prunus* rootstocks was determined by isolating and sequencing twelve *S*-alleles obtained by amplifying the second intron of *S-RNase* with consensus primers. Deduced polypeptide sequences showed over 97% identity with *S-RNases* from other *Prunus* species, such as sweet cherry, almond and Japanese plum, identifying previously described *S1* and *S4* alleles, among others. Results and their usefulness for breeding purposes will be discussed.

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33.- Is the optimal light really an optimal light for plants?

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When we grow *Arabidopsis* plants in the lab, we normally use 130-200 $\mu\text{mol}/\text{m}^2/\text{s}$ of light to mimic natural conditions. But we are missing something important and the problem is not the intensity but the quality, because the light that we are measuring in the lab is not the same as the sunlight, and what we see as white light, actually it is mostly green, and very little blue and red light which are useful for photosynthesis. Then, we are growing our plants under low light, and not optimal light. In this work I present a simple experiment showing the different behavior of *Arabidopsis* growing at sunlight, fluorescence light (“optimal light”) and using LED light as a excellent alternative, and the result is astonishing.

Aknowledge: Proyecto núcleo milenio NC130030, Proyecto PAI 82140040

34.- Analysis of DNA methylation polymorphisms in sweet cherry varieties (*Prunus avium*) with different chilling requirement.

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Chilling requirement is a critical phenological trait that controls the timing of vegetative bud break and floral bloom, and impacts on the climatic distribution of fruit trees. The chilling requirement in sweet cherry (*Prunus avium*) in Chile ranges from 600 to 1000 chilling hours (temperatures $\leq 7.2^{\circ}\text{C}$). Chilean winters have become warmer during the past years. As a consequence, sweet cherry trees that haven't been able to fulfill their chilling requirement present impaired flowering, which negatively affects fruit production. Although the molecular basis of the chilling requirement trait are not well understood, evidence from other plant models shows that epigenetic mechanisms may play a role in temperature-modulated plant developmental responses. In this work, genomic DNA from floral buds of overwintering sweet cherry trees of four varieties with different chilling requirements (Royal dawn, Glen red, Regina and Kordia), was examined for changes in DNA methylation patterns, using the experimental approach MSAP (Methylation-Sensitive Amplified Polymorphism). Polymorphic fragments were differentially present among the studied varieties according to their chilling requirement, which can be further validated as molecular markers for the trait. These results additionally indicate that epigenetic mechanisms are involved in this temperature-modulated process.

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35.- Uncovering genetic factors associated with leaf and seed alkaloid content in yellow Lupin (*Lupinus luteus* L.)

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State of art and aim: Plant secondary metabolites are part of the communication system used for higher plants to interact with the environment. Lupin alkaloids belong to this group of compounds, and high consumption of them, has a detrimental effect on human and animal nutrition. The generation of low alkaloid varieties has facilitated the use of lupins in human and animal diets, and satisfied food industry standards. However, it has also increased susceptibility to herbivores and transmission of aphid-borne viruses and bacteria. This bitter-pest resistance/sweet-food industry balance has generated a complex challenge for lupin breeders. Thus, generating breeding tools to aid the efficient manipulation of alkaloids could significantly increase the generation of better adapted good nutritional quality varieties. Several research initiatives carried out at the Agriaquaculture Nutritional Genomics Center (CGNA) have allowed the identification of QTLs associated to alkaloids; however, there is still uncertainty of what gene(s) are directly involved in the biosynthesis of these compounds. The main goals of this study are to genetically dissect the natural variation of alkaloid content in leaf tissue and mature seeds on a diverse set of *L. luteus*, narrow down alkaloid associated genomic regions and ultimately, find candidate genes involved on alkaloid biosynthetic pathways. Results and discussion: Alkaloid content was measured, in leaves and mature seeds, from a diverse yellow lupin core collection during two seasons using HPTLC technology. Lupin accessions were genotyped using a set of ~300 molecular markers (SSRs, EST-SSRs, SNPs and INDELs). Association analyses uncovered several genomic regions associated to lupinine, spartein, and gramine alkaloid content in both lupin tissues. Currently, we are carrying out bioinformatic analyses to target candidate genes, such as lysine decarboxilase, and key enzymes on the quinolizidine pathway, as an attempt uncover yellow lupin genes behind the production of these plant compounds.

FONDECYT Project 3140064), And The CONICYT Regional/GORE Araucanía/CGNA/R10C100.



36.- Functional characterization of the biosynthesis of eicosapentaenoic acid in *Nannochloropsis oceanica*

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Long chain polyunsaturated fatty acids (PUFA or ω 3 fatty acids) have generated a great deal of interest because its beneficial characteristics associated to human consumption. However, the availability and sustainability of primary sources (oceanic fish) has been severely compromised due to overfishing and environmental contamination. It is imperative to find new safe sources of these fatty acids that can also have a positive effect on the environment. Moreover, fish produced from aquaculture has grown exponentially, with more than half of global fish production in 2013. Fish naturally obtain ω 3 fatty acids from oceanic microalgae, but these microorganism are poorly present in this type of culture. Nowadays, the industry is reutilizing almost 80% of the ω 3 fatty acids in fish feedstock, leaving only 13% for human consumption. This is why we propose to go back to primary ω 3 fatty acids producers: microalgae. Within the latter we found *Nannochloropsis oceanica*, which is a natural producer of the ω 3 fatty acid EPA (eicosapentaenoic acid). These microalgae produce high lipid quantity, and nuclear homologous recombination techniques have been described in order for genetic manipulation. Our main objective is to determine which genes are involved in EPA production. Bioinformatics analysis of *N. oceanica* genome has allowed us to determine putative genes involved in these pathways, and preliminary experiments suggest that ABA could play an important role in the production of these fatty acids. Over-expressing lines of ω 3 genes have been made, which should help us clarify how ω 3 fatty acids are produced.

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37.- DNA fingerprinting of limachino tomato accessions

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Limachino tomato is an emblematic local variety cultivated by tomato growers of the Limache Valley of the Valparaíso until the 1970 decade. The use of this variety has suffered a decline due to its replacement by more productive tomato cultivars. At present, INIA is conducting an initiative to promote its reintroduction, valorization and protection. Thirteen accessions named Limachino and collected in 1960 (1 accession), 1980 (6 accessions) and 2015 (6 accessions) are currently available. A molecular analysis using 17 highly polymorphic SSR markers has been conducted to efficiently discriminate Limachino plants from other similar varieties and to characterize and make profitable use of this traditional tomato variety by small tomato growers. Molecular characterization of eight accessions by eight SSRs showed that these accessions are very closely related. All loci are homozygous and only one locus was polymorphic. Polymorphism was only found within plants in one accession, revealing some degree of contamination. Analysis with remaining SSR loci and the inclusion of genetic material of different origins will allow the identification of a specific genetic pattern for Limachino variety.

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38.- Genetic structure based on est-ssr: a promising tool for fruit color selection in japanese plum (*Prunus salicina* L.) breeding programs

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Japanese plum is one of the most important stone fruit crops worldwide. Molecular markers are extremely useful tools in plant genetics and breeding. Currently, very few markers are available in Japanese plum hindering the implementation of Marker-Assisted Selection (MAS). Next-generation sequencing allows the study of genes involved in traits of interest, facilitating the development of strategies based on QTLs and GWAS conducted with data from genotyping-by-sequencing (GBS). RNA-seq is a accurate and sensitive tool for quantification of gene expression. Transcriptome studies facilitate the understanding of metabolic pathways, allowing the suggestion of transcription factors associated with traits of agronomic interest. They have also enabled the development of simple sequence repeats (EST-SSR) and single nucleotide polymorphism (SNPs) two of the most common markers used in MAS. Currently few studies have focused on developing EST-SSR markers considering both, genes expression levels and specific transcription factor from metabolic pathways. In this work, we report the first set of EST-SSR markers for *P. salicina* developed from specific genes associated to fruit color by anthocyanin accumulation. Our objectives were (i) to develop a set EST-SSR related to the synthesis and regulation of flavonoid pathway genes, (ii) determine the genetic structure in *P. salicina* germplasm using GBS, (iii) validate and evaluate the potential of EST-SSR as molecular markers for presence or absence of anthocyanins accumulation.

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39.- Direct organogenesis from petiole explants treated with colchicine in *Actinidia deliciosa* (A.Chev.) C.F.Liang & A.R.Ferguson

Santander, M. A., Rivas , C., Pertuzé, R., Aros, D.,Infante, R., Muñoz, C., Producción Agrícola, Ciencias Agronómicas, Universidad De Chile.

Colchicine is the most common substance used to induce polyploidy in plants. Polyploids are useful in plant breeding for several reasons, including the facilitation of wide crosses and the increase in organ size, a trait that is of interest in many fruit species. In kiwi fruit, large fruit size is a character seek by breeders, and many efforts have been made to try to induce polyploidy in commercial cultivars. This study aimed to determine the best colchicine concentration to be used for the in vitro induction of polyploidy in *Actinidia deliciosa*. The first step to do this was to find a culture method that enables the direct organogenesis from petiole explants. A culture media consisting of the Murashige & Skoog (MS) macro and micronutrients supplemented with 3.5 mg•L⁻¹ BAP, 0.5 mg•L⁻¹ IAA and solidified with 7.5 g•L⁻¹ of agar plant TC (Phytotechnology Inc.) was used. To determine the best colchicine treatment, leaf petiole segments of 0.5 cm were immersed in a solution made with the MS salts, but containing 0.0-0.1-0.2 and 0.3% colchicine for 4 h. Treated explants were rinsed with sterile distilled water and planted in petri dishes containing 30 mL of the culture medium previously described. Petri dishes were incubated in a growth chamber at 21±1°C and a 16/8 light/dark photoperiod for 60 days and evaluated for explant survival and the number of shoots arising from them. No significant differences were found in any of the parameters analyzed. Neither was found damage attributable to exposure to colchicine. Average explant survival was 85%, and number of shoots per explant, 0.8, with no statistical differences among treatments. We are in the process of determining the ploidy of the emerging shoots.

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40.- Identification of *lyce* gene mutants to potentially increase β -carotene content in durum wheat (*Triticum turgidum* l.var. *durum*) grain through tilling

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Increasing β -carotene (vitamin A precursor) content in durum wheat grains is important to improve pasta quality and its nutritional value. *LYCE* (Lycopene Epsilon Cyclase) is a key enzyme that diverts the carotenoid biosynthetic pathway into two metabolic branches. Studies in other species show that altering the expression of *LYCE* genes increases the flux towards the β - β branch, accumulating higher levels of β -carotene. Our objective is to identify *LYCE* point mutations created with EMS (Ethyl Methane Sulphonate) that could potentially increase β -carotene content in durum wheat grain through TILLING (Target Induced Local Lesions IN Genomes). For this purpose, a Kronos TILLING mutant population constituted by 1,300 individuals from the University of California in Davis was used. Due to the tetraploid nature of the durum wheat genome, genome-specific primers for *LYCE-A* and *LYCE-B* genes were designed and validated. These primers amplified a fragment of 1,760 bp, targeting exon 4 through exon 10 within the *LYCE* gene. To simplify the TILLING procedure and decrease costs, fragment 1 was digested with CJE (Celery Juice Extract) and visualized on 2% agarose gels. First, 6X mutant pools were identified, which showed cleavage products and then 2X pools were made to identify mutant individuals. *Lyce* mutants were sequenced and evaluated with BLOSUM 62, SIFT and PSSM algorithms. Substitutions W437*, P334L and G368R in *LYCE-A* and P405L, G352R and T393I in *LYCE-B* predicted to affect protein function. For genotyping, markers for the identification of these mutations were designed. These new *LYCE* alleles can potentially be incorporated and enrich durum wheat breeding programs and be useful for conducting functional genomic studies.

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41.- Relationship of *phytoene synthase psy-a1* and *psy-b1* alleles with semolina yellowness in durum wheat (*Triticum turgidum* L. var. *durum*)

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Phytoene synthase (*Psy*) is a key gene influencing semolina (milled, coarse durum wheat product used as raw material for pasta production) yellowness. Specific molecular markers have been developed to identify some of the known allelic variants of *Psy-A1* (*Psy-A1a*, *Psy-A1l*, *Psy-A1o*) and *Psy-B1* (*Psy-B1a*, *Psy-B1b*) associated to semolina yellow color. This study was undertaken to assess the genetic variability existing for *Psy1*, and to evaluate the relationship between genotype and phenotype, in two contrasting durum wheat populations formed mostly by American breeding lines (BL population), and a collection that included 155 Mediterranean landraces and 20 modern cultivars (M population). The BL population showed h^2 values over 0.93 for two indicators of yellowness [i.e. semolina yellow pigments absorbance (SYPA), and b^* values that were strongly correlated ($r = 0.95$, $P < 0.0001$)], reflecting the strong genetic control of semolina yellowness. Compared to the BL population, the M population showed greater variability in *Psy-A1* and *Psy-B1* allelic frequencies, but only *Psy-A1* exhibited significant differences in yellowness. This suggests that using molecular markers associated with *Psy-A1* (i.e., *Psy-A1_STS* and *YP7A-2* jointly) for marker assisted selection can be sufficient and valuable to improve grain yellowness. The presence of the *Psy-A1l* allele was associated with the highest values of semolina yellowness, whereas the presence of the *Psy-A1a* allele was associated with the lowest values for this trait in the M population. Fourteen genotypes carrying high-yellowness *Psy1* allelic variants were identified in both the BL and the M populations. These can be used as parents for increasing yellowness in durum breeding programs.

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42.- *In vitro* propagation of Chaura (*Gaultheria pumila*), a biotech approach for its domestication.

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Gaultheria pumila is a Chilean native species belonging to the Ericaceae family, it is distributed from Central Chile to the Antarctic Region and used to grow between 400 and 2500 msl. The species is very interesting as a potential source of antioxidants, as well as a very resistant species to be cultivated in extreme zones. For instance, it would be a very good target to be domesticated and to provide farmers with a new productive alternative. To start *in vitro* cultures of the species, different disinfection protocols were evaluated, using seasonal sprouts collected under natural conditions. Washing with NaClO twice during 40 min and 25 minutes at 1% and 2% led to the highest disinfection efficiencies as well as the best survival rates. Also, the effect of the media composition as well as plant growth regulators on plant recovery, shooting and explants survival was also studied. Zeatin riboside added into the Woody Plant Growth medium was most efficient than the rest of PGRs studied. Zeatin concentration also influenced the efficiency of shooting, since 1 mgL⁻¹ in the basal medium allowed the highest formation of shoots and plant recovery. Plants developed under this condition showed a normal development under *in vitro* conditions and were able to be multiplied four to six weeks after *in vitro* establishment, reaching up to 5 cm of plant height as average.

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43.- Expression of genes related to kernel growth at pre- and post-anthesis in sunflower (*Helianthus annuus*)

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The main components of crop yield are the number of grains per unit area and the average weight of grains. To understand the genetic and environmental clues determining both yield components is critical for further increasing yield potential of grain crops. Knowledge gained recently on physiological, genetic and biochemical mechanisms affecting grain weight has been noticeably. However, most of these achievements were in model plants as *Arabidopsis*, while the knowledge of key mechanisms controlling grain weight in grain crops remains elusive. The objective of this research was to evaluate the expression of genes related to grain size during the growth of flowers and during grain filling in sunflower as a model crop. Two sunflower genotypes contrasting in grain size and weight were sown in a Split Plot Design with three replicates at the “Estación Experimental Agropecuaria Austral (EEAA)” of the Universidad Austral de Chile in Valdivia. Ovaries and grains (divided in pericarp and embryo) were sampled at pre and post-anthesis, respectively. RNA was extracted from sampled ovaries, pericarp and embryo between R3 stage and harvest three times a week. Strand cDNA was synthesized by reverse transcription. Orthologous genes in sunflower GW2 (RING-type E3 ubiquitin ligase-like), FW2 (fruit-weight2.2-like), AP2 (EREBP-like), SBT (Subtilase-like), and a QTL for grain weight in sunflower were isolated and their expression was evaluated. The analysis of results showed different expression of genes across the phenological stages in both pericarp and embryo tissues. Interestingly, putative genes associated with grain dynamics envisaging as promising for a better understanding of mechanisms controlling grain size.

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Poster II

Abiotic Stress

1.- Physiological response of an indica rice cultivar to periods of High Night Temperature (HNT)

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Nighttime temperatures have increased in rice regions because of the Climate change in Colombia during last years, causing a reduction on the rice grain yield. The aim of this study was to estimate the effect of different periods of high nighttime temperature (HNT) on the physiological behavior of an indica rice cultivar that is widely cultivate. Ninety six rice plants of cv. F60 were grown in glasshouse conditions during thirty days. The climatic conditions in the glasshouse were the following: a natural photoperiod of 12 hours and the day/night time temperature at 33/24 °C, respectively. After this period of time, a group of forty eight were placed into growth chamber at 30°C during the nighttime for four hours during 4, 8, 12, and 16 days, respectively; in order to apply HNT treatments. The remainder group of plants was maintained in the glasshouse and was considered the control treatment (CT). Results showed the leaf photosynthesis, Fv/Fm ratio and Leaf pigments content decreased at 30°C, meanwhile, leaf respiration and intercellular CO₂ concentration increased. These results allow suggesting that these physiological variables may be useful to evaluate the tolerance of rice plants to HNT in plant breeding programs. These differences are not constant and change among the periods of time, showing major differences in the early days.



2.- Expression of a cuticle protein gene (*eceriferum 1*) in *Eucalyptus* spp. in response to cold acclimation.

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Eucalyptus is one of the most widely used genera in global commercial plantation timber industries. In Chile, the potential productivity of the *Eucalyptus* plantations is high, but maximum rates are rarely achieved because of freezing sensitivity limitations. The overwinter survival of evergreen trees relies on their ability to develop increased freezing tolerance in response to low but non-freezing temperatures, and adaptive response, known as cold acclimation. The cold acclimation mechanism relies on transcriptome modifications that trigger physiological and metabolic changes. This work describes the analysis of gene expression of ECERIFERUM 1 (*CER1*) of two *Eucalyptus* species (*Eucalyptus globulus* and *Eucalyptus nitens*) and their hybrid by qRT-PCR after cold acclimation compared to a control condition. The CER1 protein has been proposed to function in the decarbonylation of aldehydes to alkanes, components of wax cuticular. The results indicate that the CER1 transcription is down-regulated by cold stress compared to a control condition and is differentially expressed in different species and the hybrid.

Acknowledgment: Proyecto Fondecyt iniciación 11121559 “mRNA-Seq platform for the frost tolerance analysis of *Eucalyptus globulus*, *Eucalyptus nitens* and the hybrid *E. nitens* x *E. globulus*”.

3.- Evaluation of physiological parameters in blueberry varieties under osmotic stress

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In many cultivated species, one of the most important factors limiting production is water deficit. It is perhaps not surprising that plants exhibit a variety of strategies to reduce or avoid desiccation, such as osmolyte accumulation, stomatal closure and reduced photosynthetic activity, among others. Blueberry is a fruit with high antioxidant capacity that is widely cultivated in Chile mostly for export. So far, most stress research in this species has focused on cold acclimation, and little information exists on physiological and molecular responses to drought stress. In order to evaluate the physiological responses of six blueberry varieties (Biloxi, Sharpblue, O'Neal, Elliott, Bluegold and Brigitta) under osmotic stress, we simulated drought stress conditions with polyethylenglycol-6000 at 20% and 40% w/v. We measured chlorophyll fluorescence and concentration, relative water content and anthocyanin concentration. The results showed differences among blueberry varieties in response to osmotic stress conditions at 40% PEG-6000. This experimental approach presented here will be useful for defining which varieties are tolerant or susceptible to osmotic stress as well in selecting new varieties with drought tolerance.

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4.- Relationship between cultivars, fruit development stage and transcript levels in fruit cherry cracking tolerance.

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Rain-induced cracking causes great economic loss in many cherry-producing areas. Recent research has shown that early cuticle crack development can be correlated with the length of fruit growth phases, which are different between cultivars with contrasting degrees of cracking tolerance. The cuticular membrane is the primary barrier to water and thus their properties can be involved in tolerance to this problem. Moreover, the flexibility of fruit cell walls may also play an important role, allowing greater elongation under conditions of abrupt increase of the fruit surface. In order to better understand the relationship between cherry fruit cracking and gene expression, the transcriptional patterns of nine genes related to cell wall modification and cuticular wax biosynthesis were analyzed during three key stages of fruit growth, using two contrasting cultivars: 'Kordia' (tolerant) and 'Bing' (susceptible). The transcription levels of PaWINB, wax synthase, PaKCS1, PaKCS6, PaLACS2, PaGAT4/8, and β -galactosidase, showed higher levels in 'Kordia' than in 'Bing' during the first stage of fruit development, suggest that they are genes involved in conferring cracking tolerance, likely due to their function in cuticle deposition during early stages of fruit development. In contrast, transcription levels of expansin (PaEXP1) were higher at fruit ripening in 'Kordia' than in 'Bing', which would allow for a faster growth rate in this tolerant cultivar. Transcript profile of PaLTPG1 decreased during fruit development, with higher levels in 'Bing' than in 'Kordia' suggesting no relation with cracking tolerance.

5.- Evaluation of growth and root morphology using contrasting potato genotypes to water stress

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Potato (*Solanum tuberosum* L.) is considered sensitive to water stress, with significant reductions in yield from moderate levels of water deficit, due to its root system is limited and shallow. The plasticity of growth and root development in response to changes in available soil moisture provides the opportunity to explore the natural variation and identify the beneficial characteristics of roots to improve productivity of potatoes. Consequently, the aim of the present research was to evaluate the growth and morphology of roots in potato using susceptible and tolerant genotypes to water stress under semi controlled rizhotrons at greenhouse. To achieve this objective, two stages were done. The first stage used three tolerant genotypes to water stress and three susceptible. At the second stage, two contrasting genotypes from first stage were use and in both steps were used two treatments: water stress and control.

