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M E E T I N G P R O G R A M

Wednesday October 14th

12:00 - 14:45 **Registration and poster set up.**

15:00 – 15:15 **WELCOME** (Meeting Organizer - Dr. Andrés Zurita)

15:15 – 16:15 **OPENING SEMINAR**

Dr. Daniel Klessig, Boyce Thompson Institute for Plant Research, Ithaca, New York, USA **"SA, methyl salicylate, and systemic acquired resistance – the plot thickens"**

16:15 – 18:20 **ORAL SESSION 1**
Chairs: Herman Silva / Lorena Norambuena

16:15 **Carvallo, L.,** Herrera, A., Blanco, F. and Holuigue, L. **TGA transcription factors mediate glutaredoxin GRXC9 gene activation by salicylic acid in *Arabidopsis thaliana*.** Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

16:35 **Cofee Break**

17:20 **Defilippi, B.G.**[1,2], Manríquez, D.[1] and González-Agüero, M.[1,2]. **Aroma: an integrative approach for understanding and improving a complex quality trait.** 1. Instituto de Investigaciones Agropecuarias (INIA-La Platina). Santa Rosa 11610, La Pintana, Santiago. bdefilip@inia.cl 2. The Plant Cell Biotechnology Millennium Nucleus (PCB-MN).

18:00 **Gaete-Eastman, C.**, Fuentes, L., Figueroa, C., Valdenegro, M., Balbontín, C., Herrera, R.,Moya-León, M.A. **Molecular characterization and substrate preference of an alcohol acyl-transferase isolated from mountain papaya fruit (*Vasconcellea pubescens*).** Laboratorio de Fisiología Vegetal y Genética Molecular, IBVB, Universidad de Talca.

18:20 **Prat, L.[1],** Dominguez, A. M.[2], Agosin, E.[2], Valenzuela, P.[3] and Silva, H.[1] **The Fragaria chiloensis carotenoid cleavage dioxygenase 1 gene contribute to the formation of the aroma compound 3-oxo-α-ionol.**1. Plant Functional Genomics & Bioinformatics Lab and Millennium Nucleus in Plant Cell Biotechnology (PCB). Universidad Andrés Bello República 217, 837-0146, Santiago. 2. Centro del Aroma, Pontificia Universidad Catolica de Chile. 3. Fundación Ciencia para la Vida.

18:40 **Fuentes, P.** and Stange, C. **The effect of development and light upon the expression levels of carotenogenic genes and a structural analysis of the *lcyb* promoter**. Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

19:00 **Welcome Cocktail – Cheese and Wine**

21:00 **POSTER SESSION (Odd number panels)**

Thursday October 15th

07:30 - 8:30 **Breakfast**

09:00 - 11:00 **ORAL SESSION 2**
Chairs: Gastón Muñoz/ Lee Meisel

9:00 **Dr. Ykä Helariutta,** Institute of Biotechnology/Department of Biological and Environmental Sciences, University of Helsinki, P.O. Box 56, FIN-00014 University of Helsinki, Finland, yhelariu@mappi.helsinki.fi. **"Integration of hormonal and genetic regulation during vascular morphogenesis in *Arabidopsis*".**

10:00 **Dr. Rodrigo A. Gutiérrez,** Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile. **"Post-transcriptional regulatory networks in the nitrogen response of *Arabidopsis thaliana*".**

10:40 **Cofee Break**

11:00-12:20 **ORAL SESSION 3**
Chairs: Loreto Holuigue/ Erwin Krauskopf

11:00 **Obrecht, A.,** Araya, C., and Paneque, M. **Sir2 a new player in dormancy regulation in *Arabidopsis thaliana*.** Facultad de Ciencias Agronómicas. Universidad de Chile. Santiago. Chile.

11:20 **Pérez, F. J.,** and Kühn, N. **The role of phytochromes and floral integrator genes in the transition of grapevine-buds into dormancy.** Universidad de Chile, Facultad de Ciencias, Laboratorio de Bioquímica Vegetal; Casilla 653, Santiago Chile, Email: frperez@uchile.cl.

11:40 **Ibáñez, C.[a,1],** Kozarewa, I.[a], Johansson, M.[a], Ögren, E.[b], Rohde, A.[c] and Eriksson, M. E.[a] **The Circadian Clock Controls Critical Daylength for Growth, Cold Response during Dormancy and Bud Break after Dormancy in *Populus*.** [a] Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, SE-901 87 Umeå, Sweden. [b] Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, The Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden. [c]Department of Plant Growth and Development, Institute of Agricultural and Fisheries Research, 9090 Melle,

Belgium. [1]Actual address: Instituto de Biología Vegetal y Biotecnología. Universidad de Talca. cibanez@utalca.cl.

12:00 **Almada, R.D. [1*]**, Cabrera, N.E.[1], Peña-Cortés, H[2], Ruiz-Lara, S[1] and Gonzáles E.[1]. ***VvCO* and *VvCOL1*, two grapevine constans-like genes, show a diurnal expression pattern under controlled conditions and belong to a multigenic family.** Instituto de Biología Vegetal y Biotecnología, Universidad de Talca.[1], Universidad Técnica Federico Santa María[2]. *email: ralmada@utalca.cl.

13:00- 14:45 **Lunch**

15:00 - 17:10 **ORAL SESSION 4**
Chairs: Alejandra Moya/ Marlene Rosales

15:00 **Sánchez, E.,** Pinto M., Hinrichsen, P. **Characterization of the expression of *Elip*-like genes on grapevine (*Vitis vinifera* L.).** Instituto de Investigaciones Agropecuarias, La Platina. Santiago, Chile.

15:20 **Casanueva, X.[1-2],** González-Agüero, M.[1-3], Aliaga, R.[4], Aravena, A.[4], Hinrichsen, P.[1] **Identification of genes related to ovule development in stenospermocarpic table grape (*Vitis vinifera* L.).** [1]Instituto de Investigaciones Agropecuarias, La Platina, Santiago, Chile. [2] Universidad Andrés Bello, Santiago, Chile. [3]The Plant Cell Biotechnology Millenium Nucleus (PCB-MN). [4]Laboratorio de Bioinformática y Matemática del Genoma. Centro de Modelamiento Matemático. Universidad de Chile. Santiago.

15:40 **Sáenz-Diez, D[1].,** Olivares D [1, 2]., Calvo F[1]., García de Cortazar V[1]., and Pinto M.[1, 2] **Photosynthetic characterization of grapevine (*Vitis vinifera* L.) leaves developed under different light conditions.** [1/] Universidad de Chile [2/] Instituto de Investigaciones Agropecuarias – INIA.

16:00 **Cofee Break**

16:30 **Santiago, M.,** Romero, M. P., Sanhueza, D., and Silva, H. **The *LEA18* gene family is essential for gametes development in *Arabidopsis thaliana.*** Plant Functional Genomics & Bioinformatics Lab and Millennium Nucleus in Plant Cell Biotechnology (PCB).Universidad Andrés Bello, República 217, 837-0146, Santiago.

16:50 **[1]Bascuñan, L.** [2]Goday A. and [1]Bravo L.A. **LEA proteins in different organs of *D. antarctica* subjected to abiotic stress**. 1. Laboratorio de Fisiología Vegetal, Universidad de Concepción, Concepción, Chile. 2. CSIC, Instituto de Biología Molecular de Barcelona.

17:10 Dinamarca, J.[1*], Gutierrez, A.[1*], **Sandoval, A.[1]**, Villarroel, R.[2], Alfredo Herrera Estrella, A.[3] and Gidekel, M.[1,4]. **Differentially expressed genes induced by cold and UV-B in *Deschampsia antarctica*.** [1]Laboratorio de Biología Molecular Aplicada, Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera, Temuco, Chile. [2] VIB Department of Plant Systems

Biology, Ghent University, Belgium. [3]LANGENBIO, Centro de Investigacion y Estudios Avanzados, Irapuato, Mexico. [4]VentureL@b-Knowledge Center for Business, Universidad Adolfo Ibañez, Santiago, Chile.

18:00 **ROUND TABLE**

Contributions of research in plant biology to enhance the country as a leading food producer.

Mr Andrés Sáez R. Chief Technical Officer, M&V S.A.
Ms María Elena Boisier. Director of FONDECYT Program, CONICYT

21:00 POSTER SESSION (Even number panels)

Friday October 16th

07:30 - 8:30 **Breakfast**

09:30 - 11:50 **ORAL SESSION 5**
Chairs: Virginia Garretón/ Andrés Zurita

9:30 **Dr. Michael Handford**. Carbohydrate metabolism in *Arabidopsis thaliana*. Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile, Santiago.

10:10 **Urbina, D. C.,** Meisel, L. **Citokinin and sucrose modulate ε Adaptin role in plant cell division.** Núcleo Milenio en Biotecnología Celular Vegetal, Laboratorio de Genética Molecular Vegetal del Centro de Biotecnología Vegetal, Universidad Andrés Bello(daniurbina@gmail.com)

10:30 **Salinas, C.,** Cardemil, L. **Acemannan and fructan from aloe barbadensis miller (aloe vera) undergo structural modification during water stress.** Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

10:50 **Cofee Break**

11:10 **Pieringer, H.[1],** Rasmussen-Poblete, S.[1,2], Krauskopf, E.[1,2]. **Heterologous expression of the gene *Egbor1* of *Eucalyptus globulus* in *Saccharomyces cereviseae.*** [1] Departamento de Cs. Biológicas, Universidad Andrés Bello, MIFAB, Santiago. [2] Fundación Ciencia para la vida, Santiago.

11:30 **González, W.[1]**, Morales, S.[1], González-Nilo, F. D.[1], Ruiz-Lara, S[1], Ingo Dréyer, I.[2]. **Characterization of voltage regulation, pH regulation and role under abiotic stress conditions of KAT1 and SKOR potassium channels from *Arabidopsis thaliana*.** [1]Universidad de Talca, [2]Universität Potsdam.

11:50 **Mathias, M.[1],** Montenegro, A.[1], Peñaloza, E.[1], Soto, B.[1], and Zúñiga, J.[1,2]. **Growth patterns of Near Isogenic Lines for the aluminum tolerance *TaALMT1* gene in wheat grown in phosphorus-deficient and high aluminum content soils.** [1] Instituto de Investigaciones Agropecuarias INIA-Carillanca, Casilla 58-D, Temuco, Chile. [2] Corresponding author, E-mail: jzuniga@inia.cl.