Morphological analysis of the root was evaluated using photographs, which were analyzed with RootSnap software. It determined the number of roots, total roots length, average long roots, total area roots, total volume roots, average root diameter, average of root volume and average area of roots. In addition, biomass and plant growth traits were made. It used multivariate statistical analysis, and also compared with their respective controls. Genotypes displayed significant variation in growth and morphology of roots in susceptible genotypes regarding to the tolerant, that it correlated with growth and biomass.

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6.- Over-expression of *Daucus carota* lycopene β -cyclase gene (*DcLCYB1*) enhances abiotic stress tolerance by a positive feedback in carotenoid, gibberellin and chlorophyll pathways in *Nicotiana tabacum* plants.

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Carotenoids are isoprenoid pigments widely distributed in nature. In plants, carotenoids are synthesized in plastids and participate in photosynthesis, photoprotection, phytohormone biosynthesis and in the prevention of oxidative damage under abiotic stress conditions. Carotenoids, as well as chlorophylls and gibberellins share the same precursor, geranyl-geranyl-diphosphate (GGPP) enabling a crosstalk between these pathways. One of the most important enzymes involved in carotenoid biosynthesis is lycopene β -cyclase (LCYB) that catalyzes the conversion of lycopene into β -carotene. In *Daucus carota* (carrot), two *LCYB* paralogs have been reported (*DcLCYB1* and *DcLCYB2*). *DcLCYB1* is required in carotenoid biosynthesis across the whole plant; however, it is preferentially expressed in leaves. In this work, heterologous expression of *DcLcyb1* gene in tobacco results in a general increase in total carotenoids, β -carotene and chlorophyll content. Furthermore, *DcLcyb1* expression resulted in increased gibberellin (GA4) content. This was reflected with an increase in plant height, leaf area, biomass and early flowering phenotype in transgenic lines. Deeper molecular analysis showed an up-regulation of key carotenoid (*NtPSY1*, *NtPSY2* and *NtLCYB*), chlorophyll (*NtCHL*) and gibberellin (*NtCPS* and *NtKS*) endogenous genes, as well as those involved in the biosynthesis of isoprenoid precursors (*NtGGPPS* and *NtDXS2*). Moreover, these transgenic lines showed enhanced survival rate under salt stress and high-light stress tolerance. Taken together, we proposed *DcLCYB1* as a candidate gene with biotechnological applications to potentiate plant fitness by an increase in carotenoids, gibberellins, chlorophylls and abiotic stress tolerance.

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7.- Heterologous expression of carrot *DcPsy1* and *DcPsy2* in *N. tabacum* produce an increase in carotenoids and improved tolerance to salt stress

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Carotenoids are isoprenoid pigments derived from the secondary metabolism. In plants, carotenoids are synthesized in plastids where they contribute to light-harvesting for photosynthesis, protect against photooxidative damage and favor tolerance to abiotic stress conditions such as salinity. Carotenoids are also precursor for the plant hormone abscisic acid (ABA), involved in plant development and abiotic stress responses. The phytoene synthase (PSY) enzyme, which catalyzes the first committed step in carotenoid synthesis, is a key regulatory point. Interestingly, in *Daucus carota*, the two paralog *DcPsy1* and *DcPsy2* genes present differential expression pattern, being *DcPsy1* preferably expressed in mature leaves while *DcPsy2* is mostly expressed in roots during development. Heterologous expression of *DcPsy1* and *DcPsy2* in *N. tabacum* causes an increase in carotenoids and chlorophyll levels and changes in endogenous carotenogenic gene expression. Transgenic *DcPsy1* and *DcPsy2* lines with 1.5 to 2.8 fold increase in carotenoids showed from 1.6 to 2.3 fold increase in fresh weight respect to control plants, after chronic NaCl treatment. Moreover, an enhanced response to acute salt treatment in terms of decreased electrolyte leakage and an increment in ABA accumulation was observed in transgenic lines. These results suggest that the PSY-mediated salt tolerance is associated with the carotenoid antioxidant property and the increase in ABA content.

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8.- Effect of long-term salinity stress on carotenoid biosynthesis in *Solanum lycopersicum* fruits.

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Tomato is a major horticultural crop worldwide that lately has been considered a functional food by consumers, since its fruits contain lycopene, a carotenoid pigment with antioxidant activity. Unfortunately, desertification and urbanization had led to use non suitable for agriculture soils, challenging scientist to find more efficient ways to grow crops without losing yield and quality during long-term abiotic stress. In this study, we characterized fruit quality in response to long-term salt stress in *Solanum lycopersicum* cv. Micro Tom plants. We show that plants irrigated with different salt treatments (0, 40, 80, 120 and 160mM NaCl) presented differences in fruit quality parameters, including caliber, fresh weight and total soluble content. In addition, changes in transcript levels of genes associated with carotenoid biosynthesis pathway were observed, leading to an increase in total amount of lycopene in fruits of salt stressed plants. Interestingly, tomatoes from plants irrigated with 160mM showed an earlier coloration change in comparison with other treatments. These findings suggest the potential of salinity to obtain tomatoes with higher levels of carotenoids.

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9.- Carrot AREB/ABF and Alfin-like transcription factors bind to *Daucus carota* phytoene synthase 2 (*PSY2*) promoter

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In plants, carotenoids are synthesized in plastids and act as accessory pigments in light harvesting and protect cells against photo-oxidation. Furthermore, carotenoids are precursors of phytohormones such as abscisic acid, which is involved in dormancy and abiotic stress defense. A key point in carotenoid biosynthesis regulation is the production of phytoene from geranylgeranyl pyrophosphate, reaction catalyzed by the enzyme phytoene synthase (PSY), the first committed step in the pathway. *Daucus carota* (carrot) accumulates large amounts of carotenoids, mainly α - and β -carotene, in the modified root only in darkness. In carrot, two *PSY* paralogs have been described, of which *PSY2* would be the mainly responsible for the biosynthesis of carotenoids in the storage roots. Here we show by *in silico* analysis that only *PSY2* promoter possesses ABA-responsive elements (ABREs), suggesting the presence of an abscisic acid responsive complex. In agreement with the proposal, only *PSY2* expression is induced by ABA in carrot seedlings. Using a yeast one-hybrid assay we found 3 sequences belonging to the AREB/ABF transcription factor family and 2 belonging to the Alfin-like protein family (Factors involved in stress tolerance and development) that bind to the *D. carota PSY2* promoter and activate the transcription of reporter genes. Finally, the expression analysis of these genes in response to ABA treatment, showed a significant induction of *AREB3* in roots that correlates with *PSY2* expression.

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10.- Association genetics of wood chemical composition and growth traits, and transcriptional validation of candidate genes in *Populus trichocarpa* subjected to abiotic stress conditions

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An increase in the consumption of distinct energy resources is expected for the coming years. That is driving the search of new biofuel sources, including lignocellulosic biomass from forest trees to obtain bioethanol. However, the efficiency of the production processes depends on several biomass characteristics, as such as growth rate and chemical composition of wood. The possibility of using marginal lands, not utilized for agricultural purposes, to produce bioethanol, supposes the ability of trees for coping with multiple abiotic stress conditions. Under those conditions, molecular mechanisms can be regulated differentially, triggering distinct phenotypic responses in trees. In order to know the expression patterns associated with genes underlying growth and chemical components of wood, under stress conditions, we apply an approach based on association genetics and gene expression to study the response of *Populus trichocarpa*. We identify Single Nucleotide Polymorphism (SNPs), within a suite set of 40 genes involved in the biosynthesis of cellulose and lignin, significantly associated with growth and wood traits. Association population comprised 460 clones established in a common garden. Then, we compared the transcript abundance of two key candidate genes (*4CL2* y *CesA1A*) in leaves of plants grown hydroponically under copper excess and gravitational stress, during 6 weeks. One hundred and six significant associations were identified among SNPs and traits. Finally, the transcriptional analyses indicated that there were not significant differences in transcript levels for genes.

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11.- Assessment of biochemical parameters in *Colobanthus quitensis* growing in substrate contaminated with Cu⁺² ions.

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Copper is an essential micronutrient for plant growth, involved in the oxidation-reduction processes, however, slightly higher concentrations, than those required for growth, affect various biochemical and physiological processes leading to induced cell death. *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) is one of two species of native vascular plants that colonized maritime Antarctic and also distributed latitudinal and altitudinal from southern Mexico. Their habitats are always extreme, so this species has developed; molecular, biochemical and physiological mechanisms that allows for survival in its ecosystems, and is considered a model plant for studies of adaptation to abiotic stress. The aims of this study was to evaluate the changes in biochemical parameters induced by three concentrations of Cu ions (II) (0 (control), 0.1 and 0.5 mM) in two populations of *C. quitensis* growing in contaminated substrates. The content of MDA and proline was significantly higher in the “La Marisma” population. The enzymatic activity of catalase and the content of photosynthetic pigments (chlorophyll a and b) were greater in the “Conguillio” population. While carotenoids content and enzyme activity of guaiacol Peroxide were not significantly different. The different responses in both *C. quitensis* populations may be due to phenotypic plasticity or the development of geo-ecotypes as mechanisms of adaptation of the species to the extreme conditions of their range of distribution.

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12.- Functional analysis of putative copper binding sites in the *Populus deltoides* Kunitz trypsin inhibitor 3, a protein related with copper stress tolerance

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Poplars (*Populus spp*) have been proposed as candidate species for phytoremediation of heavy metals. Copper is an essential micronutrient for plants and a common pollutant in some countries. In previous studies, genes encoding Kunitz trypsin inhibitor (KTI) proteins were significantly upregulated in the roots of plants exposed to copper excess. However, the specific function of KTIs under heavy metal stress is scarcely known. In order to determine the role of this protein in the context of the mechanisms of copper tolerance, we focused this study on the functional characterization of the *Populus deltoides* KTI3 protein (PdKTI3). PdKTI3 was isolated and sequenced, and its protein structure was modeled. The predicted structure showed two putative copper binding domains, suggesting the ability of the protein for chelating this metal. Both sites were mutated in silico without effects on the three-dimensional structure of the protein. Then, PdKTI3 was modified by site-directed mutagenesis and used to complement a copper sensitive *Saccharomyces cerevisiae* strain (*cup2Δ*). The evaluation of the effect of mutations on its metal tolerance is in progress and will be presented in the meeting. We also analyzed the sub-cellular localization of PdKTI3 by transient expression in onion cells. The PdKTI3:GFP product was detected all over the cell, without an organelle-specific pattern. The obtained results suggest that PdKTI3 would be part of the defense mechanisms induced by copper stress, performing a role as a chelating protein.

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13.- Validation of reference genes for real-time qrt-pcr normalization during cold acclimation in the hybrid *Eucalyptus nitens* x *Eucalyptus globulus* to analyze the gene expression of the cold inducive genes elip.

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Due to its fast growth and fibre quality, *Eucalyptus* is the most commonly planted hardwood in the world. The expansion of *Eucalyptus* plantations is limited to climatic regions. Some species belonging to this genus are particularly vulnerable to freezing injury. The ability to survive freezing temperatures is reached after the exposition to low but non-freezing temperatures, a process known as cold acclimation, involving changes in gene expression. Quantitative Real-time qRT-PCR method is a reproducible and sensitive tool to quantify gene expression with high specificity in the detection of low levels of cDNA, however are necessary housekeeping genes with stable expression for analysis. This work study, the gene expression stability of four housekeeping genes, two previously validated for *Eucalyptus nitens* (FLP and EIF4- α) and two of *Eucalyptus globulus* (UBC and EF1- α) in the hybrid *Eucalyptus nitens* x *Eucalyptus globulus*. The results confirm the importance of selecting an appropriate number of reference genes for an accurate and reliable normalization of ELIP gene expression demonstrating that ELIP gene is differentially expressed at cold acclimation and shown differences in the transcript accumulation among the two species and their hybrid.

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14.- Salt stress response in lettuce type lollo rosso and lollo bionda grown in hydroponic system.

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Nowadays there is a limited water resource available for the vegetable production, and in the other hand there is an interest for the consumers to increase the consumption of fresh vegetables that are rich in health beneficial compounds. The plants exposed to abiotic stress conditions respond with an over production of radical oxygen species (ROS). These species generate damage in plants which are protected increasing the synthesis of antioxidant compounds (phenolic compounds). The main objective of this work was to evaluate the application of nutrient solutions made with salt water (0, 25, 50, 75 and 100 mM NaCl) in the hydroponic culture of two lettuces: Lollo Bionda (cv. Levistro, green) and Lollo Rosso (cv. Carmolí, red). At harvest, the following parameters were evaluated: yield, total phenolic compounds (TP) and antioxidant capacity (AC). In both cultivars, it was not observed a significant difference in the fresh weight among treatments. However, there was an increase in the dry mass with the salt raise in the nutrient solution. In the Levistro cv. was observed an increase in TP and AC in all salt treatments, especially in the highest concentrations. The AC increased in the Carmolí cv. grown with salt in all the different concentrations but there was no a significant difference among concentrations. Finally, green leave lettuces had greater response than red.

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15.- Functional characterization of class II TGA transcription factors in response to abiotic stress in *Arabidopsis thaliana*.

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Plants along evolution have developed complex mechanisms to respond to environmental stress, due to their characteristic of sessile organisms. The hormone salicylic acid (SA) is an important regulator of the plant stress responses. Relevant elements in the SA-signaling pathway are the class II TGA transcription factors (TGA2, TGA5 and TGA6). These proteins have a redundant function in the regulation of genes involved in defense and detoxification. Nevertheless, their role in the defense response, and particularly in constraining oxidative stress, has not been studied yet. In this work, we functionally characterized mutant plants in the transcription factors TGA2/5/6 (*tga256*) under stress conditions that accumulate reactive oxygen species (ROS), with the objective to determine their importance in the plant defense response. To address this point, wild type (WT), *tga2/5/6* mutant plants and two independent mutant lines complemented with a construction that expresses TGA2 gene under the control of a strong promoter (*tga256/pUBQ:TGA2-V5*) were evaluated in their responses to stress treatments with methyl viologen and UV-B radiation. We found that *tga256* mutant plants are less tolerant than WT plants, and this phenotype is complemented in TGA2 overexpressor plants. Furthermore, we evaluated the expression of defense and detoxification genes in the mentioned genotypes under UV-B treatments. These results suggest a key role of TGA2/5/6 transcription factors as a node for redox regulation in response stress in plants.

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16.- The first intron of H⁺-pyrophosphatase vacuolar of *e. globulus* (evp1) has an effect in the tissue-specific expression of gus in *A. thaliana*.

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In Chile, forestry is the second most important industry in our economy, representing *E. globulus* 21% of the commercial species grown. At present, the availability of optimal plantation sites is low, so an option would be to use land located in the north of Chile. However, this land characterizes by high salt content and low water availability. Sodium is toxic for the cells if it reaches high intracellular concentrations. A method by which cells resist this high concentration it through Na⁺ compartmentalization in the vacuole using a proton gradient. Two proteins play a key role in the generation of this gradient: vacuolar H⁺-ATPase and vacuolar pyrophosphate (VP). The overexpression of vacuolar pyrophosphate increases plant's tolerance to saline and osmotic stress. Our laboratory has isolated the vacuolar pyrophosphate gene of *E. globulus*. This gene has a promoter region of approximately 2000 bp, and an ORF made up of 5 exons and 4 introns, with the first intron being 10 times bigger than the other three introns. Furthermore, it has been reported than large introns near the 5' end of a gene are usually related to an increase of gene expression. To test this, we developed different constructs that contain the promoter with and without the intron, using a reporter gene (GUS). We established that while the promoter was capable of regulating the GUS expression in the root tip and trichomes, the construct with the intron and promoter drive GUS expression to pollen and the root tip.

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17.- The transcription factors bzip17/bzip60 plays an important role in salt response in *Arabidopsis thaliana*.

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In plants, abiotic and biotic stress leads to the accumulation of misfolded proteins in the Endoplasmic Reticulum (ER) triggering the Unfolded Protein Response (UPR). As signal transducers of this response, membrane-associated transcription factors known as bZIP28, bZIP17 and bZIP60, move to nucleus where they promote up-regulation of ER associated genes. Recently, bZIP17 and bZIP60 have been associated with the response to salt stress suggesting that UPR is associated to this response. Interestingly, salt stress does not lead to the accumulation of UPR-responding genes or the activation of the IRE1/bZIP60 branch of the UPR. Moreover, the relationship between bZIP17 and bZIP60 in this process has not been yet determined. Using simple and double mutants on bZIP17 and bZIP60, we showed that treatments with different concentrations of NaCl, impair the growth of *bzip60* mutant plants but not *bzip17*, interestingly, *bzip17bzip60* mutants rescued the growth of *bzip60*. Also, we used RNA-seq to identify stress responsive genes associated to bZIP17 or bZIP60 under salt stress. We identified differentially expressed genes on *bzip17* or *bzip60* mutant plants, none of these were regulated by both of these transcription factors, but in *bzip17bzip60* mutants, some genes recovered the expression level. In addition, we identified transcription factors that might interact with bZIP17 or bZIP60 by yeast-two hybrid analysis. We found that transcription factors associated to different abiotic stresses interact with bZIP60 or bZIP17. These results suggest that bZIP17 and bZIP60 could participate in the response to salt stress in a same or different signaling pathway. These transcription factors could interact with other transcription factors in the regulation of gene expression under salt stress conditions

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18.- Biochemical mechanisms of tolerance to copper in *Colobanthus quitensis* growing *in vitro*.

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Colobanthus quitensis is a vascular species inhabiting extreme environments describing a wide, both latitudinal and altitudinal, geographical distribution. Within its habitat a number of converging abiotic factors hinder the survival of any other species. It is therefore of interest to study the biochemical mechanisms that allow it to tolerate adverse conditions in its environment, such as high concentrations of copper. Three biochemical markers (proline content, quantification of photosynthetic pigments and lipid peroxidation (MDA)) were used on inland *C. quitensis* populations in order to evaluate the effect, of two periods, of the metal in its ability to spread *in vitro* using three concentrations (0 μ M, 100 μ M and 500 μ M of CuSO_4). It is postulated that *C. quitensis* has tolerance mechanisms that persist high concentrations of Cu. In the first evaluation there was an increase in proline content, though a decrease in the second period was observed. Both chlorophyll and carotenoids content decreased in seedlings, as the metal concentration increased in the middle of the cultivation. MDA content increased dramatically during the second assessment compared to the first. The main differences were observed in the highest concentration. This is one of the first studies showing evidence of the dynamics of tolerance mechanism to heavy metals in *C. quitensis*.

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19.- Effect of salt stress (NaCl) on antioxidant capacity in fruits of two tomato genotypes: wild type and cherry cultivar

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According to the FAO, approximately 20 % of the current 230 million ha of irrigated land is salt-affected. Salt stress in plants produces an excessive generation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals. The trial was conducted to determine the effect of salinity stress (NaCl) on lycopene (LYC), b-carotene (b-CAR), oxidized (DHA) and reduced (AsA) ascorbic acid, oxidized (GSSG) and reduced (GSH) glutathione, dehydroascorbic acid (DHA) and antioxidant enzyme activity of ascorbate peroxidase (APX) and superoxide dismutase (SOD) in fruits of two tomato genotypes: wild type (*Solanum chilense* Dun.) and cherry (*Solanum lycopersicum* var. *cerasiforme* L.). Both tomato species were grown in a greenhouse under a hydroponic system at two NaCl concentrations: 40 and 80 mM, representing two different saline stress treatments, and compared to the control at 0 mM NaCl. LYC and b-CAR were determined through high performance liquid chromatography (HPLC) stationary reversed phase. Ascorbic acid, glutathione and antioxidant enzyme activity (APX and SOD) were also determined by spectrophotometer analysis. Results showed the absence LYC and b-CAR in wild tomato. On the other hand, cherry tomato showed amounts of LYC similar to those previously reported for different tomato varieties, increasing their values when salinity increased while b-CAR contents were reduced suggesting a regulation of the these compounds when salt stress occurs. In addition, we also show the effect of salinity on the levels of ascorbic acid and glutathione composes that determine in part the mechanisms of free radical oxygen detoxification within the fruit. Results suggest that the regulation of antioxidant compose synthesis and antioxidant enzyme activity in wild tomato genotype is different to cherry genotype, thereby opening interesting opportunities for plant breeding in order to obtain new commercial varieties.

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20.- Transcriptional analysis and structural characterization of expansins involved in the molecular response to inclination in radiata pine

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Plants have the ability to reorient their vertical growth when they are exposed to inclination. In nature, conifer trees develop compression wood in response to gravitropic stimuli in the lower side of the stem. The genes involved in this phenomenon are still unknown. Several studies have identified genes encoding proteins named expansins, which are involved in cell expansion process among other developmental biological processes in vegetal organisms. Therefore, to provide new evidences relating these proteins in the inclination response in radiata pine seedlings, cDNA libraries from different times of inclination, generated by RNAseq technology, were analyzed. From those libraries, 6 putative sequences were identified and classified as expansins. Its differential transcript abundance observed in FPKM values was validated by qPCR analysis. Results showed increased transcript abundance in the lower side of stem in five genes and a repression of one in response to the gravitropic stimulus. Additionally, to gain insight about the protein structure of pine expansins and its mechanism of action at molecular level, the structures of proteins were built by comparative modeling methodology. An open groove on the surface of the proteins was observed. This open groove serves as the polysaccharide binding site and it is composed of conserved residues. We evaluate the interaction with cellulose octamer as ligand by molecular docking simulation and the results shown differences in the protein-ligand interaction mode. These differences in the binding energy interaction can be explained by changes in some residues that generate differences in electric charges in the open groove surfaces. Finally, the data is congruent with a probable role of expansin proteins in the disassembling and remodeling of the cellulose matrix during the reorientation response to the inclination stress.

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21.- Analysis of germination, vegetative growth and methylation patterns of *A. thaliana* Col. under WiFi radiation exposure.