12:20 – 12:50 **Closing Ceremony**

ORGANIZATIONAL SESSION – V Plant Biology Meeting

13:00 **Lunch**

OPENING SEMINAR

INVITED SPEAKER

SA, methyl salicylate, and systemic acquired resistance – the plot thickens

Daniel Klessig

Boyce Thompson Institute for Plant Research, Ithaca, New York, USA

For over a century naturalists and scientists have observed that plants which survive an initial pathogen attack often develop enhanced resistance to subsequent infections. Kenneth Chester in his 1933 review of 200 publications covering this phenomenon termed it physiological acquired immunity (Q. Rev. Biol. 8:275-324). Systematic studies by Frank Ross in the early 1960's demonstrated that prior infection of tobacco plants by Tobacco Mosaic Virus (TMV) enhanced resistance in the distal systemic tissue to subsequent challenge by TMV or other pathogens, which he termed Systemic Acquired Resistance (SAR; Virology 14:340-358). Kuc and others showed that development of SAR required movement of a signal made in the primary infected tissue through the phloem to the distal systemic tissue more than a quarter of a century ago (Phytopathology 69:753-756, 1979). Our studies on salicylic acid (SA) – mediated signal transduction have shown that methyl salicylate (MeSA) is a critical phloem-mobile signal required for SAR in tobacco (Science 318:113-116, 2007). MeSA is biologically inactive; it is converted by the MeSA esterase activity of salicylic acid-binding protein 2 (SABP2) to SA, a key hormone for activating host defenses to many plant pathogens. Results of grafting studies indicate that SABP2's MeSA esterase activity is required in systemic tissue. A mutation, which destroys SABP2's SA-binding activity and the resulting feedback inhibition leading to unregulated MeSA esterase activity, compromises SAR if expressed in primary infected tissue that generates the SAR signal. MeSA levels increase in primary infected leaves, phloem exudates from these leaves and systemic leaves of control plants but not in these tissues of transgenic tobacco expressing the unregulated SABP2 in the primary infected leaves. SAR also is blocked when SA methyl transferase, which synthesizes MeSA from SA, is silenced in primary infected leaves. Current studies suggested that MeSA is also an SAR signal in Arabidopsis (Plant J. 56:445-456, 2008) and potato. However, a recent Plant Cell (21:954-971, 2009) paper from Jürgen Zeier's group argues that MeSA is not a mobile SAR signal in Arabidopsis. They employed two different KO mutants in Benzoic acid-SA Methyl Transferase 1 (BSMT1I) and found that although these mutants were unable to produce elevated levels of MeSA after infection, they were still able to develop SAR. In contrast, we have found that a similar KO mutant in BSMT1 has suppressed levels of MeSA and is compromised for SAR. Moreover, SAR can be restored in the bsmt1 KO by treatment with MeSA or phloem exudate from infected wt, but not bsmt1 KO, plants. In addition, recently several groups have identified lipid-derived mobile SAR signals. These include azelaic acid (Science, 324:87-91, 2009) and a diterpenoid (Jyoti Shah per. comm.). In summary, SAR is a complex process that involves multiple mobile signals. These signals appear to interact directly or indirectly with each other and may be used during different developmental stages of the plant.

ORAL SESSION 1

TGA transcription factors mediate glutaredoxin *GRXC9* gene activation by salicylic acid in *Arabidopsis thaliana*.

Loreto Carvallo, Ariel Herrera, Francisca Blanco and Loreto Holuigue.

Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

Salicylic acid (SA) accumulation in plants is induced in the onset of defense responses against biotic and abiotic stress. Recently in our laboratory we reported that GRXC9 gene, coding for a glutaredoxin with antioxidant function, is rapidly activated by SA in *Arabidopsis*. We are interested in elucidate the mechanism of transcriptional activation of this gene by SA. In silico promoter analysis of the GRXC9 gene identified two SA responsive as-1-like elements, which have been found in several plant promoters activated during defense. In this work we have used a combination of tools to elucidate the function of these elements. Mutants in the TGA 2/5/6 subclass of transcription factors showed impaired GRXC9 activation by SA. In vivo reporter assays, with constructs containing the full length, deletions and mutants of GRXC9 promoter controlling the expression of GUS reporter gene allowed to investigate the contribution of as-1-like elements. Also, we used yeast two- hybrid assays to study the interaction between TGA transcription factors. Finally, we assessed the association of TGA transcription factors to GRXC9 promoter in seeding exposed to SA by Chromatin Immunoprecipitation (ChIP) assays. Our results indicate that SA activates the transcription of GRXC9 gene by a mechanism involving TGA transcription factors and as-1-like elements found in the promoter.

Supported by FONDECYT-CONICYT (1060494), Núcleo Milenio de Genómica functional de Plantas (P06-009-F).

INVITED SPEAKER

Aroma: an integrative approach for understanding and improving a complex quality trait

Defilippi, B.G [1,2]., Manríquez, D[1]. and González-Agüero, M[1,2].

1. Instituto de Investigaciones Agropecuarias (INIA-La Platina). Santa Rosa 11610, La Pintana, Santiago. bdefilip@inia.cl

2. The Plant Cell Biotechnology Millennium Nucleus (PCB-MN)

Flavor composition has been defined as a complex attribute of quality, in which the mix of sugars, acids, and volatiles play a primary role. In addition to the four basic flavors (sweet, sour, salty, and bitter) that humans can recognize in fruits and vegetables, aroma has an important influence on the final consumer acceptance of the commodity. Fruit aroma is determined by a complex mixture of a large number of volatile compounds including alcohols, aldehydes, and esters. During fruit development, especially at ripening, there are many changes of these metabolites caused by their synthesis, transport or degradation. In terms of volatile biosynthesis, several studies have been performed identifying and characterizing the most important genes and encoded enzymes involved in aroma-related volatiles; however, research in the mechanisms of regulation or modulation is still limited. In order to understand some of these issues, we have been extensively working on aroma modulation during fruit ripening, especially by considering species in which aroma is a key quality attribute, such as apple, apricot, melon, among others. Our results indicate that the interaction between environmental and biological factors must be integrated in order to understand the underlying mechanism of aroma during fruit development (Fondecyt 1060179).

Molecular characterization and substrate preference of an alcohol acyl-transferase isolated from mountain papaya fruit (*Vasconcellea pubescens*)

Gaete-Eastman, C., Fuentes, L., Figueroa, C., Valdenegro, M., Balbontín, C., Herrera, R., Moya-León, M.A.

Laboratorio de Fisiología Vegetal y Genética Molecular, IBVB, Universidad de Talca.

Aroma of a fruit is a complex attribute determined by different volatile compounds of low molecular weight. Aroma is considered of great importance as it determines fruit quality and influences the final acceptance of consumers. In mountain papaya fruit (*Vasconcellea pubescens*) aroma is one of the most important attribute due to its exceptional organoleptic quality. In this fruit, esters are the most representative volatiles compounds due to their abundance and great sensory impact. Esters are synthesized by the enzyme alcohol acyl-transferase (AAT) by means of a transacylation of an acyl-CoA to an alcohol. To determinate the relation of AAT with the volatile pattern of mountain papaya fruit, the transcript accumulation of *VpAAT1* and enzymatic activity were analyzed. During ripening of mountain papaya fruit a high increase in the production of some esters was observed, which was coincident with the increase in enzymatic activity and *VpAAT1* transcript accumulation. The *VpAAT1* cDNA was expressed in yeasts, and the recombinant enzyme can utilize a variety of substrates, showing the characteristic preference for an acyl-transferase gene member of sub-family III. These results suggest that the generation of esters during the ripening of the mountain papaya fruit is related to the presence of AAT and substrate availability.

Acknowledgements: We are grateful to the PBCT_CONICYT Anillo ACT-41, Postdoctoral PBCT PSD17 and PSD61 Projects for financial support.

The Fragaria chiloensis carotenoid cleavage dioxygenase 1 gene contribute to the formation of the aroma compound 3-oxo-α-ionol

Loreto Prat[1], Ana Maria Dominguez[2], Eduardo Agosin[2], Pablo Valenzuela[3] and Herman Silva[1]

[1]*Plant Functional Genomics & Bioinformatics Lab and Millennium Nucleus in Plant Cell Biotechnology (PCB). Universidad Andrés Bello República 217, 837-0146, Santiago.*
[2]*Centro del Aroma, Pontificia Universidad Catolica de Chile.* [3]*Fundación Ciencia para la Vida.*

Aroma volatiles contribute in a large extent to the overall sensory quality of the fruit. Research during the last decade has been dedicated to identification of volatile compounds present in cultivated strawberry but the study of aroma composition in *Fragaria chiloensis* (L.) Mill. (native Chilean white strawberry) is still limited, although its aroma is very characteristic and pleasant. The volatiles of *F.chiloensis* were extracted by using liquid-liquid extraction and evaluated by gas chromatography-olfactometry (GC-O) and aroma extract dilution analysis (AEDA). We detected 65 odorants by GC-O. Upon diluting the original extracts in AEDA, we identified 17 odorants with high dilution (FD) factors of 10,000. We were able to identified for the first time in *Fragaria* specie the odorant 3-oxo-α-ionol (4-(3-hidroxi-1-butenil)-3,5,5-trimetil-2-ciclohexanona), which has a floral and citric odor and had a FD factor of 1,000. This compound belongs to the non-isoprenoid family, which probably is derived from carotenoids. A search for a gene putatively responsible for the cleavage of carotene into 3-oxo-α-ionol was carried out in Fragaria chiloensis EST database (MIFAB) yielding a sequence (FcCCD1) highly similar to other plant carotenoid cleavage dioxygenase genes. Expression of the gene was studied by real-time PCR at different tissues and development stages of *F. chiloensis* fruits. FcCCD1 mRNA was detected in all tissues tested. The highest transcript levels were detected in flower tissues. Also the transcript amount increased throughout the ripening stages, reaching maximal value at the two last stages of maturity of the fruits.

This research was supported by PBCT R-11, Millennium Nucleus in Plant Cell Biotechnology (PCB) ICM P06-065-F and UNAB DI-51-06/R.

The effect of development and light upon the expression levels of carotenogenic genes and a structural analysis of the *lcyb* promoter.