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Several studies in the area of plant biology indicate that non-ionizing electromagnetic radiation influence on various aspects of plants. The results vary, depending on the intrinsic factors of the species in question, type of radiation and exposure time. Studies suggest that low-frequency radiation (0-10⁵ Hz) have a stimulating effect, especially at the stage of germination, while high frequency radiation (10⁶-10¹⁰ Hz) tends to have a negative effect, both in germination and plant development. Radiofrequencies (RF) and Microwaves (MW) belong to the latter range, and is where most wireless communication devices emit, such as WiFi (frequency 2.45 x 10⁹ Hz). The great development of telecommunications in recent decades has raised the alarm of a possible “electromagnetic pollution” that may cause long-term damage to living organisms. Thermal effects were first studied and the current legislation in the world is based on them, but today the controversy is centered on the potential damage caused by non-thermal effects. The aim of this study was to analyze the germination and vegetative development of *A. thaliana*, as well as, the methylation patterns of genomic DNA extracted from leaves under exposure to wireless radiation from a household router. Germination of 100 seeds was monitored for 14 days under 24 h of continuous exposure, and vegetative development was monitored at 21 days. Methylation patterns of genomic DNA were analyzed in exposed and control leaves from the last experiment, using Methyl-Sensitive Amplification Polymorphism (MSAP). Results indicate that germination is not affected by the presence of WiFi radiation, while foliar and root biomass of seedlings exposed reveal important differences to the control seedlings. These results were contrasted with epigenetic patterns.

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22.- Effect of gibberellic acid on cracking and gene expression in sweet cherry fruits

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Sweet cherry production is presented as a good business in Chile, because the country has managed to position itself as the second largest exporter of cherries, being in a privileged position as an off-season cherries supplier in the North Hemisphere markets. One of the most important problem associated with the production corresponds to fruit cracking which is mainly caused by rain fall during preharvest. In this regards, there are several practices used to reduce it, including the application of gibberellic acid (GA_3), which is commonly used in the cherry orchards to delay harvest time and increase firmness, showing also effects on the decrease of cracking. In this work, several parameters of fruit quality were assessed at ripening. Also cracking was evaluated *in vitro* as well as in the field. Expressions of genes involved in the wax/cuticle biosynthetic pathway (*Cer6*, *Cer4* and *ABC transporter*) and in cell wall modification (*Expansin* and *XET6*) were analyzed at fruit color change and fruit ripening. Three sweet cherry varieties with different tolerance to cracking were used: Kordia, Regina and Lapins. Four doses of GA_3 (0, 10, 20 and 30 $mg \cdot L^{-1}$) at two developmental stages (translucent green and straw-yellow) were applied. GA_3 applications reduced cracking susceptibility by 6% in 'Lapins', 7% in 'Regina' and 15% in 'Kordia'. Additionally, GA_3 applications increased the expression of *Cer4*, *Expansin* and *XET* in 'Lapins' and decrease them in 'Kordia' at the ripe stage of development. However, an increase of *Cer6* expression using 10 $mg \cdot L^{-1}$ of GA_3 at the straw-yellow stage was observed in 'Lapins'. Based in our analysis we can conclude that GA_3 applications reduced the incidence of cracking and also trigger a change in the expression of genes related with cell wall modification and wax/cuticle biosynthetic pathway.

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23.- Small intrinsic protein (sips) family in two *Prunus* rootstocks under waterlogging stress.

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An oxygenated root atmosphere is essential for plant growth. However, in field may occur a hypoxic condition due to waterlogging problems by poor drainage, where water saturates the soil pores and, as consequence, gases are displaced which limits the oxygen availability. *Prunus* rootstocks are classified as hypoxia-sensitive, although a tolerance degree among genotypes has been reported. In plants, waterlogging limits the water absorption thus leading to an internal water deficit. The water flow is majorly modulated by proteins called aquaporins, which facilitates the transport of water through the cell. These proteins, belonging to the membrane intrinsic proteins (MIP) family, have six hydrophobic α -helices forming a pore in the membrane and have two characteristic motifs (NPA and the constriction region, aromatic/arginine (ar/R)). These transmembrane proteins can be classified in different subfamilies: PIP, TIP, NIP, SIP, XIP and LIP. SIP (Small Intrinsic Protein) subfamily is characterized by its shorter N-terminal tail in comparison to the rest of aquaporins. We identified three genes of SIP subfamily in the *Prunus persica* reference genome. qRT-PCR analyses evidenced differences in the transcript levels of these genes between a root hypoxia tolerant- and sensitive- genotype of *Prunus* rootstocks under waterlogging stress. *In silico* studies show the formation of two subgroups (SIP1 and SIP2) with variations in their motifs, which could give special characteristics to this subfamily. In addition, changes in the promoter region could be the reason for the differential transcriptional activity of *SIP* genes detected in contrasting genotypes of *Prunus* rootstocks under root hypoxia by waterlogging.

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24.- Differential water stress induced senescence in three quinoa genotypes growing under two levels of nitrogen

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Grain filling is one of the most drought-sensitive stages during plant growing cycle and drought stress accelerates senescence and shortens the grain filling period reducing grain yield. In the same way nitrogen deprivation accelerates senescence even under well watering conditions. *Chenopodium quinoa* Willd. is an Amaranthacean plant able to grow under many abiotic stresses with an extraordinary capacity of nitrogen stores in their seeds. We hypothesized that drought tolerant genotypes of Quinoa present higher yield performance because are able to delay stress-induced senescence, retaining assimilatory and photosynthetic capacities under drought conditions. Three genotypes from central (Faro and Udec9) and south of Chile (BO78) were grown at two levels of N conditions: High nitrogen (HN, 146 Kg/hc) and low nitrogen (LN, 100 Kg/hc). After 2 weeks of flowering initiation, watering was 70% reduced (WS) to the end of the life cycle. Measurements were performed after 14 days of water treatment. Differential N-supplementation induced changes in photosynthesis, protein and chlorophyll content in Udec9 and BO78 genotypes. Under WS, BO78 exhibited the lowest photosynthesis level without differences between N conditions. Respect to the effect of drought in N assimilation, NR transcript was maintained in Faro in both HN and LN, while was reduced in Udec9 and BO78. These results are related with the stronger maintenance of proteins in Faro leaves respect to Udec9 and BO78. Finally, Faro presented lowest penalty on yield under LN and LN/WS, and shows significantly higher Nitrogen-Use-efficiency with respect to Udec9 and BO78 without reduction on N per seed. Concluding, Faro is able to delay stress-induced senescence maintaining a greater carbon and nitrogen assimilation under water scarcity and LN allowing to maintain yield. This study will contribute to breeding programs with focus on the development of varieties adapted to diverse drought conditions and nitrogen deficiency.

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25.- *PaKCS6* expression and wax accumulation in different sweet cherry cultivars affect tolerance to cracking.

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Sweet cherry is an important fleshy, non-climacteric fruit growing in temperate regions worldwide. In Chile the planted surface has grown exponentially in recent years due to a higher demand in international markets. One of the major losses in the industry is fruit cracking that has been reported in many sweet cherry varieties. The molecular/genetic factors involved in the differential tolerance of different cultivars of sweet cherry to cracking are still unknown. The cracking is mainly caused by rain fall during the harvest period, promoting water absorption through the skin by increasing the osmotic pressure and its volume inside the fruit, triggering the disorder. One component that can act as a barrier is the cuticle that corresponds to a semipermeable membrane principally formed by waxes. We hypothesized that genes involved in wax biosynthesis could be good molecular markers to be used in the selection for varieties tolerant to cracking. RNA-seq was performed at two different stages of fruit development. Expression levels of genes involved in the wax biosynthetic pathway were compared. An increase in the accumulation of transcripts level for some genes as *PaKCS6* (key gene for elongation of alkanes), *PaLACS1* (long-chain acyl-CoA synthetase 1) and *PaLTPG1* (Glycosylphosphatidylinositol-Anchored Lipid Transfer Protein) was observed during the fruit ripe stage of a more tolerant cultivar (Regina). In addition, components of the cuticle like alkanes, triterpenoids and sterols show a higher accumulation by GC-MS in “Regina” compared to Bing, a less tolerant cultivar. qPCR analysis confirmed the results of the RNA-seq. An increase of *PaKCS6* was observed in “Regina” versus “Bing”, after an in vitro cracking test. We conclude that an increase of *PaKCS6* could be involved in the triggering of wax accumulation at the ripe stage of sweet cherry showing tolerance to cracking.

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26.- Transcriptional profiling in response to cold acclimation in *Eucalyptus nitens* and *Eucalyptus globulus*

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In Chile, *Eucalyptus globulus* is the second cultivated species for the forest industry. However, the sensitivity of this species to low temperatures limits its productivity and distribution. In colder areas is replaced with *Eucalyptus nitens*, a frost tolerant species. Transcriptional profiling studies using RNA-seq and differential expression analysis *in silico* have offer a deeper insight to understand the molecular mechanisms of cold response. This study shown a comparative transcriptomic analysis in response to cold acclimation in *E. globulus* and *E. nitens*, to identify the molecular mechanisms controlling cold acclimation that could explain the differences in the cold tolerance. The transcriptional profiling was compared in non acclimation (NA) and two cold acclimation conditions; to chilling temperatures (CABF) and freezing temperatures (CAAF). Comparing differentially expressed genes (DEG) at different cold acclimation stages in both species allowed to identified exclusive DEGs for *E. nitens* and *E. globulus*. Gene ontology analysis (GO) for two different gene clusters showed differences in GO tems represented for biological process (BP), cellular component (CC) and molecular function (MF). Among unique GO terms, like “Metabolic process” for *E. globulus*, and “oxidation reduction” for *E. nitens* in BP. These differences in DEG and gene ontology terms associated to *E. nitens* and *E. globulus* suggest that *E. nitens* developed a different mechanism for cold acclimation which could explain the frost tolerance.

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27.- Expression analysis of, *1-sst* and *6G-fft*, two key genes involved in the synthesis of fructans in *Aloe barbadensis* Miller.

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Fructans have been considered for many years reserve polysaccharides that consist mainly of fructose units linked to a terminal glucose or to a terminal fructose. Previous reports have shown that these polysaccharides can protect the integrity and function of cell membranes and proteins during dehydration caused by either drought or low temperatures. Different plant species are known to synthesize and accumulate fructans in response to these abiotic stresses. One of these plants is *Aloe barbadensis* Miller, also known as *Aloe vera*, a monocot CAM plant adapted to grow in arid environments. Previous results from our group have shown that *Aloe vera* plants have higher concentrations of fructans when irrigated with 50% and 25% field capacity (FC) than 100% FC irrigated plants. Glycosidic linkage analysis confirmed an increase of neo-fructans in its leaves, a highly branched sub-type of fructans. Considering our previous results, we have analyzed the expression of two crucial genes involved in the initial fructan biosynthesis in *Aloe vera*; *1-sst*, sucrose: sucrose 1-fructosyltransferase responsible for the formation of the trisaccharide 1-kestose and *6G-fft*, fructan: fructan 6G-fructosyltransferase which synthesizes the trisaccharide 6-kestose. Preliminary PCR results indicate the presence of both genes, *1-sst* and *6G-fft*, in *Aloe vera*, while RT-qPCR analysis shows an increase expression of *1-sst* in plants irrigated with 50% and 25% FC. By using MALDI-TOF-MS we have also detected a significant increase in the degree of polymerization of *Aloe vera* fructans in plants irrigated with 25% FC compared to those irrigated with 100% FC.

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28.- Water stress induced by mannitol in a wild tomato relative *Solanum sitiens*.

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Solanum sitiens is a xerophyte species and endemic of Antofagasta Region in northern Chile. This species inhabit some of the driest parts of the Atacama Desert. This member of the *Solanaceae* family is located in a very narrow range of elevation (2,500-3,000 m). *S. sitiens* has a high potential economic value due to its extreme aridity tolerance; however, this characteristic still has not been studied. Mannitol has been used in several studies for its capacity to induce in vitro water stress, and according to its concentration determines different water potential expressed in MPa. This study aimed to determinate the tolerance level in water stress induced by different mannitol concentrations. The concentrations were correlated with the osmotic potential in the culture media. In vitro 3 cm shoot cuts were used. The explants were established into glass tubes with 20 mL of Murashige & Skoog (MS) and exposed to the following treatments T0: 0% (0 MPa), T1: 0.15% (-0,4 MPa), T2: 0, 2% (-0,5 MPa) T3; 0.3% (-0,7 MPa) of mannitol. Plant survival was observed and T2 0, 2% showed the higher values compared to T0.

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29.- Molecular characterization of biosynthesis pathway and quantitative determination of endogenous jasmonic acid in two *Prunus* rootstocks under waterlogging stress

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Phytohormones play essential roles in the plant signaling and responses under several abiotic stresses. The effect of abscisic acid (ABA), salicylic acid (SA) and ethylene has been extensively investigated. Jasmonic acid (JA) is mainly related to regulation of the plant defense against pests and pathogens, but its role under abiotic stress is not clearly elucidated. The hypoxic stress, caused by waterlogging, by poor drainage of soil and/or due to inefficient irrigation practices, is one of the most important causes of crop losses. Several species of the *Prunus* genus, used as rootstocks, are particularly affected by waterlogging stress. To evaluate the role of JA on the response to hypoxic stress in *Prunus* rootstocks, a waterlogging treatment of two genotypes with contrasting response to hypoxic stress was performed. Quantitative PCR analyses were conducted to determine the gene expression level of the isoform of genes coding for main enzymes involved in the JA biosynthesis [*lipoxygenase* (LOX), *allene oxide synthase* (AOS), *allene oxide cyclase* (AOC) and *oxophytodienoate reductase 3* (OPR3)] and jasmonic acid carboxyl methyltransferase (JMT), a key enzyme for the jasmonate-regulated plant responses, in root samples of both genotypes. In addition, it was performed an analysis of hormonal levels in roots and leaves of the *Prunus* rootstocks under waterlogging. Quantification of JA and its conjugated form, Jasmonoyl Isoleucine (JA-Ile), were performed. JA role in *Prunus* rootstocks response under waterlogging stress and the relation between gene expression level and hormones accumulations are discussed.

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30.- Transcriptomic analysis of *Solanum peruvianum* and *Solanum lycopersicum* in response to drought stress.

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Solanum peruvianum is a wild tomato that inhabits the Atacama Desert. Under this environment *S. peruvianum* suffer several stresses such as drought, cold, salinity and high radiation. Tomatoes are the most cultivated vegetables in the world with an important economic value. This crop is very sensitive to the water deficit affecting severely its productivity and fruit size. Genome-scale gene expression profiling studies will provide a complete knowledge in transcriptional regulation mediated by abiotic stress in this tolerant specie. In this research we investigated the genes differentially expressed in leaves of *S. peruvianum* accession Q958 and *S. lycopersicum* var. Money maker under two severities of drought stress, PEG 5% and PEG 10%. They were also compared with the expression of genes during treatments with ABA and Ethylene. In both drought treatments we have identified more than 5000 significant differentially expressed genes (DEG) for both species. For *S. peruvianum* considering a fold change (FC)=2 and FDR=0,05 we identified 440 and 918 DEG during 5% and 12% PEG respectively. 65 genes were upregulated during PEG 5% compared with 488 induced by PEG 12%. Contrarily, the number of genes upregulated in *S. lycopersicum* was higher in PEG 5% (199) compared with PEG 12% (128). The main pathway modified by drought stress for both species were related with sugar, aminoacid, lipids and mevalonate biosynthesis. Gene ontology enrichment studies showed that the most important signal response were ABA, Ethylene, Salicylic acid and Jasmonic acid. These results suggest that while similar signaling can be involved in the expression of genes under drought stress, this regulation is modulated differentially between *S. peruvianum* and *S. lycopersicum* depending of severity of the drought.

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31.- Functional characterization of salicylic acid-inducible genes coding for GSTs and GRXs in the defense response to stress in *Arabidopsis thaliana*.

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Plants are constantly exposed to biotic and abiotic stress conditions that increase the production of reactive oxygen species (ROS). Plants survival depends on a complex balance between the production and detoxification of ROS. Salicylic acid (SA) is a key hormone in the establishment of the defense response to stress, being essential for the production and also for the contention of the oxidative burst needed to establish the defense responses. Still needs to be elucidated if this contention is due to a modulation of the redox state, the induction of antioxidant genes, or both. Using the genetic coded sensor GRX1-roGFP we evaluated the antioxidant role of SA in the modulation of the redox state *in vivo*. Then we performed an extensive analysis of available microarray databases to determine the expression patterns of antioxidant genes *GLUTATHIONE-S-TRANSFERASES* (*GSTs*) and *GLUTAREDOXINS* (*GRXs*) under different stress conditions where SA is involved as a signal. We selected 4 *GSTs* and 2 *GRXs* genes and confirmed their expression patterns under stress conditions using real-time PCR. We used mutant or silenced plants for the selected *GSTs* and *GRXs* genes and evaluated their relevance to overcome different stress conditions such as treatments with methyl viologen, and UV-B radiation. Our results indicate that SA induces the expression of a set of *GSTs* and *GRXs* genes in a temporal specific manner and that they are important for the contention of oxidative damage produced by these abiotic stress, suggesting a particular role for them in controlling ROS accumulation in the plant defense responses.

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32.- Salt stress response triggers activation of the jasmonate signaling pathway leading to inhibition of cell elongation in *Arabidopsis* primary root

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Salinity is a severe abiotic stress that affects irrigated croplands. Jasmonate (JA) is an essential hormone involved in plant defense against herbivory and in responses to abiotic stress. However, the relationship between the salt stress response and the JA pathway in *Arabidopsis* is not well understood at a molecular and cellular level. In this work we investigated the activation of JA signaling by NaCl and its effect on primary root growth. We found that JA-responsive *JAZ* genes were upregulated by salt stress in a *COI1*-dependent manner in the roots. Using a JA-Ile sensor we showed that activation of JA signaling by salt stress occurs in the meristematic zone and stele of the differentiation zone. This activation was dependent on JAR1 and proteasome function. We also found that the elongation zone (EZ) and its cortical cells were significantly longer in JA-related mutants (*AOS*, *COI1*, *JAZ3* and *MYC2/3/4* genes) compared with wild-type plants under salt stress, revealing the participation of the canonical JA signaling pathway. Noteworthy, osmotic stress, a component of salt stress, inhibited cell elongation in the EZ in a *COI1*-dependent manner. We propose that salt stress triggers JA-Ile accumulation in the roots leading to activation of the JA signaling pathway followed by transient inhibition of cell elongation in the EZ. Therefore, we demonstrate a crosstalk mechanism between the salt stress response and the JA pathway that regulates root growth in *Arabidopsis*.

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33.- Oxidative damage induced the wheat (*Triticum aestivum* L.) activation of antioxidant enzymes during drought and oxidative stress.

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Drought stress is one of the most important abiotic factors that affect the crops in the world. The wheat (*Triticum aestivum*) is the most cultivated cereal in Chile reaching at 37% of cultivated land surface. The production of wheat is associated to dry lands, where the pluviometry is lowest during spring and summer. Previously we have identified several accessions from germplasm collection of wheat with contrasting tolerance to drought. The aim of the present research was determinate the role of antioxidant enzymes in the drought tolerance of four contrasting accessions of wheat. They were germinated and seedling of seven day old were used for drought and oxidative stress (Paraquat) treatments. The measurement of enzyme activity was measured at 48 and 72 hours then of start the treatments for ascorbate peroxidase (AsPX), Glutation reductase (GR), Guaiacol peroxidase (GP) and Superoxide dismutase (SOD) that were evaluated in the leaves of plant seedling. These measurement were correlated with the relative water content (RWC), lipid peroxidation (MDA) and chlorophylls content for the treatments and combinations. The results were analyzed for the treatments using ANOVA. The drought stress affected severely the chlorophyll content and RWC. At the same time the combination of drought and oxidative stress induced the higher increase of lipid peroxidation compared with the drought or oxidative stress separately. The peroxidase activity (POX) was increased by drought stress but it was mainly induced then of 72 hours of drought or drought-paraquat treatments. The activity of SOD, GR and AsPX was induced by the treatments from 48 hours without significant differences at 72 hours. It was correlated with the increase of MDA and reduced chlorophyll content suggesting a enzyme activity regulation mediated by oxidative damage.

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34.- Identification of the stomatal behavior of three grapevine varieties *Vitis vinifera* L. from a commercial vineyard in the Cachapoal valley.

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A challenge for sustainable agriculture is to produce more, with less water. Therefore, further understanding on the responses of cultivated plants to water shortages is needed, particularly on the stomatal responses and the efficiency of the water use. Grapevine varieties have evolved in different climates developing divergent stomatal responses to water deficit. We studied the stomatal responses to water stress of three varieties of *Vitis vinifera* L.: Syrah (S), Cabernet Sauvignon (CS) and Carmenere (C). Plants were irrigated with 12 Lh⁻¹ (T1) or 4 Lh⁻¹ (T2) drip emitters per plant, from veraison until harvest, resulting in 180 mm and 60 mm of water for the period, respectively. Stem water potentials were higher in T1 than T2 plants, but lower levels were found in CS and C compared to S. No differences were observed in leaf water potential (Ψ_{leaf}) between irrigations treatments however, CS and C shows higher levels than S and C. Also, a poor correlation between transpiration and VPD occurred in CS and C compared to S, but S show a lower stomatal conductance responses to VPD than CS and C. Stomatal conductance values in sunlight exposed leaves of S varied up to a lesser extent compared to CS and C. Different from S, a high correlation between E as well as A, and stomatal reduction occurs in C and CS. A negative correlation between WUEi and Ψ_{leaf} is observed in CS and C, and no correlation at all occurs in S. We concluded the anisohydricism may become a handicap for adjusting WUEi upon water scarcity.

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35.- Drought responses of naturalized grapevine (*Vitis vinifera*) as rootstocks for northern Chile.