Paulina Fuentes and Claudia Stange

Laboratorio de Biología Molecular Vegetal,
Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

Carotenoids are isoprenoid compounds which have important functions in plants and animals. For this reason, the carotenogenic pathway and its regulation have been widely studied and almost all the genes and enzymes involved are now known. Studies in different plant models indicate that the principal point of regulation occurs at the transcriptional level and that light is its main activator. Carrot (*Daucus carota*) is a dicotyledonous plant which accumulates high amounts of carotenoids, especially β-carotene, in its modified root, which develops in darkness. Therefore, this plant is an interesting model to study the regulation of the carotenogenic pathway in the absence of light. By means of quantitative RT-PCR we determined that the expression of *psy1, pds, zds2* and *lcyb* increases between 7 (*zds2*) and 56 fold (*pds*) during leaf development. In the modified root, the expression of all genes, except *zds1*, increases between 2 fold (*psy1*) and 12 fold (*lcyb*) positively correlating with development and carotenoid content, measured by HPLC. The expression of *psy2*, *zds1* and *zds2* in 4-week old leaves and of *pds* and *zds2* in 12-week old leaves is higher than the expression in leaves exposed to darkness for 48h. Light inhibits the normal development of the modified root and causes an increase in the expression of all genes in young plant roots. However, at 12 weeks all genes are more highly expressed in roots developed in darkness, correlating with carotenoid levels. Given that *lcyb* is the most highly expressed gene during modified root development, we obtained 1500 bp of its promoter by means of GenomeWalker. Bioinformatic analysis indicates the presence of light and gibberellic acid response elements in the sequence.

ORAL SESSION 2

INVITED SPEAKER

Integration of hormonal and genetic regulation during vascular morphogenesis in *Arabidopsis*

Ykä Helariutta, Anthony Bishopp, Ana Campilho, Jan Dettmer, Satu Lehesranta, Annakaisa Elo, Kamil Ruzicka, Kaisa Nieminen, Anne Honkanen, Juha Immanen, Sedeer El-Showk, Hanna Help, Raffael Lichtenberger, Robertas Ursache.

Inst of Biotech/Dept of Bio and Env Sci, University of Helsinki, P.O. Box 56, FIN-00014 University of Helsinki, Finland, yhelariu@mappi.helsinki.fi

Vascular plants have a long-distance transport system consisting of two tissue types, phloem and xylem. The cell lineages of the root vascular cylinder harboring phloem and xylem and the intervening procambial tissue originate from stem cells near the root tip. We and others have taken a combination of genetic and genomic approaches to understand how the specification of vascular cell lineages is determined at a molecular level. We have recently demonstrated that in *Arabidopsis*, cytokinin phytohormones negatively regulate protoxylem specification, a "default" identity. AHP6, an inhibitory pseudophosphotransfer protein, counteracts cytokinin signaling in a spatially specific manner, allowing protoxylem formation in this domain. On the other hand, APL, a MYB coiled-coil-type transcription factor has a dual role in promoting phloem differentiation and in repressing protoxylem differentiation. Recent progress in understanding the molecular control of vascular tissue specification by cytokinins and APL together with other regulatory pathways will be presented.

INVITED SPEAKER

Post-transcriptional regulatory networks in the nitrogen response of *Arabidopsis thaliana*

Rodrigo A. Gutiérrez

Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile.

One of the most striking examples of plant developmental plasticity to changing environmental conditions is the modulation of root system architecture in response to N supply. Despite the fundamental and applied significance of understanding this process, the molecular mechanisms behind nitrate regulated changes in developmental programs are still largely unknown [1]. Small RNAs (sRNAs) have emerged as master regulators of gene expression in plants and other organisms. In plants, sRNAs are involved in the post-transcriptional repression of important genes that regulate plant growth and development. Previous microarray analysis of global gene expression responses to N treatments suggested that microRNAs may be involved in N responses [2]. To evaluate the role of sRNAs in mediating the nitrate response at a global scale, we identified sRNAs that are regulated by nitrate treatments in *Arabidopsis* seedlings using next generation sequencing technologies (454 and Illumina). Bioinformatics analysis of the sequence data identified nitrate-regulated microRNAs and other sRNAs in *Arabidopsis*. Detailed analysis of one nitrate-responsive microRNA:target regulatory module, revealed a coordinated regulatory feedback loop that is induced by nitrate and repressed by N forms produced by nitrate reduction. To understand the functional role of this nitrate regulatory module for plant development, we analyzed root system architecture changes in response to nitrate treatments in mutant plants of the microRNA target and overexpressor plants of the microRNA. Our results indicate that this microRNA:target is a novel N-responsive regulatory module that controls root system architecture in response to external and internal N availability in *Arabidopsis*.

References

1. Vidal, E. and Gutiérrez, R.A. (2008). A systems view of nitrogen nutrient and metabolite responses in *Arabidopsis thaliana*. Curr. Op. Plant Biol. 11, 521-529.

2. Gutiérrez R.A., Lejay L., Chiaromonte F., Shasha D.E. and Coruzzi G.M. (2007). Qualitative network models and genome-wide expression data define carbon/nitrogen-responsive molecular machines in *Arabidopsis*. Genome Biology 8, R7.