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One of the main restrictions that affect grapevine productivity is water availability and seasonal drought occurring in most of the world's producing regions, a condition that is especially increasing in semiarid regions, which have experienced severe declines in rainfall in recent years. The aim of this work was to determine tolerance of naturalized grapevine rootstocks and to analyse morpho-physiological responses, plant performance and targeted gene expression of genotypes tested in water stress. We assessed five genotypes: G25 and G32 (Copiapó valley); G57, G65 and G70 (Huasco valley), from our latitudinal gradient collection "GermoVidNor" (18 ° SL to 32 ° SL). The genotypes were established at complete random block design with three replicates at field conditions in two sites from Coquimbo Region (Vicuña & Las Cardas), where moderate and severe stress treatments were applied, along with full irrigation as control. Measurements were performed over several seasons using firstly own-rooted and then grafted isohydric and anisohydric cultivars (Cabernet Sauvignon & Syrah respectively). Both morphometric and functional parameters measured resulted in enhanced performance of G65 under severe stress in own-rooted plants. Gene expression was highly variable for each season, suggesting that initially up-regulated genes shifted to down-regulation progressively: gene expression such as *GDH*, *NECED1* and *NECED2*, *AP2/EREBP*, *VvNAC1* and *AUXIN INDUCED*, aquaporin (*TIP2.1* and *PIP2.2*) were measured. Interestingly, genotype performances were modified when cultivars with contrasting hydric regulation were grafted on them, suggesting a rootstock-based regulation of drought responses over the cultivars used as scions.

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Biotic Stress

36.- Effect of the use of tomato INIA rootstock on defense mechanisms grafted plant antioxidants limachino variety during infection *Pseudomonas syringae* pv. tomato

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Pseudomonas syringae pv *tomato* (*Pst.*) is one of the most harmful bacteria for tomato production, causing significant economic losses due to diseases produced both in fruit and leaf tissues. To develop these pathogenic infections *Pst* is provided with several virulence factors that trigger different mechanisms of plant defense. The use of rootstocks is an effective solution for the disease control, especially in sensitive tomato varieties like a local Limachino tomato, which it is highly affected by environmental conditions such as salinity, drought and pathogens, decreasing their productivity. Several studies include the production of H₂S during pathogenic infections, which also has been demonstrated that compounds can induce antioxidant enzymes and flight levels (leaves and stems). In addition, the activity of *DES1*, the enzyme most probably responsible for the bulk of H₂S production is increased in infected plants. The aim of this study is to determine the effect of tomato rootstock INIA on the defense response of the grafted plant variety Limachino to *Pseudomonas syringae* pv. *Tomato* by synthesis of H₂S in roots and leaves. Growth parameters and damage produced by the bacteria will be assessed in the plant. Furthermore, the plant physiology and major antioxidant enzymes activities in the defense mechanism will be studied. The plant defense response through the antioxidant mechanism in the aerial part (graft) will be determined. H₂S synthesis and the expression of genes encoding enzymes involved in synthesis of this molecule will be analyzed at graft and rootstock.

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37.- Genetic improvement and molecular characterization of *Vitis vinifera* resistance to fungal pathogens *Botrytis cinerea* and *Erysiphe necator*

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Grapevine (*Vitis vinifera* L.) is one of the most important fruit crops worldwide and is greatly affected by a large number of pathogens that cause diseases in pre- and post-harvest periods. This plant is highly susceptible to *Botrytis cinerea*, a necrotrophic fungus that obtains nutrients from dead tissue, and *Erysiphe necator*, a biotrophic fungus that feeds on nutrients obtained from living tissue. Due to the agronomic importance of *V. vinifera*, it is essential to introgress resistant traits against these pathogens and to understand how plants respond to the infection. The aim of this work is to generate *V. vinifera* plants with greater resistance to *B. cinerea* or *E. necator* by traditional breeding and to characterize at the molecular level the defense responses against each pathogen. For this, experimental crosses were made using plants with greater level of resistance and susceptible plants of commercial interest. Thus, varieties with lower susceptibility to infection by *B. cinerea* or resistance to *E. necator* were selected by phenotypic analysis. The selected plants were evaluated through biochemical and histological strategies in order to go deeply into the defense response induced by each kind of pathogen. Our preliminary results indicate that resistance against *B. cinerea* is mainly due to mechanical barriers, while resistance to *E. necator* is related to an effector triggered immunity or ETI response. This work will contribute to the study of plant-pathogen interactions in agronomically important crops.

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38.- Evaluation of antioxidant enzymatic activity of recombinant glutaredoxins GRXC9 and GRXS13 from *Arabidopsis thaliana*

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As plants are constantly exposed to stress conditions, they are equipped with a variety of mechanisms to tolerate and survive to these conditions. One of them is the antioxidant capacity to constrain the increase in production of reactive oxygen species (ROS) that occurs after biotic and abiotic stress. In fact, plant cells are equipped with various antioxidant enzymes that contribute to maintain a reduced intracellular state. Glutaredoxins (GRXs) are low-molecular-weight oxidoreductases that play an important role in redox regulation in eukaryotic and prokaryotic cells. They use glutathione (GSH) as electron donor to catalyze different reactions: direct reduction of peroxides or dehydroascorbate (DHA) or regeneration of other antioxidant enzymes such as peroxiredoxins, by reduction of disulphide and glutathionylated thiol groups. We are interested in elucidate the enzymatic activities of two GRXs from *Arabidopsis*, GRXC9 and GRXS13, that previously we reported are involved in the stress defense response mediated by salicylic acid (SA). These enzymes belong to the CC-type GRXs (also called ROXYs) that are exclusive and represent the most numerous group of GRXs in higher plants, for which the enzymatic activities have not been determined. We expressed and purified recombinant versions of both GRXs from *E. coli*. Currently we are evaluating the enzymatic activities of these GRXs, to explain their role in protect plants against oxidative stress.

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39.- Study of the *Actinidia* spp. defense response against *Pseudomonas syringae* pv. *actinidiae* infection.

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Kiwifruit is a plant species originally from China which has been recently introduced and commercially exploited in several countries. Italy, New Zealand and Chile are the leaders in the exportation of this fruit. Finding new varieties with improved organoleptic and yield features have been the main focus of the kiwifruit industry. Since 2008 the kiwifruit industry is being severely jeopardized because the disease produced by the bacteria *Pseudomonas syringae* pv *actinidiae* (Psa), which produces big economic losses worldwide. Therefore, finding kiwifruit varieties with improved resistance to Psa has turned into a crucial trait to pursuit. This disease produces the progressive death of all the plant tissues, inhibiting growth and fruit development. In this work we evaluated the physiological and molecular response of kiwifruit varieties to Psa. We introduced 10 kiwifruit varieties to *in vitro* culture to produce clonal plants grown under controlled conditions and evaluate in them the Psa effects. To characterize the molecular components of the plant response, we identify and assessed the expression of marker defense genes in 3 varieties. We have amplified and sequenced several putative defense genes and housekeeping genes from these varieties and comparing the sequences we observed a high conservation among the genes from the different varieties evaluated. We also evaluated the callose deposition after Psa attack as a parameter of the PTI response; we detected callose deposition 48 hpi with Psa in the cultivar KC5.

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40.- Wrky iid transcription factors regulate the plant immunity acting as repressors in a Ca²⁺/calmodulin dependent manner in *Arabidopsis thaliana*.

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When plants recognize a pathogen, disease resistance responses activate the expression of defense genes, among them PR (*Pathogenesis Related*) proteins that are secreted to counteract the pathogen proliferation. The high demand of PRs secretion generates endoplasmic reticulum (ER) stress and triggers the Unfolded Protein Response (UPR), which leads to an increased synthesis of chaperones to restore the ER homeostasis. This response requires a fine-tuning of the UPR genes expression to avoid programmed cell death. Recently, we described the role of WRKY IID transcription factors (TFs) as repressors involved in the early and transient kinetics of UPR genes (WRKY7, -11 and -17). Interestingly, the WRKY IID present a CaM binding site. The WRKY IID/CaM interaction was analyzed *in vivo* using BiFC experiments in tobacco leaves, showing that WRKY TFs interact with CaM in a Ca²⁺ dependent manner. Moreover, we co-infiltrate tobacco leaves with fusions of WRKY to GFP and CaM to RFP which allow us to observe its subcellular location and its correlation with calcium dynamics. To further characterize the WRKY IID/CaM interaction, its complex was analyzed *in silico* using advanced molecular simulation methods, such as homology modeling, molecular dynamics and MM-GBSA. This combined experimental/theoretical approach allow us to analyze the effect of substituting selected residues in WRKY TFs, determined *in silico* to play a role in the complex stabilization. Our data suggest that Ca²⁺ is able to modulate WRKY7, -11 and -17; in a CaM dependent manner uncovering a connection between ER calcium dynamics and UPR during the establishment of plant immunity.

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41.- Differentially expressed genes detection through relative rt-qpcr in meeker variety *Rubus idaeus* plants in the Maule region.

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ToRSVinfected *Rubus idaeus* reduces production and its fruits' organoleptic quality, financially affecting producers. To determine the differential gene expression in response to ToRSV infection contrasted against infection-free plants, 13 starters were analyzed through RT-qPCR; two correspond to the actin and histone reference genes. The other starters refer to genes related to the defense of ToRSVinfected *Nicotiana benthamiana* plants (Dardick, 2007). For the ToRSV infection-related gene expression analysis, healthy and infected Meeker variety plant leaves were used. Reactions were performed in LineGene9660 (Bioer) equipment, using SYBR Green as fluorophore. Relative expression analysis was determined through comparing the interest gene expression to a reference gene (actin). Relative expression values of the interest genes were calculated using $2^{-\Delta\Delta Ct}$. In the analyses of the expression levels differences in the Meeker variety it was impossible to verify the expression of pathogenesis-PR and protein synth genes, in healthy and infected plants. The interest genes expression was observed in lower rates than the actin gene. Chloroplast-photosynthesis was the gene with the greatest expression. The methodology and results will be used for standarizing other genes in Heritage, Amira, Amity and Chilliwack varieties, which will provide a view on how the infection kinetics develops. Additionally, this methodology will provide a primary focus of which genes are present in the *Rubus idaeus* viral infection defense system.

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42.- Molecular characterization of the virulence of Chilean isolates of *Pseudomonas syringae* pv. *actinidiae* in kiwifruit

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Pseudomonas syringae pv. *actinidiae* (Psa) is the kiwifruit pathogen responsible for causing the bacterial canker disease, which is destroying entire kiwifruit orchards and causing extreme economic losses worldwide. Chile is the third country in the production of kiwifruit after Italy and New Zealand, and we are not free of this outbreak. Psa has been classified into four groups, being the Psa 3 (also called Psa-V) the most virulent form. Although many efforts have been done to understand the high virulence of this bacteria, little is known about the molecular mechanisms behind the Psa biovar 3 virulence. In order to contribute to the understanding of the molecular mechanisms of the infection in Chile, we characterized fifteen Chilean Psa isolates identified and kindly facilitated by the Servicio Agrícola Ganadero de Chile (SAG). We confirmed, in terms of microbiological behavior and molecular analysis, that fourteen out of fifteen isolates were indeed Psa. We also evaluated whether the Psa isolates were able to do swimming and swarming movements, which have been associated to the virulence of some pathogens. On the other hand, to evaluate the influence of the effector proteins on Psa virulence, we have site-directed mutated two Psa isolates in three putative effectors conserved in *Pseudomonas* species. The Psa mutants were then inoculated in a kiwifruit variety to assess infection parameters in comparison to the wild type isolates.

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43.- Method for tosv detection through absolute rt-qpcr in *Rubus idaeus* plants from the Maule region

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The tomato ringspot virus (ToRSV) affects *Rubus idaeus*. Infected plants present certain symptoms indicating the presence of said virus but, in most cases, there is no specific infection sign (asymptomatic plants) which makes more sensitive molecular analyses necessary. This study is based on ToRSV identification in *Rubus idaeus* plants through absolute RT-qPCR using specific primers designed in an early stage from sequences obtained from the gene bank (DQ641947.1; KM083894.1; GQ141528.1; GQ141527.1; GQ141525.1; KP759299.1). The obtained amplicon was inserted in a cloning vector with which the calibration curve was built. Used dilutions were 1:5, 1:10, 1:25, 1:50, 1:100, 1:150, 1:200 (3 repetitions/dilution). The obtained curve was $Y = 41,08 - 3,48X$, $R^2 = 0,99$. Transcript levels obtained from evaluated varieties were from 4 to 574 ng/ μ L for the Coho, Chilliwick, Heritage, and Meeker varieties. Genetic distance analyses determined a high variability in the sequences; therefore, specific starters were developed again for Heritage, Meeker and Amity varieties. Obtained amplicons were of 170 bp in Heritage, 130 bp in Meeker and two amplicons for the Amity variety of 180 and 110 bp. New standard calibration curves were built. The results of this study show that transcript levels of the virus were detected in 50% of the raspberry plants in the Heritage, Meeker and Amity varieties when compared to the infection-free plants. The developed method is efficient in ToRSV detection; hence, it can be used as certification method for virus-free plants in greenhouses that wish to spread high sanitary quality plants.

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44.- Evaluation of antifungal activity against *B. cinerea* and structural identification of biotransformation extracts of terpenylated phenols

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Terpenylated phenols are compounds from plants and animals, which possess multiple biological activities, including antioxidant and cytotoxic properties. Their applications may be extended to the industrial and to agriculture and pharmaceutical sectors. The aims of this study were to generate and characterize terpenylated phloroglucinols derivatives by biotransformation with the fungus *Gibberella fujikuroi*. The biotransformation for the preparation of potential agrochemicals, such as mono and di-alkylated (geranylated and prenylated) phloroglucinol derivatives, allows using reactions under milder conditions, eliminating side reactions and decreasing strongly the environmental impact. Six molecules obtained by biotransformation were characterized by NMR. The molecules have a structural pattern that indicates to the oxidation of the side chains or alkyl chains causing separation of the aromatic ring. *In vitro* cytotoxicity tests indicate that the extracts obtained from extraction with organic solvents with higher polarity (from liquid culture medium after 17 days biotransformation) possess significant cytotoxic capacity against an important phytopathogenic fungus, *Botrytis cinerea*, achieving almost 100% of growth inhibition. The compounds applied to plant tissue (leaves of *Solanum lycopersicum*) infected with the fungus showed high fungal inhibition.

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45.- Global gene network to assess molecular mechanisms involved in N-modulation of plant susceptibility to pathogens in *Solanum lycopersicum*.

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Nitrogen (N) is a key macronutrient for crop production and the major limiting factor of plant growth, development and yield in agricultural fields. Thus, intensive crop production relies on a large input of this fertilizer. Previous studies have shown that nitrate availability may have significant effects (both positive and negative) on the plant capacity to defend against pathogens. Nevertheless, the mechanisms underlying the connection between N and defense responses are poorly understood. Employing a genomic approach, in this study we analyze and characterize the plant defense response at different concentrations of nitrate in tomato plants infected with *Botrytis cinerea*. For this purpose, global gene expression in tomato plants (*Solanum lycopersicum* cv. *Micro-tom*) grown at different nitrate conditions infected with *B. cinerea* was analyzed using the Gene Chip Tomato Genome Array (Affymetrix). A gene network of differentially expressed genes was constructed, allowing the identification of several gene interactions. Among these, we identified several transcription factors associated with the pathogen response modulated by nitrogen availability, supporting the role of the plant N status in the defense response. These results are contributing to a better understanding of the interaction between plant nutritional signals and the plant response to biotic stress.

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46.- Characterization of gene expression in response to *Botrytis cinerea* infections in *Solanum lycopersicum* under contrasting nitrogen availability.

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Nitrogen (N) is the main limiting nutrient for plant growth and yield in agriculture, with important effects in different developmental programs, including leaf growth, senescence, root system architecture and flowering time. In addition, the plant N nutritional status also influences its ability to respond effectively to pathogens. Many agronomical reports indicate that plant N availability had an impact on the incidence of crop diseases. Although significant progress has been made in the characterization of defense-response genes, the molecular mechanism underlying the connection between N and pathogen responses are poorly understood. In this work, we evaluated *Solanum lycopersicum* defense response to the necrotrophic fungus *Botrytis cinerea* under contrasting N regimes. To identify potential mechanism mediating the plant defense response associated with N nutrition, we analyzed change in gene expressions related with N metabolism and defense response in these plants. These results are contributing to a better understanding of the convergence points between plant N status and defense response against *B. cinerea*

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47.- Role of cell wall and intercellular polymers during plant-aphid interaction

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The green Peach Aphid (*Myzus persicae*) is a plant feeder insect that colonizes more than 400 species and can transmit over 100 viral diseases, thus, it is considered one of the most important agricultural pests worldwide. Aphids represent a major challenge for plant responses due to the low tissue damage related to its particular feeding strategy where a slender stylet intercellularly probes until a single sieve element is reached and penetrated. It is known that short-term responses to phloem sap feeding insects by plants include sieve element sealing and callose deposit synthesis. However, little attention has been paid to cell wall and intercellular matrix during aphid-plant interaction. Therefore, our study focuses on the investigation of cell wall and intercellular polymers, such as callose and pectins, at an early colonization stage because at this point aphids constantly test different plant tissue until a rich nutrient phloem segment is reached. Moreover, this time period represents a critical step where explorer aphids can choose or reject potential feeding hosts. Our results with Col-0 *A. thaliana* demonstrated significant changes in pectin related enzymes and polymers during the first 5 hours of aphid – plant interaction, as the global activity of pectin methylesterases (PMEs) significantly increased, and pectin methylesterification decreased. Additionally, we found a behavioral response of the aphid which did not prefer to feed on PME inhibitor (PMEI) *A. thaliana* mutants. This is consistent with the idea that a more rigid pectin polymer limits insect feeding.

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48.- Identification of differentially expressed genes in raspberry (*Rubus idaeus* var. Amira) in response to tomato ringspot virus (torsv)

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Rubus idaeus is one of the most important agronomical species in the Maule region. It concentrates around 57% of the Chilean production that is mainly exported overseas. Some of the current industry challenges are related to the dispersal of diseases producing decrease in productivity and quality of the fruit. In order to identify potential candidate genes associated with tomato ring spot virus (ToRSV) infection, we identified differentially expressed genes in raspberry infected by ToRSV. *De novo* RNA-seq of blackberries under different conditions (i.e., control and infected by ToRSV) was performed using Illumina short-read sequencing. Following assembly and removing of redundant sequences, 68,853 protein-coding sequences were predicted. Around 66.54% and 71.27% of those genes were annotated using PFAM and GO databases. To identify differentially expressed genes, we aligned high quality-filtered reads from each condition against this reference transcriptome. From this analysis, we identified many differentially expressed genes (DEGs), which are mainly associated with immunity and response to external stimuli. These genes are involved in the blackberry defense mechanism directly or indirectly through different pathways. Detailed analysis of some of the DEGs by qPCR corroborated our RNA-seq results, suggesting these genes play key roles in blackberry response against pathogens. Further studies on the functionality of these differentially expressed genes will provide valuable information on the immune mechanisms in blackberry, and the role of the differentially expressed genes during ToRSV infection. Our data provide novel insights into the innate immune response against ToRSV challenge in *R. idaeus* (var. Amira), for which prior genomic information was limited. This new sequence information will improve the knowledge of this important and healthy fruit, providing an invaluable new tool for biological research.

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Poster III



Metabolism

1.- Synthesis induction of alkaloids in *Rhodophiala pratensis* bulbs using precursors and elicitors

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Previous studies in several species of *Amaralidaceae* family allowed identify that furthermore of its ornamental potencial, have great value in pharmacological area, attributed to alkaloids contents, which have proven effectiveness in the treatment of neurodegenerative diseases, particularly galanthamine derivatives as possessing inhibitory activity against acetilcolinoesterasa, enzyme involved in the development of Alzheimer's disease, showing a prolonged, selective and reversible activity. One of the species belonging to this family is *Rhodophiala pratensis*, it is a bulbous plant native from Chile, currently considered as a vulnerable species, and no present chemical studies related to alkaloids of the generous *Rhodophiala*. However for sustainable use of this resource, the development of technologies that allow acceptable performance in alkaloids obtaining projecting future production is necessary. In this study independent assays of alkaloids optimization were employed, using the elicitor sugar in three concentrations (0, 30, 60 and 90 g L⁻¹), MeJa (0, 25 and 50 µM), and the precursor acid transcinámico (0 and 250 mg L⁻¹) and finally F-alanine + L-tyrosine (0 and 250 g L⁻¹, respectively) in bulbs *R. pratensis ex situ*. The expected results is to quantify the alkaloids ex situ, determining the treatment that does increase yields and whether they are higher or lower compared to previous results of *in vitro* culture.

2.- Increasing the sink/source ratio in *Prunus persica* (L.) batsch alters photosynthetic rate dependent on stomata closure and sugar accumulation in source leaves

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The sink/source balance is determinant in carbon partitioning within the plant, impacting yield of fruits trees. Thinning is an agronomical practice that consists in changing the ratio between adult leaves and fruits, source and sink organs respectively. Decreasing fruit load on the tree leads to an increase in fruit diameter, fruit weight and soluble solids content in the remaining fruits. However it has been reported that a reduction in sink organs may result in decrease of photosynthetic process by negative feedback due to final products accumulation in leaves, photoinhibition and increase on stomatic resistance. The aim of this work was to study the effect of sink/source balance in physiological parameters of *Prunus persica*. Differential thinning was performed in two nectarine varieties, 'Magique' and 'Red Pearl', early and late harvest varieties respectively. Fruit development kinetics as well as physiological parameters as chlorophyll fluorescence, gas-exchange, and chlorophyll and sugar composition of leaves were determined during the whole season. Photosynthesis rate was decreased in thinned trees and seemed to be related associated to stomatic limitation and sugar accumulation in leaves. The most abundant sugar detected in leaves was sorbitol that is the main sugar exported by leaves of *Prunus persica*. On the other hand, fluorescence parameters and chlorophyll content remained constant in leaves of both varieties with different thinning treatments in the whole season. We suggest that increasing sink/source ratio by thinning leads to stomata closure, and this is associated to an excess of sugar that are not exported and remains accumulated in leaves of thinned trees.

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3.- Genetic improvement of microalgae for biodiesel production

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Continued use of fossil fuels is not sustainable, and is expected to decline in a near future. Due to this, we need to seek alternative sustainable sources of biofuels from renewable raw materials, both economically and environmentally efficient. A promising alternative to fossil fuels is the production of biodiesel, of which there are a variety of sources. Among these, one of the most auspicious are microalgae, due to their excellent lipid production, high growth rate and scalability, compared to other crops. Of all the species of algae that have been studied so far, including *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum*; microalgae from the group *Nannochloropsis* are probably one of the best candidates, mainly because of its high rate of biomass production, their ability to accumulate large amount of lipid (up to 60 % of its dry weight), ease to scale cultures and the possibility of genetic improvement via homologous recombination, among other features. The focus of this work is to optimize the lipid biosynthesis pathway in *Nannochloropsis* in order to improve the production of biodiesel. To do this, we will study the overexpression of *AtWRI1* and *NoKASIII* genes, both genes involved in the lipid production in *Arabidopsis thaliana* and *Nannochloropsis oceanica*, respectively. Additionally, we will transform *Chlamydomonas reinhardtii*, as it represents an excellent model in order to study lipid metabolism due to the facility to make genetic changes and the comprehensive set of resources that are available. We aim to develop new microalgae lines suitable for biodiesel production.

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4.- Methyl jasmonate regulates expression of key transcription factors involved in early jasmonate response and other related to flavonoid biosynthesis in strawberry fruit.

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The relationship of jasmonates (JAs) and the accumulation of proanthocyanidins (Pas) and anthocyanins in fruits remains unclear. MYC transcription factors (TFs) are involved in early JAs responses and have shown to positively regulate the expression of other TFs and flavonoid biosynthetic enzymes in Arabidopsis. On the other hand, the ternary regulatory MYB-bHLH-WD40 protein complex (MBW) regulates target genes of the flavonoid pathway. We analyzed the effects of 100 μ M methyl jasmonate (MeJA) during 0.25, 0.5, 1 and 6 h treatment on the transcript levels of MYCs-, flavonoid biosynthesis- and MBW-codifying genes in strawberry (*Fragaria x ananassa*) fruit at the white stage. MYC1 and MYC2 exhibited an increase in their expression levels in MeJA-treated fruits at 0.25 h and 6 h. In addition, anthocyanidin synthase (ANS) showed an increase of expression in MeJA-treated fruits at all evaluated times. Furthermore, FabHLH3, FabHLH33A and FabHLH33B TF genes showed an increase in their expression levels only at 0.25 h of MeJA treatment. The same pattern was observed in expression of ANR (a gene directly involved in PAs biosynthesis). Remarkably, no significant changes in gene expression were observed for MYB TFs, suggesting that JAs could regulate the flavonoid biosynthesis in strawberry fruit through the upregulation of bHLH gene expression.

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5.- Expression of sorbitol dehydrogenase from grapevine (*Vitis vinifera*) in *Nicotiana tabacum* and *Arabidopsis thaliana*

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Depending on the plant species, sucrose and/or different sugar alcohols (polyols) are produced as primary products of photosynthesis. Among polyols, sorbitol is one of the molecules involved in processes of micronutrient mobility, protection against free radicals and abiotic stress. This sugar alcohol is translocated via phloem to sink organs and transformed into fructose by the enzyme sorbitol dehydrogenase (SDH, E.C. 1.1.1.14) mainly in plants of the Rosaceae and Plantaginaceae families. However, VvSDH is an orthologous sequence found in grapevine (*Vitis vinifera*, Vitaceae) which possesses 75-86% amino acid identity with known SDHs (e.g. from tomato and apple), and *Arabidopsis thaliana* (Brassicaceae) also possesses a known SDH enzyme. *Arabidopsis* *sdh-* mutants develop normally under standard growth conditions, but exhibit greater resistance against drought than wild-type plants, indicating a role for this enzyme, and polyols, in abiotic stress tolerance in species which do not translocate sorbitol in the phloem. In order to determine the function of VvSDH, we constructed a suitable binary vector, and expressed VvSDH-His transiently in leaves of *Nicotiana tabacum* (tobacco). We tested the functionality of this vector by RT-PCR and by western-blotting (anti-His), after RNA and protein extractions, respectively. Subsequently, we stably transformed wild-type and mutant (*sdh-*) *Arabidopsis* with VvSDH-His, obtaining multiple transgenic lines which will be subjected to drought treatments in order to determine the role of the enzyme *in vivo*.

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6.- Microarray analysis and comparison of transcriptional regulation over-expressing a *Pinus radiata* d. don mads10 in Arabidopsis

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The response to inclination in trees is a widely studied phenomenon, but the molecular mechanism involved are still unclear. A MADs box transcription factor was found differentially expressed in response to inclination (2 hours after treatment) in *Pinus radiata* D. Don. We overexpressed this MADS10 gene in *Arabidopsis thaliana* and identified up and down regulated genes using the Affymetrix AraGene chip. A total of 1211 genes were modulated, with 689 genes up regulated and 529 genes down regulated. Interesting candidate genes were validated by qRT-PCR. Biologicals processes were analyzed using the BioMaps tool available from VirtualPlant. An overrepresentation of transcripts belong to response to stimulus was observed, mainly response to starvation, cell communication, regulation of biological process. MapMan was used to complement and create a general view of differentially expressed genes. Genes involved in the synthesis of cell wall were mainly down regulated and genes involved in secondary metabolism were up regulated. Based on these results, we propose MADS10 modulate expression of genes involved in important steps from the phenylpropanoid and lignin biosynthesis pathways, such as PAL, 4CL and CCOAMT.

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7.- Identification and *in silico* analysis of flavonoid transporters abcc in radiata pine seedlings exposed to inclination

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In the gravitropic response, one of the most accepted theories relates differential accumulation of auxin to induce reorientation. Flavonoids have been identified as polar auxin transport blocker, influencing plant architecture. Young radiata pine seedlings were exposed to inclination and RNAseq libraries were built from extracted RNA at both stem sides. We identified several encoding sequences for ABCC-like transporter proteins, which are related to intracellular flavonoids transport. Multiple local alignments were performed considering reported ABCC transporters from several model plants and radiata pine sequences. Three sequences showed the highest identity score to ABCC genes and, their transcript abundance was estimated by FPKM value. Interestingly, the three transporters genes showed a differential transcript accumulation in a time and spatial manner. Deduced amino acid sequences showed characteristic ABC-family conserved domains and the phylogenetic analysis classified these three proteins into the specific clade of ABCC transporter members. One of these sequences was selected for modeling based on their FPKM values and the high coverage of the crystal structure of ABC used. A model of PrMRP2 was obtained by homology modelling where 99.1% of residues were categorized in favorable regions (including most favorable, allowing and generous regions). Moreover, the model obtained showed the highly conserved characteristic regions of ABCC transporters, which was evident after multiple alignment analysis. The structure features 12 transmembrane regions and 2 ATP binding regions. The protein binding site for different flavonoid ligands was identified.

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8.- Micropropagation and determination of the antioxidant capacity of the *Leptocarpha rivularis*.

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“Palo negro” is a native shrub of southern Chile, originally called Cüdu-mamëll (in Mapudungun) Scientific name is *Leptocarpha rivularis* and belongs to the Compositae family, classified as Asteraceae. The secondary metabolites that characterize this family are acetylenics, sesquiterpene and sesquiterpene lactones. (Martínez et al, 2006) The antioxidant capacity has been determined for leaves and stems from *Leptocarpha rivularis*, a plant that grows in rainforests in the south of Chile. The methodologies used were: ORACFL (oxygen radical absorbance capacity - fluorescein), DPPH (2,2-diphenyl-2-picrylhydrazyl). Despite the great interest in *Leptocarpha rivularis* due to the wide variety of secondary metabolites with biological activity that produces, until now there no report describing a regeneration system or micropropagation through tissue culture. In this work we described the first protocol for disinfection and in vitro establishment of this plant from material collected in field near Temuco. For that, the plant material was previously washed with water and commercial detergent to remove soil traces and disinfected with an antifungal solution (Captam 2.5 g/L), followed by ethanol 30% v/v and commercial bleach (NaOCl 1%). Post-disinfecting explants were placed on 2% sucrose half-strength Murashige and Koog medium (MS) and incubated in a growth chamber at 18-22°C. Although there were a significant percentage of the explants contaminated with fungi (30%) we were successful in achieving the growth and rooting of plants in vitro. Based on these results, nowadays, we are improving the disinfection protocol as well evaluating hormonal combinations for more efficient micropropagation.

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9.- Analysis of antioxidant activity of the aqueous extract prepared from *Nertera granadensis* (MutisL.t.) druce.

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This study focuses on the analysis of the antioxidant activity of *Nertera granadensis*, species belonging to Rubiaceae family, which has predominantly cosmopolitan pantropical distribution. The antioxidant activity was determined by capturing the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). The total polyphenols concentration was also determined. It was evaluated the antioxidant capacity for both radicals calculating the decrease percentage of absorbance radical. For polyphenols evaluation was used the Folin-Ciocalteu method to determine the total concentration phenols in the aqueous extract of *N. granadensis*. In the antioxidant assays with DPPH and ABTS, a positive activity was observed, resulting in a maximum decrease of 53,47 % and 54,70 %, respectively. However, in previous studies of other autor (1) with methanolic *N. granadensis* extract, was obtained a higher antioxidant activity, with a maximum decrease of 79,11 % with DPPH and 87,99% with ABTS. The content of polyphenols in gallic acid equivalent was 133.044 µg/g of extract. (1) Ruminot, F. 2015. Determination of antioxidant and antimicrobial activity of methanol *Nertera granadensis* extract (Mutis ex L.f.) Druce. Departamento de Ciencias y Tecnología Vegetal, Universidad de Concepción, Campus Los Ángeles. Chile.

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10.- Study of Stevia genes involved in sweetener synthesis using model plants.

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Steviol glycosides produced by *Stevia rebaudiana* have several properties, besides sweetness, they offer therapeutic benefits, as they are anti-hyperglycemic, anti-hypertensive, anti-inflammatory, among others. Sweeteners synthesis is carried out from mevalonate, sharing the early steps of the biosynthetic pathway leading to gibberellins. The key step of steviol synthesis occurs at the specific hydroxylation of *ent*-kaurenoic acid, which is catalyzed by an enzyme belonging to the plant cytochrome P450s superfamily. Later on, successive glycosylations produce the final sweeteners. This work aims to study the hydroxylase and the glycosidase function leading to the sweeteners compounds from *S. rebaudiana* in model plants, such as *Solanum lycopersicum* and *Citrus sinensis*. Previously, tomato and orange plants were transformed with the specific cytochrome P450 enzyme from *Stevia* and were characterized using molecular techniques and by chromatography to detect sweeteners. We found that tomato plants produce Stevioside, one of the intermediates, but not the sweetest molecule: Rebaudioside A. In orange, this work is still in progress. To date, the coding region for one of the glycosyl-transferases from *Stevia* (SrUGT) was synthesized according with the published sequence. Then, we generated an expression vector directing the expression of SrUGT coding region under control of the CaMV35S promoter. The final construct, was used to transform transgenic lines overexpressing cytochrome P450 enzyme from *Stevia* and wild-type tomato plants. Currently, a further characterization of transgenic lines is carry on in order to determine the production of Rebaudioside A.

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11.- Effect of the abscisic acid (ABA) on the grapevine (*Vitis vinifera* L.) buds respiration rate and its role on the dormancy transition

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Grapevine bud dormancy is induced under short-day photoperiod (SD) and characterizes by a temporary growth arrest of the meristematic tissue within the latent bud which allows them to cope with adverse winter conditions. In this study we show that during the transition from active growth to dormancy, respiratory rate of buds strongly decreases and the expression of genes coding for enzymes of mitochondrial electron transport chain (mETC) such cytochrome c (VvCITC), cytochrome oxidase (VvCOX6a), alternative oxidases (VvAOXs) and NADH alternative dehydrogenases (VvANDs) is also repressed. The dormant buds are less sensitive than active growing buds to stimuli that normally increase the respiration like high temperatures and glucose treatment. All the evidence suggests the presence of a metabolic or respiratory inhibitor within the dormant buds. Absciscic acid (ABA) has been postulated as a key regulator in the induction of grapevine bud dormancy. Here, we observed that the SD induces the expression of VvNCED3 gene, coding for the enzyme 9-cis-epoxycarotenoid dioxygenase, which is a key enzyme in ABA biosynthesis. Moreover, ABA treatment reduces the respiration rate and the expression levels of mETC genes in actively growing buds. Besides, ABA increases the expression level of VvSnRK1 gene, which encodes for an enzyme belonging to a family of protein kinases known for its role as energy sensors, promoting stress tolerance and survival. We postulate that ABA induces dormancy by repressing the respiration rate of grapevine buds.

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12.- Evaluation of the antioxidant activity and phenolic compounds in the fruits of *Cestrum parqui* (L'HER)

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Based on their biodiversity and secondary metabolites content, native plants provide a rich source of antioxidants with potential uses in the biomedical, nutraceutical and food industry. On the search of new plants with antioxidant activity, this study focused on the shrub *Cestrum parqui* (L'Hér, Solanaceae), commonly known as palqui, which is characterized by tolerate harsh conditions, growing in dense masses across central Chile, crowding out other species and with extreme toxicity to farm animals, attributed to alkaloids presence. The phytochemical studies on this plant have been focused on their leaves, already known by the mapuches for their febrifuge, anti-inflammatory and healing effects. In the present study the phenolic composition and antioxidant activity of its fruits were evaluated from methanol extracts and compared with other native antioxidant-rich fruit, maqui (*Aristotelia chilensis*). The antioxidant activity was measured using radicals capture techniques DPPH and ABTS and calculated with reference to the reaction signal given by Trolox solution (Trolox equivalents, TE). The results for palqui of the inhibitory activity equivalent to ABTS were 64.55 ± 1.15 μmol of TE/g of fresh fruit and 134.31 ± 0.70 μmol of TE/g in the case of DPPH, comparable for those found in maqui fruits (67.17 ± 3.06 μmol of TE/g of fresh fruit and 123.23 ± 1.09 μmol of TE/g). The total concentration of polyphenols, determined using the Folin-Ciocalteu method, was 500.49 ± 18.46 mg eq. galic acid (GAE)/ 100 g fresh fruit for palqui and 470.22 ± 33.90 mg GAE/ 100 g fresh fruit for maqui. For both fruits the high antioxidant activity and content of total polyphenols observed, correlates with a high content of total anthocyanin and flavonoid concentration. The results obtained open the use of this plant as a novel source of antioxidant compounds and anthocyanins for biotechnological applications.



13.- Does sugar matter? The role of glucose in grape berry ripening

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Grapevine fruit development can be divided into three stages: the formation stage, the lag phase (veraison), and the ripening stage; during which physiological and biochemical changes occur that allow cell differentiation and the accumulation of different solutes. Interestingly, during veraison, a fast glucose and fructose accumulation begins together with anthocyanin biosynthesis allowing the color change of berries, which remains during the whole ripening process. The anthocyanin accumulation together with other processes like size increase, turgor, acidity decrease and hormonal variations, allow the full berry development. Despite there is widespread knowledge about berry grape development, the nature of the signal that initiates and facilitates the coordination of the ripening process still remains unknown. Sugars have been studied not only as an energy source, but as a signaling molecule able to control gene expression. It has been demonstrated that glucose and fructose induce anthocyanin biosynthesis in cell cultures of grape. So, in order to determine which is the signal that initiates and regulates the ripening in grape berry, this work is mainly focused on understanding the role of glucose and fructose during berry development, due to the increment of these molecules in the beginning of this stage. Genetic and phenotypic analysis was used in order to determine the degree of ripening of the berry. The results of this work allowed us to demonstrate the importance of glucose in the regulation of the beginning of ripening, because pre-veraison berries treated exogenously with glucose matured 20 days earlier than fruit treated with water. Moreover, sugar treatment of cell cultures of grape showed that the expression of the MybA1 gene, which is essential for anthocyanin biosynthesis, is induced in response to glucose.

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14.- Evaluation of the elicitor effect of *Arthrospira maxima* (Spirulina) extracts on model plants

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The use of agrochemicals in agriculture causes detrimental effects on the environment, including the release of greenhouse gases, contamination of water, air and soils, and a decrease in biodiversity. In recent years, interest has grown on the pursuit of new sources of plant nutrition, such as organic fertilizers and biostimulants that could contribute to a more environmentally friendly agriculture and to assure food safety to world population. In this study, the use of lyophilized extracts of *Spirulina* is proposed as a plant nutrition source in order to improve agronomic traits and stress response, since it contains up to 70% protein (dry weight), representing an important source of organic nitrogen, together with other essential compounds such as fatty acids, vitamins, minerals and polysaccharides, among others. Cyanobacteria represents a major source of polysaccharides, and it has been demonstrated that exopolysaccharides have elicitor effects on plants, increasing the accumulation of a variety of phytochemicals. These compounds enhance plant protection and resistance to freeze, dehydration, and plagues of different nature. We have found that the addition of *Spirulina* on *Arabidopsis thaliana* and *Lactuca sativa* in in vitro cultures stimulated the vegetative growth through water accumulation. Moreover, the addition of *Spirulina* on *Solanum lycopersicum* grown in greenhouse showed an increased vegetative growth. Plant productivity was evaluated and in vitro assays were made to assess plant performance treated with *Spirulina* under stress conditions. Furthermore, we analyzed the expression of marker genes representing the phenotypes observed and important physiological processes within the plant.

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15.- Comparision of different extraction methods in the determination of the antioxidant activity from fruits of *Nertera granadensis*

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Nertera granadensis also known as Rucachucao, Coralito, is a herbaceous plant belonging to the Rubiaceae family. It is distributed in Chile between the regions of Coquimbo and Magallanes and also in Argentina (Marticorena et al. 2010). Currently in the literature, there is no evidence from the study of the fruit of *Nertera granadensis*, therefore it arises interest to investigate and determine properties of this plant. It is for this reason that in this work different methods were tested with lyophilized fruit, fresh fruit, extract obtained by maceration and extract obtained with Soxhlet extraction. The antioxidant activity of the fruit extracts were quantified by both DPPH (1,1-diphenyl-2-picryl-hydrazyl) and ABTS⁺ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) scavenging assays. The best extraction method was the Soxhlet extraction. In these assays the antioxidant effect could not be determined from the lyophilized fruit, since the outer husk of the fruit was impossible to dissolve in the tested solvents (methanol, ethanol). The disadvantage of the maceration method was the obtention of a little extract quantity. For assays with DPPH and ABTS⁺, IC₅₀ values (concentration of the extract able to inhibit by 50% the absorbance of the radical) measured were 8.16 and 1.208 mg / mL, respectively. Antioxidant effect results were expressed as Trolox equivalents, and gallic acid equivalents for ABTS + and DPPH assays, respectively.

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16.- Biological effect of extract obtained by maceration with methanol from *Dendroligotrichum dendroides*

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Dendroligotrichum dendroides is a moss belonging to the family Polytrichaceae and it grows in Chile from Arauco to Magallanes province. It exists evidence of antimicrobial and antioxidant activity in a variety of mosses, therefore in this study an extract prepared by maceration at room temperature was evaluated, through DPPH (1,1-diphenyl-2-picryl-hydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) to determine antioxidant activity. The IC_{50} values were 11,985 mg/ml and 0,966 mg/ml with DPPH and ABTS⁺ assay, respectively. One gram of extract is equivalent to $19,610 \pm 2,900$ mg of Trolox (ABTS⁺ assay) and 35.38 ± 2.40 mg of gallic acid (DPPH assay) and the polyphenol content in gallic acid equivalents was 49.1603 ± 3.4245 mg / g extract. In the kinetic assay a better and more stable result was observed with ABTS⁺ cation in the extract obtained with maceration method, in comparison with results obtained with Soxhlet, method (Aceituno, 2013). The antioxidant effect of the extract obtained with Soxhlet extraction (Aceituno, 2013) exceeded the effect of the extract obtained by maceration at room temperature, with a small difference in percentage of inhibition with DPPH radical. Moreover, the methanol extract obtained by maceration method from *Dendroligotrichum dendroides*, presented antibacterial effect, obtaining a inhibition against *Staphylococcus aureus* strain, with a concentration of 50 mg/ml and the inhibition was 0.34 cm more than the control. These results are of interest for extracting compounds from plants by maceration at room temperature, because this method reduces energy costs and other materials. Aceituno Valenzuela, Uri. I. (2013). Determinación de actividad antioxidante y antibacteriana en extracto metanólico de *Dendroligotrichum dendroides* (Brid. Ex Hedw) Broth. (Memoria de título).

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Post-harvest Molecular Physiology

17.- Glycosylation is important for the activity of FcXTH1, an enzyme related to softening of *Fragaria chiloensis* fruit.

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Fruit softening is related to cell wall disassembling. Changes in the hemicellulose fraction have been reported during ripening of *Fragaria chiloensis* fruit and therefore we suggested that FcXTH1, a xyloglucan endotransglycosidase/ hydrolase (XTH) enzyme, might play a key role in this process. XTHs may have two activities: transglycosidase (XET) and hydrolase (XEH). FcXTH1 contains a conserved N-glycosylation site adjacent to the predicted catalytic residues. The aim of this work was to study the effect of glycosylation on FcXTH1 activity. For this, FcXTH1 was cloned and heterologous expressed in *Pichia pastoris*. The recombinant purified protein was found to be active and displayed both XEH and XET activities, with 10-12 times higher XET than XEH activity. The optimal pH and temperature for both activities were 5.5 and 37°C. Both activities were stable at 4°C as more than 50% initial activity remained after 5 days. A K_m value of 29.5 μM was determined for XGO (xyloglucan oligomer). The deglycosylation of FcXTH1 by the treatment with PNGase-F did not affect maximum activity but reduced the stability of the enzyme, and more importantly, induced changes in kinetic parameters of XTH activity (higher K_m value). In conclusion, glycosylation of FcXTH1 affects its substrate affinity and stability.