ORAL SESSION 3

Sir2 a new player in dormancy regulation in *Arabidopsis thaliana*

A.Obrecht, C. Araya and M. Paneque

Facultad de Ciencias Agronómicas. Universidad de Chile. Santiago. Chile

Dormancy and germination processes are regulated by environmental conditions (eg light, cold, water) which generate changes in hormone levels (ABA GA ethylene). Hormone synthesis and degradation are in turn regulated by changes in gene expression. Some of the mechanisms of gene silencing are accomplished by histone deacetylation. Sirtuins are a group of NAD-dependent histone deacetylase (Group III) and its product nicotinamide acts as an inhibitor of its activity. Sir2 was the first member described in yeast, and is involved in gene silencing of rDNA, telomere and mating type loci (HM). It has been linked to longevity, genome stability and stress response in both mammals and yeast. We have identified the homologues of Sir2 in *A.thaliana* and studied the effect of molecules that modify the Sir2 activity and its relationship to the regulation of *A.thaliana* seeds germination. SIR2.2 is located in nucleus and has deacetylase activity. Deacetylase inhibitors delays germination in *A.thaliana* seeds at concentrations below those known to inhibit early seedling development**.** The study of mutants Sir2.1 and Sir2.2 allowed us to discriminate the contribution of each at germination. Deletion or inhibitions of SIR2.2 affect germination time drastically. This nuclear homologue would be silencing of genes is involved in germination transforming it into a new player in the germination process. Financed by FONDECYT-2007-11070234.

Contact: M. Paneque, Laboratory of Environmental Biotechnology and Bioenergy. Department of Environmental Sciences and Natural Resources. Facultad de Ciencias Agronómicas. Universidad de Chile, Santa Rosa 11.315, La Pintana, Santiago, Chile. Tel. (+56 2) 978 5863. Fax (+56 2) 978 5929. E-mail: mpaneque@uchile.cl.

The role of phytochromes and floral integrator genes in the transition of grapevine-buds into dormancy

Francisco J. Pérez and Nathalie Kühn

Universidad de Chile, Facultad de Ciencias, Laboratorio de Bioquímica Vegetal; Casilla 653, Santiago Chile, Email: frperez@uchile.cl

Abstract. Phytochromes (Phys) and floral integrator genes play a critical role in photoperiodic dependant process such as flowering in *Arabidopsis*, tuberization in potato and seasonal growth cessation and dormancy in poplar (*Populus trichocarpa*). In grapevines, photoperiod drives the entrance of buds into dormancy and modifies the expression profile of *VvPHYA* and *VvPHYB* in leaves. To analyze the role of floral integrator genes and phytochromes in inducing photoperiod controlled entry of grapevine buds into dormancy and whether this transition is a mere consequence of a decision taken in the leaf or in the buds or in both organs. Here, we studied the dynamic of *VvPHYA* and *VvPHYB* and grape homologues of *Arabidopsis* floral integrator genes *CO* (*VvCO*), FT *(VvFT*) and *SOC1 (VvMADS8*) in field grown grapevine leaves throughout daily cycles under decreasing photoperiod. Moreover, expression analysis of these transcripts was also carried-out in grapevine-buds on a daily basis before, during and after the critical-day for dormancy transition. The expression of both *VvPHYs* in latent buds and the SD-induced repression of *VvPHYA* suggest that latent buds can perceive directly photoperiod and that *VvPHYA* might play a key role in the transition of buds into dormancy. Moreover, the SD-induced downregulation and transient repression of *VvFT* and *VvMADS8* in leaves and in buds seems as a necessary step for the transition of grapevine buds into dormancy.

The Circadian Clock Controls Critical Daylength for Growth, Cold Response during Dormancy and Bud Break after Dormancy in *Populus*.

Cristian Ibáñez[a,1], Iwanka Kozarewa[a], Mikael Johansson[a], Erling Ögren[b], Antje Rohde[c], and Maria E. Eriksson[a]

[a]*Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, SE-901 87 Umeå, Sweden.*

[b]*Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, The Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden.*

[c]*Department of Plant Growth and Development, Institute of Agricultural and Fisheries Research, 9090 Melle, Belgium.*

[1]*Actual address: Instituto de Biología Vegetal y Biotecnología. Universidad de Talca.*

cibanez@utalca.cl

In temperate regions deciduous species need to time their vegetative growth period to occur in spring and summer, and to remain dormant under non-permissive conditions such as drought or cold. In *Populus* cold acclimation and the onset of dormancy is decided by daylength and there is a critical daylength (CDL) below which it occurs. Typically, growth cessation and bud set are notable physiological responses to daylength shortening and is required for cold acclimation and full winter dormancy. The exit from dormancy is temperature controlled and manifested as bud break. Since the circadian clock controls daily and seasonal processes in other species we set out to probe its role in the seasonal regulation of dormancy. We obtained and characterized transgenic trees with reduced levels of the putative hybrid aspen (*Populus tremula x tremuloides*) orthologs of the clock components LATE ELONGATED HYPOCOTYL (PttLHY1, PttLHY2) and TIMING OF CAB EXPRESSION1 (*PttTOC1*). In this study we show that they constitute regulatory clock components and control the CDL setting. We also found that *PttLHY1/2* are necessaries for the timing of bud break and full winter hardiness. We propose that *PttLHY1/2* and *PttTOC1* equally contribute to the control of growth cessation, while *PttLHY1/2* is also critical for temperature sensitive processes during dormancy.