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18.- Identification and characterization of a fruit-specific NAC transcription factor (FcNAC1) from *Fragaria chiloensis*.

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Chilean strawberry (*Fragaria chiloensis*), an octoploid species ($2n = 8x = 56$) is the mother of the commercial strawberry (*Fragaria × ananassa* Duch.). This fruit has been characterized since possess different organoleptic attributes as intense flavor, attractive color and pleasant aroma. Nevertheless, fast fruit softening is one of the main problems. We have identified the differential expression of a fruit specific NAC gene. The full length was obtained and isolated encoding 334 aminoacids. This sequence contains the highly conserved NAC domain and is subdivided into 5 sub-domains (A to E). Phylogenetic analyzes showed high evolutionary proximity with others NAC transcription factors related to secondary wall formation. The analysis of transcription profile at different fruit stages of maturation (C1, C2, C3 and C4) showed an increase in the rate of transcripts level between at stage C3 and C4. This gene is also expressed in flower and stem, but low level of transcript is accumulated in roots, stolon and leaves. A structural model of NAC was built by comparative modeling, showing the highly conserved NAC domain in the amino terminal region composed of a small group of β sheets twisted antiparallel. This β sheets is compacted by α helix on one side and a short helix on the other side. In addition, the NAC domain has a rich positive charged residues area, which would be involved in the interaction with DNA. The involvement of NAC during fruit softening and senescence is still unknown, and these data allow us to understand a biological process of particular interest.

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19.- Peach fruit molecular and physiological response to exogenous application of cytokinin during development

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Peach (*Prunus persica*) fruits have a sigmoidal growth pattern that is regulated by plant hormones, including cytokinin. After floral bloom, there is an exponential growth phase that is associated with high endogenous cytokinin levels and an increase in pericarp cell division. Subsequently, the endocarp lignifies, endogenous cytokinin levels decrease and pericarp growth rate is reduced. A second exponential growth phase follows the lignification stage. Then, an increase in ethylene triggers fruit ripening. In order to determine the molecular and physiological effects of cytokinin on peach fruit, exogenous application was performed in both field and laboratory settings. Transcriptomic analyses of peach fruits treated exogenously with cytokinin (trans-zeatin) in a laboratory setting, at different stages of fruit development, revealed a higher number of cytokinin responsive genes in the lignification stage of development, as well as a significant change in genes associated with ethylene biosynthesis and perception during the fruit ripening stage. Field applications of cytokinin (Thidiazuron) revealed a significant increase fruit harvest size, increases in chlorophyll post-lignification, changes in fruit color and a delay in harvesting time. These laboratory and field studies suggest that cytokinin participates negatively in fruit ripening by altering ethylene synthesis and perception.

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20.- Transcriptional and computational study of three α -expansins involved in softening of *Fragaria chiloensis* fruit

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Fruit softening is associated to cell wall modifications produced by a set of hydrolytic enzymes and proteins. Expansins are proteins with no catalytic activity, which have been associated to fruit softening. Changes in the cellulose-hemicellulose fraction have been reported during ripening of *Fragaria chiloensis* fruit, and matched with softening. In addition, three expansin genes were identified in *F. chiloensis* with high homology to other plant α -expansins. In this work full-length sequences were obtained for FcEXPA1, FcEXPA2 and FcEXPA5, and using qRT-PCR, transcript accumulation was determined during ripening of *F. chiloensis* fruit. The results have shown a differential but overlapping expression pattern between these three α -expansins. With the aim to evaluate potential differential structures and/or substrate specificities, 3D models were built by comparative modeling methodology for each protein. The models obtained show a cellulose binding domain with a β -sandwich structure, and a catalytic domain with a similar structure to the catalytic core of endoglucanase V (EGV) from *Humicola insolens*. The catalytic HFD motif is oriented towards the central part of the open groove, conserved in all the structures, however the open groove conformation was different. Finally, molecular docking and molecular dynamics simulations evaluated the protein-ligand interaction with cellodextrin and hemicelluloses. The results shown that all expansin proteins showed favorable affinity energies for binding xyloglucan, homogalacturonan and cellodextrin as substrates, however the best stability was for expansin-cellodextrin complex. MM-GBSA analysis showed that major energy contributors for binding are van der Waals forces and non-polar terms. The data is congruent with a probable role of expansins during softening of Chilean strawberry fruit.

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21.- Jasmonate profiling and expression of its signaling components during fruit development and ripening in the non-climacteric strawberry

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The regulatory mechanisms of fruit ripening in non-climacteric fruits are complex and many hormones could be part of this regulation. Jasmonates (JAs) have been found to play a role in non-climacteric fruit ripening. In this work we showed a molecular profile of the JAs biosynthesis and signalling pathway during development and ripening of strawberry (*Fragaria xananassa*) fruit. The endogenous content of JAs were measured by LC-MS and the relative transcript levels of early genes involved in JAs biosynthesis and signalling pathway were analyzed by RT-qPCR. The 12-oxophytodienoic acid (12-OPDA) and JA levels decreased from flower until ripe stages. JA-Ile levels undergo a dramatic decline from white to ripe stage. Methyl jasmonate (MeJA) decreased towards ripening and it was not detected in ripe stage. These analytical results matched with the decrease of transcript levels of the JAs-biosynthetic genes lipoxygenase (LOX), allene oxidase synthase (AOS), jasmonate methyltransferase (JMT), jasmonic acid-amido synthetase (JAR1), methyl jasmonate esterase (MJE) and jasmonate-isoleucine hydrolase (JIH1). The JA-Ile receptor (COI1) showed a down-up-down expression pattern, and several co-repressors of the JA signaling pathway as NINJA, TPLs and HDAs displayed different expression patterns during fruit development. In contrast, the JAZ repressors and the early transcription factors MYC1 and MYC2 showed a constant decreasing pattern during fruit development and ripening. The application of MeJA during an in vitro ripening assay promoted the coloring of the fruit and significantly increased the level of JAs with a concomitantly reduction in the ABA content. These results suggest that JAs could act as important regulators of ripening-related pathways such as flavonoid biosynthesis in strawberry fruit.

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22.- Effects of exogenous methyl jasmonate and ethylene on physiological processes of *Fragaria chiloensis* fruit during *in vitro* ripening

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Previous results showed that methyl jasmonate (MeJA) induces changes during ripening of *Fragaria chiloensis* fruit, but this effect could be indirectly regulated by ethylene since MeJA upregulated ethylene biosynthetic genes. In this work, we tested this possibility by blocking ethylene perception with EthylBloc® (active ingredient 1-methylcyclopropene (1-MCP)). Large green fruits of *F. chiloensis* were *in vitro* ripened under four different treatments: 1) 100µM MeJA, 2) EthylBloc® application and 100µM MeJA, 3) 2 g/L of the ethylene-releasing agent ethephon, and 4) control (without hormones) fruits were collected at 0, 30min, 48 h, 5 and 9 days and the following physiological parameters were analyzed: weight, firmness, color, soluble solid content/titratable acidity ratio (SSC/TA), anthocyanin and lignin content. Blocking ethylene perception in MeJA-treated fruits resulted in a lower loss of firmness and weight at day 9 compared to control and MeJA treatments, and in higher anthocyanin content at days 5 and 9 compared to treatment with MeJA. Ethylene application resulted in the highest SSC/TA ratio and lignin content at days 5 and 9, respectively, and in the lowest red colouration at days 5 and 9. In conclusion, MeJA and ethylene treatments regulate different physiological processes during ripening of *F. chiloensis* fruit, being MeJA mainly associated to color and anthocyanin accumulation, while ethylene plays a more important role in promoting a high SSC/TA ratio and lignin content.

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23.- An *in silico* approaches suggests that sepallata3 (FvSEP3) transcription factor can control the expression of XTH genes by binding to cis-elements on its promoter region

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SEPALLATA transcription factors (TF) are a subfamily of MADS-box TFs and have been described as master switches during fruit development and ripening of fleshy fruits. Additionally, the fast softening of strawberries including *Fragaria vesca*, considered the model of study of the *Fragaria* genus, is a limiting step for their commercialization. Previously, we identified a MADS-box TF that is induced during ripening of *F. vesca* fruit. This TF was classified as SEPALLATA and named as FvSEP3. On the other hand, two xyloglucan endotransglycosidase/hydrolase genes (FvXTH20 and FvXTH26) involved in hemicellulose disassembling are induced during softening of *F. vesca*. Their promoters (2,000 bp) were isolated and bioinformatic analyses indicated the presence of MADS-box responsive elements (CArG-box) in both ones. As a way to explain if FvSEP3 TF plays a role in the regulation of FvXTH20 and FvXTH26, bioinformatic studies were designed. Through molecular modeling the structural model of MADS-box motif of FvSEP3 was generated, displaying a structure consisting of 2 β -sheets and 1 α -helix. By molecular docking and molecular dynamics simulation the interaction of FvSEP3 TF with specific cis-regulatory elements found in the promoter regions of the genes under study was explored. The results indicated that all interactions are favorable although cis-regulatory elements from FvXTH26 promoter have better interaction with FvSEP3 than FvXTH20. The data suggests that FvSEP3 could regulate the expression of FvXTH20 and FvXTH26 during ripening of strawberry fruit.

24.- *cis* regulatory elements present in promoter regions of structural genes of the biosynthetic pathway of anthocyanins *Fragaria chiloensis* f. *chiloensis*.

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Anthocyanins are the natural pigments belonging to the flavonoids family that give the color red, blue and violet to flowers and fruits. The pigmentation of strawberry fruits are not the exception, which also is indicative of state of maturity and quality. Anthocyanins are regulated at transcriptional level, using complex formed between transcription factors MYB, basic helix-loop- helix (bHLH) and WD-40 repeat proteins, forming the MBW complex. It has reported that the silencing of FcMYB1 by transient expression on fruits of *F. chiloensis* show a differences in gene expression of CHI, F3H, DFR and ANS, due to transcriptional activation of genes involved in the anthocyanins biosynthetic pathway. In this work, we determine the *cis* regulatory elements presents in the structural genes of the biosynthetic pathway of anthocyanins in *F. vesca* and *F. chiloensis*. For this, the promoters sequences of anthocyanin biosynthesis genes in *F. vesca* and *F. chiloensis* were checked by *in silico* and experimental techniques. We found that the promoter regions of the CHS, CHI, F3H, F3'H, DFR, ANS and UFGT in *F. vesca* genes exhibit ABA response elements (ABRE and RAV1); MYB, and bHLH. While the promoter regions of genes FcCHI and FcANS, obtained experimentally varying only the amount of MYB response elements within the promoters region in *F. vesca*. Also transcript levels of genes show an increase in their levels as the full fruit development and both genes are regulated positively by ABA treatment.

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25.- Relationship and changes on soluble sugars and organic acids metabolism throughout cherimoya development and ripening

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Fruit flavor is a crucial trait for commercial quality and consumer acceptability. Soluble sugars and organic acids are main components of fruit taste, which varies according to these metabolites type and content. Cherimoya (*Annona cherimola* Mill.) is a climacteric fruit with a unique behavior where both sugars and organic acids increases during fruit ripening. Inside the fruit, fructose and glucose are obtained mainly due to neutral invertase and sucrose synthase activity over sucrose. Both monosaccharides are phosphorylated and can be interconverted by isomerase activity. Fructose-6-phosphate forms glyceraldehyde-3-phosphate and phosphoenolpyruvate, which is used in tricarboxylic acid cycle to obtain organic acids such as citrate and malate. In this work, the correlation between sugars and organic acids in cherimoya was studied at metabolite accumulation and gene expression levels. Cherimoya samples cv. 'Concha Lisa' were collected throughout fruit development and ripening. Using degenerate primers designed from information available for other species, and then specific primers and RACE-PCR, we identified several key genes encoding putative proteins related to sugar and organic acid metabolism. Sugars and organic acids were measured by HPLC and gene expression was quantified by Real-Time PCR. Correlation between the expression of sugar-related and organic acid-related genes and the corresponding metabolites during development and ripening of cherimoya will be discussed.

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26.- Functional evaluation of *Annona cherimola* mill. citrate synthase through heterologous expression

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Acidity is an essential flavor component in fruit. Since cherimoya accumulates organic acids (specifically, malic and citric acid) during ripening; acidity becomes even more important in this fruit. In the cell cytoplasm, malic acid is synthesized from phosphoenolpyruvate due to phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH) enzymes. Later inside mitochondria, malate is converted into citrate at the tricarboxylic acids cycle by the activities of malate dehydrogenase and mitochondrial citrate synthase (mCS). Previous work from our lab in cherimoya fruit showed a correlation between citrate synthase activity, mCS transcript levels and citric acid accumulation. Thus, the main goal of this work is to functionally evaluate *A. cherimola* mitochondrial CS (AcmCS) by heterologous expression on Micro-Tom cultivar. For this purpose, AcmCS was cloned into several expression vectors for planta; which were evaluated through transient transformation in tobacco leaves, to validate mCS sub-cellular localization. In parallel, tomato explants stable transformation and somatic organogenesis was conducted. Currently, evaluations are being performed at the genetic level to establish identity of the transformed seedlings, which will be used to evaluate the effect of *A. cherimola* mCS in organic acids metabolism inside the plant and the fruit.

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27.- An integrative approach to study berry drop by using contrasting phenotypes of table grapes (*Vitis vinifera* L.).

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In table grapes (*Vitis vinifera* L.) berry drop is a problem that occurs mainly at postharvest during cold storage, affecting their commercialization. Presently, many authors suggest that it would be caused for either dissolution of the middle lamella or shared cell wall in the abscission zone. The objective of this work is to identify some indicator for berry drop by using genotypes with contrasting phenotype respect of berry drop susceptibility. To contribute to this understanding, we used two contrasting genotypes, i.e. 'Thompson Seedless' who usually shows a low incidence of berry drop, and the line P23, belonging to the INIA's breeding program, that have shown a high incidence of berry shattering at harvest and after storage, especially in bunches applied with gibberellic acid (GA₃) during berry development. Different parameters for characterizing this phenomenon were measured along fruit growth and during storage, including fruit detachment force (FDF), berry drop and gene expression. A texture analyzer was used for measuring the FDF between the berry and the pedicel. To determine the percentage of berry drop, bunches were weight before and after a standardized vertical shaking for 30-60s. This assessment showed that the P23 line showed a 43% of berry shattering after storage, being 10-fold higher than 'Thompson Seedless'. Among the variables quantified during pre- and post- harvest, the FDF decreased significantly through the progress of fruit development. Transcriptional analyses (qPCR) are currently underway, focused in the genes encoding for polygalacturonase, cellulase, pectinesterase enzymes, among others, in order to infer on the possible molecular mechanisms affecting the junction between berry and pedicel.

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28.- Lipoxygenase gene expression in Pepino dulce (*Solanum muricatum* Ait.): a preliminary study for aroma formation

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Pepino dulce (*Solanum muricatum* Ait.) has been described as a succulent, juicy and sweet fruit mainly for dessert, although some cultivars have been recommended for salad due to their higher acidity content. Pepino dulce is a diploid ($2n=24$) subtropical species, also known as melon pear, melon shrub or sweet cucumber, native species from South America specifically from The Andes area of Peru and Chile. Pepino fruit is member of the Solanaceae family, which includes many important crops such as tomato, potato, among others. Among approximately 1,500 species described in the *Solanum* genus, pepino is one of the few domesticated and cultivated for food purposes. Many aroma volatiles in fresh fruit are produced via cellular disruption due to cutting or mastication. Six-carbon (C_6) volatiles, including the aldehydes trans-2-hexenal, hexanal and cis-3-hexenal, as well as their corresponding alcohols, are produced from action of the lipoxygenase (LOX) pathway on substrates released by tissue disruption. LOX genes are classified based on function and are grouped into 13-LOX and the 9-LOX groups, which generate C_6 and C_9 aldehydes, respectively. Most of the aroma profile in fruits is determined by the 13-LOX branch of the pathway. It has been described that the aroma of pepino fruit when ripe, has a scent reminiscent of a cantaloupe melon; when immature it has a cucumber-like scent. Interestingly, C_9 compounds that have been described only for cucurbit species have also been found in pepino. We isolated RNA from pepino skin and pulp for 6 time points throughout ripening (immature to senescent stage), made cDNA and performed quantitative PCR for all LOXs. Several candidate LOX genes were cloned from heterologous and degenerated primers from closest family members such as tomato and tobacco. Gene expression data will be discussed.

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29.- Transcriptional analysis of ethylene metabolism-related genes in avocado (*Persea americana mill.*) cv. 'hass' under cold storage

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For reaching consumers, avocados are stored and shipped under cold storage conditions, and usually technologies such as ethylene inhibitors are applied for extending postharvest life. However, the response to technologies is affected by both the fruit variability observed in avocado at harvest, and the effect of low temperatures on fruit metabolism. Our objective was to elucidate the effect of cold storage and ethylene inhibition on ethylene metabolism during avocado ripening. For this goal, in the first trial fruit was harvested and stored at 5°C for 7, 14, 21 and 40 days, and then ripen at 20°C to reach a ready-to-eat stage. In a second trial, after harvest fruit was stored at 5°C for 40 days with or without the application of the ethylene inhibitor 1-methylcyclopropene (1-MCP), performed at day 0, 7, 14 or 21 during storage. At each evaluation time physiological and quality parameters were measured, such as pulp firmness, external color, pulp browning, and respiration and ethylene production rates. First trial results showed that fruit under longer cold storage periods required less time to attain the ready-to-eat stage, and a higher homogeneity in ripening was observed. In the second trial, earlier 1-MCP treatments during cold storage induced longer ripening period and higher heterogeneity among ripening avocados. In order to characterize changes in ethylene metabolism, the expression of avocado ethylene metabolism-related genes (ACS1, ACS2, ACO, CTR1, ETR1, EIL1 and EIL3) are currently under quantification by qPCR. Changes in transcript levels will be correlated to the phenology of the treatments.

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30.- Role of reactive oxygen species (ROS) and ethylene (et) as signal molecules in the production of glucosinolates in broccoli (*Brassica oleracea* L. cv. Italica) subjected to abiotic postharvest stress

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Glucosinolates (GS) are well known chemopreventive compounds. Since they are hydrolyzed into isothiocyanates, compounds that induce phase II detoxication enzymes, enhancing the antioxidant status and protecting against chemically induced cancer. Is it known that abiotic stresses such as wounding and exogenous phytohormones (ethylene and methyl jasmonate) induce GS synthesis. However, little is known about the molecular and physiological processes inducing this stress-response. Stress signaling molecules such as reactive oxygen species (ROS) and ethylene (ET) are involved on the activation of defense genes. However, their specific role in GS synthesis as a response to abiotic stress has not been elucidated. Therefore, the present work objective was to determine the role and interaction of signaling molecules (ROS and ET) in the biosynthesis of GS induced by wounding and phytohormones (ET and methyl jasmonate) in broccoli (*Brassica oleracea* L. cv. Italica). To evaluate the role of these signaling molecules, broccoli subjected to the abiotic stresses was treated with 1-methylcyclopropane (1-MCP) as inhibitor of ethylene and DPI (diphenyleneiodonium) as inhibitor of ROS biosynthesis. Broccoli florets were wounded into four pieces, and stored at 20°C for 24 h alone or in the presence of ET and/or methyl jasmonate. DPI and 1-MCP were applied individually and in combination on the stressed tissue. GS were quantified and identified by HPLC-DAD and HPLC-ESI-MSⁿ. Total GS increased about ~490% by wounding stress. This increment was reduced at ~70% by blocking the ET effect. While the use of ROS synthesis inhibitor caused a decrement about ~55% comparing to control samples. The expression of GS biosynthesis (BoCYP79B2, BoCYP83B1, BoCYP79F1, and BoCYP83A1) and hydrolysis (BoMyo, BoESP, and BoESM1) genes are being analyzed to be correlated to GS content and profiles in order to establish their relationship in GS biosynthesis.

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31.- Ethylene role in the incidence of Chilling Injury on pomegranates (*Punica granatum*) during storage

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Chilling Injury (CI) in pomegranates can appear over 45 days of confinement on low temperatures (2-4°C), generating losses on the fruit's quality, affecting on their exportation. A correlation between the apparition of CI and the increase of ethylene hormone and its precursors has been describen in non-climacteric and climacteric fruit. The aim of this research is understand the role of ehylene and its precursors against the physiological disorders that constitute CI during cold storage of pomegranates using protection systems. In the present investigation we applied different treatments to groups of pomegranates which were later stored on a cold chamber (4°C±2°C), taking samples of all treatments in days 0, 20, 60, 100 and 120 days (included 3days at 20°C). The following treatments were applied: exogenous ethylene application (0.5, 1 and 1.5 µg/ml), ethylene inhibitor 1-methylcyclopropene (1MCP) application (1µg/ml), packing in modified atmosphere X-tend bags, a combination treatment (1MCP-X-tend bags) and packing in macro-perforated bags. As control we used a group maintained at room temperature (20°C). For each sample taken we analyzed the concentrations of ethylene precursors 1-aminociclopropane-1-carboxylic acid (ACC) and 1-(malonylamino) cyclopropane acid (MACC) and the activity of the main enzymes of the route, 1-aminociclopropane-1-carboxylate synthase (ACS) and 1- aminociclopropane-1-carboxylate oxidase (ACO) using gas chromatography. The results showed an increase of ACC, direct precursor of ethylene and an activity increase of ACO, in groups treated with exogenous ethylene at 120 days. Symptoms of CI were observed on those treatments as well, suggesting a relation between CI disorders and ethylene effects in pomegranates.

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32.- The regulation of ABA biosynthesis and its effect on the softening of *F. chiloensis* fruit

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The native Chilean strawberry fruit (*Fragaria chiloensis* (L.) Mill) has potential in the international market as exotic fruit, however its fast softening limits its commercialization. As a non-climacteric fruit, the control of its ripening remains unclear, although auxin (Aux) and abscisic acid (ABA) may have a relevant role. In the commercial strawberry (*F. × ananassa*) auxin levels increase in the receptacle from fruit set until unripe large size fruit stage, declining after that, while ABA displays a constant increment during development. In this work, the transcription level of genes involved in the biosynthesis and perception of ABA were analyzed by qPCR during development and ripening of *F. chiloensis* fruit. The effect of auxin and ABA treatments on the expression of the same genes was also analyzed, in addition to some softening related genes. The data collected indicated that ABA biosynthesis and perception increases notoriously at the turning stage and remains high until the end of ripening. A high level of ABA biosynthesis transcripts was observed in the achenes. In response to auxin treatment the expression of both ABA biosynthesis genes and those involved in its perception are induced. The treatment with ABA stimulates the transcription of some softening related genes such as FcXTH1 and FcPG, nevertheless no changes were observed in FcXTH2 and FcEG. These data suggest that in developing *F. chiloensis* fruits auxins can induce ABA biosynthesis, and ABA can regulate the development of ripening of the Chilean strawberry fruit.

R. Lizana acknowledges Universidad de Talca for his doctoral fellowship. Research supported by Fondecyt 1110792 and Anillo ACT-1110 projects.



33.- Molecular characterization of a rhamnogalacturonan endolyase gene expressed during ripening of *Fragaria chiloensis* (L.) MILL.

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Fruit ripening is a coordinated event and involves several changes promoting the development of an attractive fruit to eat. Particularly, texture modifications leading to fruit softening are strongly related to cell wall metabolism, and several enzymes acting on cell wall polysaccharides have been identified. *Fragaria chiloensis* (L.) Mill. is a Chilean endemic fruit with excellent organoleptic properties, however its fast softening during ripening is responsible of its brief postharvest shelf-life. Previous research has concluded that the main cell wall polysaccharide lost during ripening are the pectins, primarily rhamnogalacturonan I (RG-I). Until now there is information about enzymes acting on RG-I side chains, nevertheless little is known about enzymes acting on RG-I backbone. From a transcriptome of *F. chiloensis* fruit a contig encoding to a rhamnogalacturonan endolyase (RGL4, Polysaccharide lyase 4 family; EC number 4.2.2.23) was identified whose FPKM values increased during its ripening. Through the catalytic β -elimination mechanism RGL4 breaks the α -1,4-glycosidic bond between rhamnose and galacturonic acid of RG-I backbone. The predicted RGL4 sequence consists of 676 amino acid residues, including a signal peptide. Two putative N-glycosylation sites are predicted. It shares 24% identity with RGL4 from the saprophytic fungus *Aspergillus aculeatus*, and also the three characteristic domains in the tertiary structure are conserved as well as the key catalytic residues (K217 and H276). Understanding the role played by this enzyme in cell wall disassembly will contribute to a comprehensive knowledge of the molecular mechanisms involved in the ripening of *F. chiloensis* fruit.

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34.- A transcriptomic approach for exploring the ripening of chilean strawberry

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The Chilean strawberry (*Fragaria chiloensis* spp. *chiloensis*) is a non-climacteric fruit with valuable traits such as aroma, pale appearance and nutritional value. However, its high softening rate leads to a short postharvest life. The edible strawberry flesh is actually an enlarged receptacle tissue. The true fruit are the numerous achenes embedded in the receptacle surface, which are responsible for producing essential phytohormones for ripening. To study the molecular mechanisms underlying the differential contribution of achenes and receptacle during ripening, a comprehensive transcriptome analysis was generated for three fruit-ripening stages: large green, turning and ripe, using whole fruit and only receptacle tissue. From these fruit materials, six Illumina HiSeq-2000 RNA-seq libraries were constructed and then sequenced. A merge 'de novo' assembly from all the libraries was performed by Trinity, obtaining 204,754 contigs with a N50 of 1,125 bp. Functional annotation by Blastx analysis revealed 103,413 contigs hit (50.5% of total) to annotated genes into references genomes of 3 species from the Rosaceae family (apple, peach, strawberry) and poplar. Additionally, Gene Ontology analyses assigned GO terms to 48.6% of total contigs. Interestingly, 20% of the sequences were classified into 'response to stimulus', which are related to stress component associated with fruit ripening. Other sub-categories highly represented are 'metabolic process', 'biological regulation', 'developmental process', 'cellular component' and 'organization or biogenesis', that probably represent sequences that have a role during fruit development and ripening. Finally, data mining of sequences annotated to GO terms classified into 'Cell wall', 'Phenylpropanoid-flavonoid' and 'Hormonal regulation' revealed a biologically meaningful reprogramming at different fruit-tissues and ripening stages.

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35.- Computational study of the glycosylation effect on FcXTH1 in protein structure and protein-ligand interaction

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Xyloglucan endotransglycosidase/hydrolase (XTH) enzymes belong to GH16 family, and use xyloglucans (XGs) as ligands. An XTH has been identified in *Fragaria chiloensis* fruit, and studies suggest that it might play a key role in disassembling of the hemicellulose-XG matrix during fruit ripening. Sequence analysis indicated a high identity between FcXTH1 and other plant XTH enzymes. The FcXTH1 sequence contains the conserved XTH catalytic motif (EIDFE) and one potential N-linked glycosylation site, which is also highly conserved in XTH enzymes from this family. To gain insight about the role of protein glycosylation for ligand interaction, the structure of FcXTH1 enzyme was built through comparative modeling methodology and then validated and refined with molecular dynamics simulation (MDS). The model structure of FcXTH1 comprises 15 β -sheets and 2 α -helices. The catalytic motif HDEIDFEFLG is oriented towards the central cavity. The NRT segment (N-glycosylation site) in FcXTH1 is contiguous to the active site. The interaction of a set of putative XG substrates and cellulose with the protein was explored using docking, MDS and MM-GBSA analysis. FcXTH1 showed favorable interaction energy with XGs and unfavorable interaction with cellulose. Nevertheless, the stability of the protein-ligand complex depends on the glycosylation state of FcXTH1: better energy interactions were observed in the glycosylated protein compared to the non-glycosylated. The data provided allow us to propose that the glycosylation of FcXTH1 is necessary for optimal protein-ligand interactions and for enzyme activity.

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36.- Sucrose modulates color development and anthocyanin biosynthesis in *Vitis vinifera* cv. “Crimson seedless”

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‘*Crimson Seedless*’ (CS) is one of the colored varieties most exported in Chile; however, the development of red color required for a commercial harvest is affected under specific environmental conditions. To improve color it is necessary the use of growth regulators such as abscisic acid (ABA) and ethylene, but its effect varies depending on the concentration and time of application. Recent studies indicate the existence of signaling molecules such as sucrose that can produce a similar effect in color. The aim of this work was to study the use of sucrose as a possible color modulator on CS berries. Sucrose treatment had a higher color development relative to the non-treated fruit, but less than the ABA-treated berries. In order to evaluate the effect of sucrose at the level of secondary metabolites, the concentration of anthocyanins in the epidermis was analyzed. Sucrose presented higher levels of anthocyanins than the control treatment. These results were consistent with the analysis of gene expression, where a differential expression of key genes of phenylpropanoid pathway: VvUFGT and VvmybA1, was observed. These two genes showed higher transcript levels than the control treatment, but lower than the treatment with abscisic acid. These results suggest that sucrose has a potential effect on the color by inducing the synthesis of anthocyanins and improving the quality of table grapes.

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37.- Application of 1 - methylcyclopropene (1-mcp) gas and its effect on the accumulation of anthocyanins and color development in *Vitis vinifera* cv 'Crimson seedless'.

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Vitis vinifera is a non-climacteric fruit since its ripening is ethylene-independent; however, the literature describes an increase in the levels of this hormone before veraison. In wine varieties it has been demonstrated that the use of 1-MCP (ethylene inhibitor) affects some quality attributes such as size, titratable acidity (TA), and color of berries. The aim of this study was to analyze the effect of the application of 1-MCP on the accumulation of anthocyanins, color development and ripening of table grape cv. 'Crimson Seedless' (CS). For this, close to veraison applications of exogenous ethylene (Ethrel, 400mL/L) and 1-MCP (SmartFresh™, 4 ppm applied in gaseous form) were made. The treatments with ethylene and 1-MCP did not affect the quality attributes of firmness, total soluble solid content and TA, being similar to the control treatment. The red color development in clusters and berries was inhibited with the application of 1-MCP, contrary to ethylene, which promoted color development by an increase in anthocyanin accumulation obtaining more colored berries. However, the ethylene effect on color development was not uniform in the grapes. In regards to the genes involved in the anthocyanin synthesis pathway, an increased expression was observed in the ethylene treatment compared to the control treatment. Coincidentally with what has been reported in the literature for wine varieties, the 1-MCP treatment in table grapes inhibits the accumulation of anthocyanins and color development delaying fruit harvest.

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38.- Expression of defense genes against *Botrytis cinerea* in *Fragaria chiloensis* fruits treated preharvest with methyl jasmonate and chitosan

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The Chilean strawberry (*Fragaria chiloensis*) fruit has interesting organoleptic properties, but its postharvest life is short due to several causes. Among them is the high susceptibility to infection by fungal pathogens. *Botrytis cinerea*, also known as gray mold fungus, causes serious postharvest losses in strawberries. In a previous work, we reported that preharvest applications of the elicitors methyl jasmonate (MeJA) and chitosan delay the decay incidence in *F. chiloensis* fruit during postharvest storage. Thus, the objective of this study was to analyze and contrast the activation of pathogenesis-related proteins (PRs)- and polygalacturonase inhibiting proteins (PGIPs)-coding genes in response to *B. cinerea* inoculation in fruits treated preharvest with the above elicitors. Gene expression analyses were carried out at 0, 2, 24, 48 and 72 h post inoculation (hpi) of fruits with *B. cinerea* spores. In general, transcriptional profiles revealed an up-regulation of the analyzed genes in both treatments at all times, with the exception of chitinase 3 (Chi3) gene. The analysis of infection evolution among treatments established the protective role of both elicitors at 48 hpi. According to this fact and the analysis of gene expression profiles, elicitor applications could reinforce defense mechanisms through the upregulation of different defense genes: while MeJA upregulates b-1,3-glucanase (BG2-1, BG2-2 and BG2-3) and chitinase 2 (Chi2) genes, chitosan upregulates of PGIP1 and PGIP2 genes. Our results showed that preharvest treatments of MeJA and chitosan protect fruit against *B. cinerea* infection during postharvest, and suggest that this protective effect is mediated by the upregulation of specific defense genes.

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39.- Role of ethylene, auxins, abscisic acid, jasmonic acid, and salicylic acid in modulating fruit responses to photooxidative stress leading to sunburn and sunscald development in apple (*Malus domestica* Borkh.) CV. *Granny Smith*

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Sunburn and sunscald, are two of the most important physiological disorder in apples (*Malus domestica* Borkh), which cause serious quality problems in pre and post harvest, respectively. Sunburn is a physiological disorder caused by excessive solar radiation and elevated temperature during the growing season, causing morphological, physiological, and biochemical changes in fruit tissues. Sunscald appears during cold storage and it has been directly correlated with sun-damage pre-harvest. The objective of the study was to study the role of indole-3-acetic acid (IAA), abscisic acid (ABA), jasmonic acid (JA), and Salicylic acid (SA) during the development of sunburn and sunscald pre-and postharvest on Granny Smith apples. The study was conducted during 2013-2014 season. Fruit tissue (peel) was extracted from fruit growing with different exposures and sunburn levels on the tree (treatments). Sampling was carried out every 15 days from 75 day after full bloom (DAFB) to 165 DAFB (commercial harvest), and then from harvest until 120 days in cold storage (1°C, >90%RH). Concentrations of IAA, ABA, JA and SA were performed using UHPLC mass spectrometry. Statistical analysis was performed using analysis of variance (ANOVA) and mean separation using Tukey test (HSD, $P \leq 0.05$). During sunburn development in the tree ABA, JA and SA concentrations were significantly higher ($P \leq 0.05$) in moderate-severe sunburn level than in the rest of the treatments. In general, IAA declined during fruit development and levels were significantly lower in sunburned fruit. During cold storage, as sunscald incidence increased with time, IAA declined and ABA significantly increased in fruit that developed sunscald. SA and JA concentrations did varied during storage. The results suggest that ABA, JA, and SA together with ethylene are modulating some of the abiotic stress defense responses, such as phenylpropanoids accumulation, in fruit under photooxidative and heat stress during sunburn development preharvest and sunscald in postharvest.

40.- *FcSEPALLA3* is able to regulate genes involved in *Fragaria chiloensis* fruit traits

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MADS-box Transcription Factors (TFs) are considered as master switches during developmental processes in eukaryotic systems. In plants they are major reproductive molecular regulators and determine floral and embryonic fates. Recently, it has been discovered in climacteric and non-climacteric fruit that MADS-box TFs play relevant roles controlling morphological and physiological characteristics during fruit ripening. Here, using molecular biology and bioinformatics approaches, we identified in *Fragaria chiloensis* fruit a SEPALLATA3 like gene (*FcSEP3*), which is up regulated during fruit ripening and is able to control the expression of several genes related to fruit traits such as softening. The *FcSEP3* TF structural model was employed in Protein-DNA interaction analysis, using CARG box elements found in the promoter regions of genes responsible of cell wall remodeling during fruit ripening. The results shown that all interactions are favorable, although cis-regulatory elements from *FcPG1* and *FcEXPA2* promoters have better interaction with *FcSEP3* compared to *FcPL1*. As a complement, *F. chiloensis* fruits were agro-infiltrated to overexpress *FcSEP3*. The results revealed that *FcPG1*, *FcPL1* and *FcEXPA2* are induced in *FcSEP3* transformed fruits compared to empty vector transformed fruits. This suggests that *FcSEP3* could directly regulate the in vivo transcription of *FcPG1* and *FcEXPA2*, while *FcPL1* could be indirectly regulated by *FcSEP3*. Future experiments such as EMSA or CHIP assays will let us corroborate this hypothesis.

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Ecophysiology of Extreme Environments

41.- Changes in the expression of photosynthesis genes associated to increase in growth temperature in *Deschampsia antarctica* plants, collected from two populations of the antarctic peninsula.

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Increase in temperature is the main effect of global warming in the Antarctic. *Deschampsia antarctica*, a vascular plant that colonize Antarctica, is affected by increase in temperature in terms of photosynthetic performance. *Deschampsia* growth in temperatures (5°C) below their photosynthetic optimum, thus an increase in temperatures could cause changes in photosynthetic performance and growth patterns. As photosynthesis is highly dependent on temperature, is possible to expect changes in the expression of genes related to photosynthesis, specifically an increase in expression at higher temperatures. According to the above, expression of five genes were assessed in plants of *D. antarctica* collected from two different locations from the latitudinal Antarctic gradient. The selected populations corresponded to King George Island, next to Arctowski Station (62°09'S, 58°28'W) and Lagotellerie island in Marguerite Bay (67°52'S, 68° 42'W). Plants were cultivated under three temperatures: 5° (growth temperature), 10°C (optimum photosynthesis temperature) and 16°C (warming temperature). Gene expression was assessed through quantitative real time PCR, using a StepOnePlus thermal cycler. Measured genes corresponded to the violaxanthin depoxidase (VDE), RuBisCO small subunit (RBS), alfa and beta subunits of RuBisCO activase (RBa, RBb). α tubulin and Gliceraldehyde-3-phosphate dehydrogenase were used as housekeeping genes for normalization of target genes through Pfaffl method. Plants collected from Lagotellerie were more responsive to temperature increase. VDE gene presented an increase in expression at 10°C, were RBb gene showed a steady increase in expression, with a higher expression at 16°C, on the other hand the small subunit of RuBisCO showed higher expression in Lagotellerie plants at 5°C. No changes in expression were observed in Arctowski plants except for the RBa gene, were an increase in the expression was found at 16°C. Finally, we can conclude that plants from Lagotellerie were more sensitive to increase in temperature that plants collected from Arctowski in terms of gene expression.

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42.- Morpho-physiological differentiation in four populations of *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) from the southern Patagonia and Antarctic

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Colobanthus quitensis (Kunth) Bartl. (Caryophyllaceae) is a native vascular plant that inhabits the Maritime Antarctic. It presents simple or branched stems with growth of distal buds that confer it the ability to form large compact cushions depending on their age. Throughout its wide distribution, ranging from southern Mexico to Antarctica, it has shown its ability to survive extreme environments becoming the subject of research focused on the mechanisms of tolerance. In this study different variables were measured in four populations of the species in order to relate their morpho-physiological state, with the influence of edafoclimatic conditions provided by its habitat. The variables analyzed were cushion length and width, the number of leaves and buds, stem diameter, foliar and root length, water content, Fv/Fm and foliar nutrient content. The populations analyzed were collected directly from natural habitats, two from the South of Patagonia ("La Marisma" -pPA and "Laredo" -pL), and two from the Antarctic island (Hannah Point -pH and Arctowski -pA). The results showed the existence of significant differences in morphological variables in the southern Patagonia plants highlighting pPA Population which presented greater vegetative development than the Antarctic plants. Regarding the physiological characteristics, it was determined that individuals from both sites survive with an amount of nutrients well below the optimal level and tend to accumulate heavy metals, highlighted in the latter the pA and pH populations.

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43.- Modulation of the relative growth rate and its components by temperature and water availability in *Colobanthus quitensis* from two spatially distant populations in antarctica

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The warming trend in Antarctic is higher in some areas, being most marked from the southern part of the Peninsula to the northernmost. This indicates that despite the fact that the plants growing in southernmost regions are exposed to lower temperatures, are also exposed to greater warming trend. If the warming is accompanied by an increase in liquid precipitation, this would eventually lead to increased water availability. Together, these two factors could affect the relative growth rate (RGR) of plants. To address this question, plants of *Colobanthus quitensis* from two populations, King George Island (KGI, 62°09'S, 50°28'W) and Lagotellerie Island (LAG, 67°52'S, 68°42'W) were cultured at field capacity (WW) and water deficit (WD), under two temperatures: 5°C and 16°C, and RGR and its components were determined. In both populations RGR was higher at 16°C, however its response to WD was different. In KGI lower RGR was determined in plant growing in WD at 5°C, however, at 16°C RGR was increased. On the other hand, in LAG independent of the growth temperature, RGR decreased always under WD. Regarding its components, in both populations the aerial biomass was maintained; however the root biomass increased in all conditions. The growth relative components indicate that at 5°C decreases in RGR due to WD was mostly associated with a decrease in physiological components (NAR) and the specific leaf area (SLA) in both populations. At 16°C the increase of RGR in KGI was determined by an increase in NAR and the leaf mass ratio (LMR), and the decrease in LAG was determined by a decrease in NAR and SLA. In conclusion, the results showed that the influences of NAR, LMR and SLA on the growth of antarctic plants will depend on the different scenarios imposed by the climate change.

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44.- Photosynthetic performance in antarctic plants: effects of *in situ* warming

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Low temperature, water availability, and short growing season are the major constrains for plant performance in the Antarctica. *Deschampsia antarctica* and *Colobanthus quitensis*, the only two vascular plant that growth naturally in Antarctica, are able to maintain its growth by maximizing photosynthesis during the growing season. Face of warming, we ask whether the in-field photosynthetic mechanisms developed by these species, and their ability to survive in extreme conditions, will be sufficient to respond to the climate change. We conducted a climate-manipulation experiment in King George Island (62° 09' S, 58°28' W) over three growing season, using passive warming systems (OTC), and the effect of warming on photosynthesis, the diffusive and biochemical limitations, and the relationship among these and plant growth was tested. Both species responded differently to the warming in the most of the parameters evaluated. The relative growth (%RG) and dry mass per area (LMA) of *D. antarctica* were not affected by warming. On contrary, in *C. quitensis*, the influence of warming led to a significant greater %RG and lower LMA. Despite *D. antarctica* presented higher net photosynthesis (A_N) than *C. quitensis*, only in this later, A_N was positively affected by warming. No differences in mitochondrial respiration at darkness were founded, suggesting possible differences in other determinants of the photosynthetic process. The leaf mesophyll conductance (g_m) displayed trends similar to A_N . Thus, the diffusion limitations were more evident in *C. quitensis* compared to *D. antarctica*. The increase of g_m in *C. quitensis* growing under warming increased significantly the CO_2 concentration at the Rubisco carboxylation site (C_c), favoring the maximum rate of Rubisco carboxylation (V_{cmax}). On the contrary, no differences were founded in *D. antarctica*. Thus, only *C. quitensis* seems to respond positively to the warming, promote higher rates of net photosynthesis and, therefore, more growth under a climate change scenario.