VvCO and *VvCOL1*, two grapevine constans-like genes, show a diurnal expression pattern under controlled conditions and belong to a multigenic family

Almada R.D. [1*]; Cabrera N.E.[1]; Peña-Cortés, H[2]; Ruiz-Lara, S[1] & Gonzáles E.[1].

*Instituto de Biología Vegetal y Biotecnología, Universidad de Talca.1, Universidad Técnica Federico Santa María2. *email: ralmada@utalca.cl*

Pioneering physiological studies through the 1960-70s identified the major environmental conditions that determine the bud fruitfulness (number of inflorescence primordia per bud) in grapevine. Temperature and light intensity are considered the most important, although grapevine bud fruitfulness is also affected by photoperiod, being greater under long days than in short days. However, the molecular basis of these photoperiod responses is unknown. In *Arabidopsis*, the day length flowering responses are mediated by the so-called "photoperiod pathway". In this pathway, *CONSTANS* gene plays a central role by mediating the circadian clock and the floral integrators. A first inspection of the grapevine genome sequence using *Arabidopsis* CO-like peptides as queries identified at least 14 putative proteins with homology to members of angiosperm CO family. The expression analysis of *VvCO* and *VvCOL1*, two grapevine *CO* family members, showed that their transcript levels fluctuate daily. An in silico search for cis-regulatory elements within the *VvCO* and *VvCOL1* promoter sequences showed the presence of motifs previously related to light responsiveness or circadian expression control in plants.

Acknowledgments: This work was funded by grants from Consorcio Biofrutales S.A. R. A. and N. C. were supported by a Universidad de Talca fellowship.

ORAL SESSION 4

Characterization of the expression of Elip-like genes on grapevine (*Vitis vinifera* L.).

Sánchez E., Pinto M., Hinrichsen P.

Instituto de Investigaciones Agropecuarias, La Platina. Santiago, Chile.

Based on comparisons to Elip (early light induced proteins) genes reported for other species, we have determined the existence of one copy of an Elip-like gene on grapevine, apparently located on linkage group 5. This gene, nuclearly encoded in every species studied, has in grapevine a nucleotidic sequence similar to their orthologous and a promoter harboring motives that would be related with the light stimulus. In order to establish the expression pattern of this gene in different plant organs an in silico transcription analysis was performed using information obtained from public libraries of cDNAs. The results showed that either in red or white varieties, the highest transcription activity was in young leaves and berries in comparison to buds and roots. Once the gene transcription pattern was determined, the evolution of the transcript expression was measured during a day-night cycle, using qPCR technique and cDNA obtained from young leaves developed under field or greenhouse conditions. Under field conditions, the results showed that transcription of the Elip gene increased after two hours of illumination, reaching a maximum at six hours. After that, transcription falls to a minimum level at the end of the photoperiod. Under greenhouse conditions the expression begin to raises two hours before the begining of the photoperiod and, like in field conditions, a decrease was verified by the end of the photoperiod. This phenomenon was not interrupted under constant light conditions, showing the typical expression pattern of a circadian rhythm.

Financed in part by Fondecyt 1070788

Identification of genes related to ovule development in stenospermocarpic table grape (*Vitis vinifera* L.)

Casanueva, X.[1-2], González-Agüero, M.[1-3], Aliaga, R [4]. , Aravena, A.[4], Hinrichsen, P.[1]

1 Instituto de Investigaciones Agropecuarias, La Platina, Santiago, Chile

2 Universidad Andrés Bello, Santiago, Chile.

3 The Plant Cell Biotechnology Millenium Nucleus (PCB-MN)

4 Laboratorio de Bioinformática y Matemática del Genoma. Centro de Modelamiento Matemático. Universidad de Chile. Santiago.

Email: xcasanueva@gmail.com

Table grape is one of the most important fruit crops cultivated worldwide, and is particulary relevant for Chilean agribusiness. The varieties most widely consumed are seedless. This phenotype, named "stenospermocarpy", is characterized by the absence of seeds and presence of seminal traces, which molecular basis is still poorly understood. For the identification of candidate genes involved in this phenotype, we are combining phenotypic analysis, QTL mapping, transcriptomic analysis and gene expression evaluation by real-time PCR. QTL mapping was based on an extensive phenotypic characterization using a reference population of 141 segregants from a 'Ruby Seedless' x 'Sultanina' crossing. The results were partially comparable with the QTLs identified in the same or other populations evaluated in previous seasons. A relevant QTL for seed and berry size was mapped on linkage group 18, where the candidate gene *VvAGL11* (SEEDSTICK), had been previously mapped. Expression analysis of this gene during berry development showed an increase in its expression after fruit set in seeded segregants, in contrast to seedless ones. On the other hand, preliminary transcriptomic analyses have identified a number of genes that are changing during development and when comparing segregants exhibiting extreme phenotypes for seed size. Some of these genes, such as *VvAP2* (APETALA2) and *VvSEP3* (SEPALLATA3), are related to ovule development and have a differential expression when comparing seeded and seedless genotypes. This information, combined with histological comparisons and complementation experiments in Arabidopsis thaliana mutants, could provide further evidence to support or reject the proposed role for these genes in table grape seed development.