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45.- Effects of temperature and water availability on relative growth rate of *Deschampsia antarctica* from two antarctic populations

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The increase in temperature and variation in water availability are two of the most important components of climate change, being the Antarctic Peninsula one of the places most affected. *Deschampsia antarctica*, is the only monocot that has been able to grow under antarctic conditions. In the actual climate change scenario, components associated to their relative growth rate (RGR) could be affected, changing their growth patterns or the modulations way of RGR. Plants from two different Antarctic populations (King George, Lagotellerie Island; KGI, LAG) were cultured at 5°C and 16°C, under field-capacity (WW) and water-deficit (WD) and RGR and their components, morphological (LAR) and physiological (NAR) were analyzed. In both populations increase temperature favors the total RGR (RGR_t), being almost two-folds higher at 16°C. However, the water conditions produced not effect on both RGR_t and total biomass. This, was significantly increased only in plants growing at 16°C. At this temperature higher differences were determined between WW and WD, mainly in aerial-biomass of plants from LAG. In both provenance and growth-conditions, the root-biomass was higher than aerial. Regarding to physiological component, plants from KGI favored their NAR in WW. This decrease could be product of the stomatal-closure and the reduction in the carboxilation substrate produced under WD. Plants from LAG increased their NAR under WD, mainly at 16°C, likely as a compensation for the reduction in the aerial-biomass. The increase temperature seems to favor the growth of *D. antarctica*, however, their responses to water availability seems to depend on the plants provenance. Thus, the mechanisms of plants from northernmost populations is to maintain their biomass-production independent on the water conditions, while plants from southernmost, reduce their biomass but increase their NAR. It's likely that under warming conditions in field will depend also on the length of the drought period and other climate factors.

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46.- Morpho-physiological response of *Colobanthus quitensis* and *Juncus bufonius* under different climate change simulation

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Maritime Antarctic is characterized by its extreme environmental conditions such as; low temperatures, low water availability and low nutrient content in the soil. Over the past 50 years global warming has caused an increase in ambient temperature in Antarctica, and has collaterally led to changes in water availability, not only due to increased of precipitation in liquid form during summer but also the melting of permafrost. This scenario of climate change will improve growth conditions, increasing the size of populations and local expansion of native species such as *Colobanthus quitensis* and *Deschampsia antarctica* as well as non-native species as reported for Maritime Antarctic. Because of this, a combination of increased temperature and water availability on morphological and physiological variables in the plants of *Colobanthus quitensis* and *Juncus bufonius*, using 4°C–H₂O (field capacity), 4°C+H₂O (40% of field capacity), 15°C–H₂O, 15°C+H₂O, 20°C–H₂O and 20°C+H₂O was evaluated. The results showed that as temperatures and water availability increased, variables such as plant length, number of inflorescences, Fv/Fm and chlorophyll content increased significantly for both species. When evaluating both species separately, it was possible to determine that the most crucial climate factor for the growth and development of *C. quitensis* was the water availability above temperature, on the other hand, the most determining factor for the growth and development of *J. bufonius* was the temperature above water availability.

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47.- Biological activity and determination of phytochemicals of *Empetrum rubrum* (Vahl ex Willd)

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Plants contain a wide variety of free radical scavenging molecules, such as flavonoids, anthocyanins, carotenoids and vitamins such natural products are rich in antioxidant activities. *Empetrum rubrum* is a subshrub, native that grows under unfavorable environmental conditions so their metabolism adjusts to these conditions, by synthesizing phenolic compounds. The antioxidant capacity was investigated by using methodologies that consider electron transfer mechanisms (ET), such DPPH[•] and ABTS^{•+} radical. The total polyphenol content by the Folin–Ciocalteu method was determined. The antifungal activity was determined by the agar dilution technique and radial growth inhibition method against the fungus *Rhizoctonia solani*. Additionally, the presence of secondary metabolites was determined by staining and precipitation techniques. In the assay using DPPH[•] a percent inhibition of 73.7% was reached at a concentration of 9 mg/ml, while in the kinetic assay a percentage inhibition of 94% at 180 minutes was reached. In ABTS^{•+} a percent inhibition of 96.6 % was reached at a concentration of 9 mg/ml. The results were expressed in equivalents of gallic acid for DPPH assay and in equivalents of trolox for ABTS^{•+} assay. The content of total phenolics was expressed as gallic acid equivalents. The extract was active against *R. solani* in concentrations of 10, 15 and 20 mg/ml. The phytochemical screening of the leaves and stems shows the presence of useful bioactive substances such as tannins, saponins, flavonoids and anthraquinones. The results showed that *E. rubrum* could be a potential source of natural antioxidants.

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48.- Photosynthetic capacity of *Deschampsia antarctica* from two population in Antarctica: diffusive and biochemical limitations

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Antarctica presents one of the most adverse conditions for plants development. However, the warming, that extends from the southern part of the Antarctic Peninsula and decreases towards the north, is changing these conditions. This could imply a relaxation of environmental limits for *Deschampsia antarctica*, the only monocot that inhabits this place. Because in field, this species grows at temperatures below their optimum for photosynthesis, increased temperature could increase net photosynthesis (A_N). To evaluate the photosynthetic capacity of *D. antarctica* in face to warming and the effect of this on the photosynthetic limitations, plants from two populations of Antarctica (King George, KGI, and Lagotellerie, LAG Island,) were cultivated (CT) and measured (MT) at different temperatures (5 °C, 10 °C and 16 °C). The CO₂ assimilation was analyzed using an infrared gas analyzer system combined with chlorophyll fluorescence. The photosynthetic performance varied between populations, both in CT response as well as MT. Higher A_N were determined in LAG at CT16°C-MS16°C (around 10 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Regarding the CT, plants from KGI showed increase of A_N only at CT16°C. However, in LAG, increased in CT as well in MS, produced increases in A_N . Respect to the photosynthetic limitations, plants from KGI showed no effect of temperature on their diffusive limitations. On the contrary, plants from LAG, showed changes in their limitations depending on the temperature. In this plants, A_N was strongly determined by the CO₂ mesophyll conductance (g_m) and in less extent by carboxylation rate of Rubisco (V_{cmax}). Thus, in *D. antarctica* the increase temperature not always imply an increment in photosynthesis. So, the positive effect of warming, in this species, seems to response in different way depending on the locations.

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49.- Effects of *in situ* warming on morphoanatomical characteristics of antarctic plants

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To survive to the severe Antarctic conditions, *Deschampsia antarctica* and *Colobanthus quitensis* have developed morpho-anatomical modification, having both xeromorphic characteristics. Much of these modifications seem to be plastic and strictly connected with the environmental. In the climate change context, the warming is already evident along the Antarctic Peninsula, where the average of annual temperature has increased about 1.5°C. It is possible that warming could produce a relaxation of environmental limits, being less severe for the plant development. The effect of warming on leaf characteristics of antarctic plants has been not evaluated in field conditions. Thus, using passive warming systems (OTC), we test the effect of warming on morphoanatomical modifications of antarctic plants. Leaves of both species growing in King George Island, inside OTC and open areas (OA) were collected and microscopically analyzed. Both species responded in different way to warming. *D. antarctica* maintained the most of the anatomical characteristics, being the mesophyll width, the cross-sectional area, epidermis width (lower and upper) and vascular bundles size similar between plants growing inside OTC and OA, however the vessels size was higher in OA. On the contrary, in *C. quitensis* all the characteristics above mentions were lower in OTC. So, in these conditions smaller leaves were developed, including vascular bundles. The stomata density was maintained in both species but, in *D. antarctica*, the open stomata was higher in OTC. Regarding to ultrastructure, in both species the cell wall width and the chloroplast number was not modified, however *D. antarctica* presented bigger chloroplasts in OA. These results agree well with previous studies, where *D. antarctica*, on contrary to *C. quitensis*, showed not response to warming in field. Thus, face climate change, the antarctic species could present different responses as in their leaf anatomy as in the physiological processes associated.

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50.- Morphoanatomical differences between antarctic plants coming from two distant populations in Antarctica

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Antarctica is characterized by low temperatures, water availability, high radiation and strong winds. Under these conditions only two vascular plant have been able to live: *Deschampsia antarctica* and *Colobanthus quitensis*. Within the Antarctica there are sectors with microclimate even harder, with lower temperatures and longer days. To withstand adverse conditions plants generate different mechanisms, being the most common those associated to the foliar anatomy. Thus, plants can reduce the epidermal contact with the atmosphere, increase the epicuticular waxes, thicken cell walls, increase the mesophyll thickness and control certain stomatal characteristics. In this study we evaluated possible differences of *D. antarctica* and *C. quitensis* growing in two spatially distant populations in Antarctica. King George (KGI, 58°28'S; 62°09'W) and Lagotellerie Island (LAG, 67°52'S; 68°42'W). Leaf samples were fixed and analyzed through optical, scanning and transmission microscopy. In the southernmost population the cross-sectional area of *D. antarctica* was higher displaying longer leaves. Its vascular bundles were bigger; however the vessels size was not different. Additionally, the cell wall width was also higher. In *C. quitensis*, an increase of the cross-sectional area was also observed in LAG; however the width mesophyll was lower. The cell wall width was higher in this population, and all the other parameters evaluated, associated to the stomata and chloroplasts characteristics, were similar between populations. However, the vascular bundle size was lower in LAG, this characteristic is described as a modification to control loss water and avoid cavitations under adverse conditions. Despite *D. antarctica* increase the cross-sectional area of the leaves and the vascular bundle size, in response of the harder conditions found in LAG, this species increase folding of their leaves, as a consequence of bulliform cells smaller. Thus, both species modified some morphoanatomical characteristics in order to withstand in a better way the climate conditions of their environment.

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51.- Temperature effect on the reproductive effort of three ecotypes of *Colobanthus quitensis*

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Colobanthus quitensis (Kunth) Bartl. is a vascular species living in Antarctica. It is a monoicous plant, with complete flowers and self-fertilization. Plants show morphological variability along its wide distribution range, along the Andean mountains stretching from the tropical Andes to the Antarctic Peninsula. Flower production in *C. quitensis* is limited by low temperature. Consequently, it is expected that warming increase *C. quitensis* flowers, seeds and allocation to reproductive organs increasing its reproductive fitness. We study the effect of temperature (5 and 11°C) on plant flowering in 3 ecotypes of *C. quitensis* (Punta Arenas, Cordillera and Antarctica). The Antarctic ecotype was the first to set up flowers (4 weeks) at 5°C, while the first ecotype that flowers at 11 °C was the ecotype from Punta Arenas (7 weeks). Despite bloom was later at 11°C, due to its faster development, the total number of flowers per plant at 25 weeks were the following: Punta Arenas, 7 at 5°C and 200 at 11°C, Antarctica, 11 at 5°C and 34 at 11°C, and Cordillera with 11 at 5°C and 31 at 11°C. However, when the number of flowers was expressed based on plant cushion area only Punta Arenas ecotype exhibited a higher allocation to reproductive organs. It is concluded that higher temperature increased the number of flowers per plant in all the studied ecotypes. Higher temperatures only increase allocation to reproductive organs in the ecotype from Punta Arenas. Therefore, warming would increase reproductive fitness of *C. quitensis*.

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52.- Relationship of genetic diversity and environmental traits in Chilean wild tomatoes

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The cultivated tomato is come from regions that cover Ecuador, Peru, Bolivia and the north of Chile in South America. The wild tomatoes growth under amply climatic conditions, from the level of the sea until 3300 masl. The wild species are characterized by a high genetic diversity, where such as *S. peruvianum* and *S. chilense* show high resistance to several abiotic stresses. The present study has as aim relate the genetic diversity of wild tomatoes from Chile with the environmental variables in their specific habitats. Here, we propose that the environmental factors have been modeled the genetic diversity present in wild tomato species. We analyzed the results of GBS sequencing for 85 genotypes belong from four wild tomato and *Solanum lycopersicum* selected from different location in the north of Chile. The final set of markers in this study (5748 SNPs) was obtained then of filtering the data with more than 10% of missing genotype rate and lower than 6% of minor allele frequency from a total of 57566 SNPs. We identified population structure, which grouped in the five studied species. *S. lycopersicoides* and *S. sitiens* showed a same cluster, while *S. peruvianum*, *S. chilense* and *S. lycopersicum* show grouping in differentiated clusters. We used spatial analysis methods (SAM) to scan the 5748 SNPs to asses a putative association with five environmental variables. The higher association of markers was founded for low temperature and higher elevation traits. The markers detected associated to environmental variables make possible understands the proportion of genome that is modeled by natural selection.

53.- Effects of temperature on the photosynthesis and their limitations in *Colobanthus quitensis* from two antarctic populations

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The Antarctic conditions are defined as hostile to the plants development. However because to the global warming, these conditions are being modified. Because the warming seems to be not uniform in the Antarctic continent, the effects on plant physiology could be different, depending on the location. *Colobanthus quitensis* is the only dicot currently present in Antarctica. Due to this species grows under temperatures lower to their optimal for photosynthesis, an increase in the temperature could result in a reduction of photosynthetic limitations, implying an improvement in the photosynthetic rates (A_N). So, under laboratory conditions, the effects of increase temperature on photosynthetic performance of *C. quitensis* collected from two populations in Antarctica (King George, KGI and Lagotellerie Island, LAG) were studied. Plants were cultivated at 5°C, 10°C y 16°C, and CO_2 assimilation was analyzed with a simultaneous chlorophyll fluorescence and gas exchange system. The increase temperature (16°C) favored the photosynthetic performance in plants from both populations (almost three-folds with respect to plants growing at 5°C), and this increase was higher in plants from KGI. The biochemical determinant, maximal carboxylation rate (V_{cmax}), increased also with temperature, in both populations. However, A_N was strongly determined by total conductance, in particular by the mesophyll conductance to CO_2 (g_m). This relationship was linear in KGI. In both populations the warming could improve the photosynthetic performance. However, due to the xeric characteristics of this species, the diffusive limitations will continue being high, limiting the photosynthesis even under warming conditions.

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54.- Effect of water availability on photosynthetic limitations of *Colobanthus quitensis*

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The Antarctic Peninsula is one of the regions that have showed greatest effects of climate change. Thus, the organisms that live there have been considered as indicators of this change. Water deficit is one of the main consequences of this change and study its effect on plant physiology may help to understand the biological consequences of warming. Photosynthesis is a physiological process highly sensitive to environmental conditions, which determine their limitations. Previous reports indicate that photosynthetic limitations of *Colobanthus quitensis*, the only Antarctic dicot, are mainly determined by diffusive limitations, mainly mesophyll conductance to CO₂ (g_m). This latter is offset by a robust biochemical machinery associated with Rubisco, allowing relatively high photosynthetic rates even under the extreme antarctic conditions. Face to the climate change scenario, we wonder if these limitations will be increased or whether this species will modify their behavior to maintain high photosynthetic rates. Thus, the effects of water availability on photosynthetic limitations of *C. quitensis* from two antarctic populations (King George and Lagotellerie Island) were evaluated. Plants were grown at 16 ° C at field capacity (FC) and 35% of FC and analyzed using an infrared gas analyzer combined with chlorophyll fluorescence system. The results indicate that water deficit causes a decrease in both diffusive and biochemical determinants. In drought, the plants reduced stomatal conductance (g_s), and consequently the g_m. This increase in resistances to CO₂ transference, reduces the substrate at the carboxylation site (C_c), decreasing the Rubisco carboxylation efficiency (V_{cmax}). Thus, the increase in photosynthesis limitations results in a lower net carbon assimilation (AN) in water deficit conditions. Given the significant reduction of photosynthesis evidenced in laboratory conditions in front of decreases in water availability, it is possible to assume that the effects of climate change could negatively affect the development of *C. quitensis* in field.

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55.- Photochemical activity of *Deschampsia antarctica*: possible effects of climate change

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The Antarctica is considered one of the most sensitive ecosystems to the effects of climate change, exhibiting a temperature increase in specific sectors of the Antarctic Peninsula, which could additionally lead to variation in the water regime. *Deschampsia antarctica* is one of two vascular plants capable of colonizing this region. The study of the effects of climate change on the physiology of this species, mainly in photosynthetic activity, is essential to elucidate potential biological consequences of warming. Considering an interaction between temperature increase and change in water availability on the photochemical and the photoprotection mechanisms, plants coming from two antarctic populations, King George Island (KGI, 62°09' S; 58°28' W) and Lagotellerie Island (LAG, 67°52' S, 68°28' O) were established at 5°C, 10°C and 16°C under irrigation (WW) and drought (WD). Different kinds of photoinhibition were analyzed through changes in maximal efficiency of PSII (F_v/F_m) and the partition light energy was evaluated across the fluorescence of chlorophyll a of PSII. At 5°C, WD produced dynamic, chronic and permanent photoinhibition in plants from KGI and only dynamic in LAG. At 16°C plants from both populations displayed chronic photoinhibition when were cultured under WD. In both populations the increase temperature favored the electron transport rate (ETR), decreasing the thermal energy dissipation (NPQ). The latter was consistent with lower zeaxanthin contents at higher temperatures. Under WD the increase temperature seems to be not positive for the ETR and neither for NPQ. It is possible that under water deficit plants display other mechanisms to solve the excess of light energy, probably associated to dissipation at reaction center level. Thus, the future climate projections could be positive for *D. antarctica* only if the water regimes are adequate for this species, where increase in the photochemical activities could results in increases the CO₂ assimilation.

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56.- Pollen record on the Fildes Peninsula (maritime Antarctica) under a climate change scenario

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Considering the close relationship that exists between pollen and vegetation, which enables identification of the possible transport routes involved in the arrival of propagules to maritime Antarctica and the current climate conditions of this ecosystem that favor anthropic activity, with greater pressure in terms of soil use and increased risk in terms of the introduction of invading species, the aim of our investigation was to perform a pollen study of topsoil from the Fildes Peninsula in three sectors subjected to different pressure from anthropic use. The sample included a heavily anthropized sector (area between the Eduardo Frei Base belonging to the FACH and the Escudero base belonging to INACH), a moderately anthropized site (Ardley Island), and a third site with low human activity (Collins Glacier). Our results indicated that for the total sampling area, no greater than 4km², the most anthropized sector presents the pollen spectrum with the greatest diversity and abundance associated with high invasion indices compared to the other sampling sites. The dominant families were Asteraceae, Brassicaceae and Chenopodiaceae. Less pollen diversity was found on Ardley Island and the Collins Glacier, and this corresponded to local vegetation. The role of humans as passive agents of potential risk of plant invasion in maritime Antarctica is discussed.

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57.- Expression analysis of *gmmt* gen involved in the synthesis of glucomannan in *Aloe barbadensis* Miller when subjected to water deficit.

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Aloe barbadensis Miller (Aloe vera) is a succulent plant physiologically adapted to arid environments, and, therefore, able to survive under conditions of water restrictions. Previous studies from our research group in Aloe vera plants to test its tolerance to water restrictions, have shown an increase in the water use efficiency with an increase in gel production when plants were irrigated with 50% and 75% of field capacity (FC). These results lead us to postulate the hypothesis that the expression of the gene encoding the enzyme glucomannan 4-beta-mannosyltransferase (GMMT) responsible for the formation of the glucomannan, the main component of the leaf gel, could increase when Aloevera plants are subjected to water deficit. For this, we generated specific primers for Aloe vera *gmmt* gene using conserved sequences that codify for the GMMT enzyme from three monocots species. By qRT-PCR we determined the levels of expression of the *gmmt* gene in plants subjected to water restrictions. Our results indicate that the amplified sequence of *gmmt* contains conserved domains and the active site characteristic of the glycosyltransferase protein family. BLAST analysis of the *gmmt* sequences suggests a high similarity to a glucomannan synthase, the CSLA9 protein. We also found that the expression levels of *gmmt* gene increased in Aloe vera plants when these were irrigated with 50% and 75% FC.

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58.- Diffusive and biochemical limitations to photosynthesis in antarctic plants from two populations in Antarctica

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Due to advances in photosynthesis modeling, a deeper understanding of the mechanisms controlling photosynthetic responses to temperatures has been accrued. However, these approaches have not been applied in field studies including antarctic species. These analyses provide useful information to develop predictive models of CO₂ assimilation and the effects of climate change, incorporating two key aspects of the photosynthetic limitations: the mesophyll conductance of CO₂ (g_m) and the biochemical limitation related with the Rubisco performance. In this study we evaluated in situ photosynthesis and photosynthetic limitations in two populations of *Colobanthus quitensis* and *Deschampsia antarctica*, King George (KGI) and Lagotellerie (LAG) Island. We modeled A_N - C_i response using biochemical Rubisco parameters determined in this study for each species. Extremely low values for g_m were estimated in both, being the highest value 0.13 mol CO₂ m⁻² s⁻¹ for *D. antarctica* LAG at 15°C. This fact provokes that the total leaf conductance (g_{tot}) was low and mainly determined by g_m . The relationship between net photosynthesis (A_N) and g_{tot} was highly significant, indicative that A_N in the antarctic plants under field conditions are limited by diffusional components. Regarding the biochemical determinants, the maximum rate of Rubisco carboxylation (V_{cmax}) tended to increase with the increase in the temperature, and *D. antarctica* showed higher values than *C. quitensis*. A high Rubisco specificity factor ($S_{c/o}$) was determined in both species. So in these species, as in many xeric species, photosynthetic rates are limited by the capacity to mobilize CO₂ into the leaf, and the mechanism to compensate this limitation, is to develop a Rubisco highly specific to CO₂.

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59.- *Prosopis chilensis* under salt stress: from germination to massive sequencing and gene expression.

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Plants living in arid environments have to cope with a plethora of stressors and the development of cellular, molecular, biochemical, physiological and genetic strategies are fundamental to survive in them. Algarrobo (*Prosopis chilensis*) is a native tree belonging to Fabaceae family, which is naturally adapted to arid stressors (salinity among them), and therefore it represents a good model for understanding woody plants adaptation to arid environments. Our aim was to identify the genotype most tolerant to salinity along the natural distribution of *P. chilensis* in Chile and then, to sequence its transcriptome for identifying putative genes involved in salt tolerance. First, we evaluated salt tolerance of several Algarrobo accessions at germination and their physiological responses. Then, using SSR microsatellite markers, we evaluated whether accessions were similar or distinct molecularly, and then, we selected the most salt-tolerant accession to sequence its transcriptome by RNA-Seq. By this approach we were able to identify more than 30.000 transcripts significantly modified by salt stress. Results obtained in each process will be discussed. We found highly conserved genes in different species such as *Theobroma cacao*, *Arabidopsis thaliana* and of course *Prosopis chilensis*. This work is giving us important information about how *P. chilensis* and other plant species can tolerate salt stress.

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