Financed by Genoma II Grant G07I-1002

Photosynthetic characterization of grapevine (*Vitis vinifera* L.) leaves developed under different light conditions

Sáenz-Diez D[1]., Olivares D [1, 2]., Calvo F[1]., García de Cortazar V[1]., and Pinto M.[1,]
2

1/ Universidad de Chile

2/ Instituto de Investigaciones Agropecuarias – INIA

In plants of grapevine cultivated under over-head trellis, leaves from the upper layers are all over the photoperiod under full sunlight condition. On the other hand, leaves from the lower layers are continuously under shade. In between, there are leaves growing under a dynamic light condition (sun-flecks). In this work we characterized the photosynthesis of leaves of cv Sultanina plants that were acclimated to these three light conditions. After two months of acclimation fluorescence measurements practiced during the day in summer time, showed that in leaves acclimated to full light, the quantum yield of PSII (ΦPSII) and the photochemical quenching (qP) were at midday at a minimum value. However both parameters totally recovered during the evening. From non-photochemical quenching (NPq) and de-epoxidation state (DEPS) values we conclude that the midday photoinhibition observed in these leaves, was not causing a permanent photodamage. Midday photoinhibition was not observed in leaves acclimated to the sun-fleck condition. These leaves showed a lower leaf temperature and a rate of net CO2 assimilation, which was similar to leaves acclimated to full light. As sun-fleck acclimated leaves represented more than 50% of the foliar area index, we conclude that in plants conducted under over-head trellis sun-fleck leaves are responsible for most of the carbon assimilated by the canopy. Leaves acclimated to shade presented a light compensation point of ± 30 µmol PAR m-2 s-1. This allowed these leaves to contribute positively to the carbon economy of the plant as most of the day the light intensity in the lower part of the canopy was over this level.

Fondecyt 1070788

The *LEA18* gene family is essential for gametes development in *Arabidopsis thaliana*

Margarita Santiago, Maria Patricia Romero, Dayan Sanhueza and Herman Silva

Plant Functional Genomics & Bioinformatics Lab and Millennium Nucleus in Plant Cell Biotechnology (PCB).Universidad Andrés Bello, República 217, 837-0146, Santiago.

To identify genes involve in the plant secretory pathway we screened for *Arabidopsis thaliana* cDNA clones that complement temperature-sensitive exocyst mutants of Saccharomyces cerevisiae. *AtLEA18B* suppressed exocyst mutants growth and secretory defects. This gene shows no sequence similarity to the exocyst components, but encodes a protein of the *Arabidopsis LEA18* gene family. This is a newly described group formed by three genes in *Arabidopsis* that are similar to the Phaseolus vulgaris *PvLEA-18* gene, all of them with unknown function. In order to study the gene function of the 3 components of the *LEA18* family we analyzed gene expression by RT-PCR in different plant tissues (caulinar and rossette leaves, stems, flowers, buds, roots and developing siliques). It was observed that *AtLEA18B* expression is ubiquitous, however *AtLEA18A* is expressed in developing siliques and *AtLEA18C* is expressed only in flowers.

We obtained a homozygous insertional mutant line for each gene. The *atlea18b/-* mutant does not show transcript accumulation. However, we observe ectopic expression in flowers of its homologue *AtLEA18A,* suggesting gene redundancy. In *atlea18a/-* mutant the expression of both *AtLEA18A* and *AtLEA18B* is not detected. Similarly, *atlea18a/-, atlea18b/-* and *atlea18c/-* mutants does not have an apparently phenotype. We decided to cross *atlea18a/-* plants with *atlea18c/-* to obtain a plant in which the expression of the three genes was disrupted. These plants have a 25% of dead, shrunken pollen grains and a seed set reduction of 50% compared to a wild type plant. This suggests an important role in gametes development.

This research was supported by Millennium Nucleus in Plant Cell Biotechnology (PCB) ICM P06-065-F.

LEA proteins in different organs of *D. antarctica* subjected to abiotic stress.

[1]Bascuñan, L. [2]Goday A. and [1]Bravo L.A.

1. Laboratorio de Fisiología Vegetal, Universidad de Concepción, Concepción, Chile. 2. CSIC, Instituto de Biología Molecular de Barcelona.

Cell dehydration and turgor loss are common consequences of environmental stresses such as high and low temperatures, osmotic and salt stress, drought etc. This is reason why LEA proteins and osmolites are commonly accumulated in response to different stressors. *D. antarctica* is the only Poaceae that has colonized the Maritime Antarctic. During the growing season this species is exposed to freezing temperatures at night, strong desiccant winds and saline sea spray which impose severe environmental constrains for plant grow. However, *D. antarctica* has developed interesting anatomical, physiological and biochemical mechanism that allow their survival in the Maritime Antarctic. It is postulated that the accumulation of diverse forms of LEA proteins is associated with the capability of this species to withstand its natural environment. Plants were subjected to low temperature (4°C) and PEG (-1.2 MPa) for six days. Their physiological status was assessed by PSII-fluorescense measurements. Leaf and crown were collected and protein fractions were analyzed by SDS-PAGE. Different LEAs were detected using antibodies raised against LEA2 (anti R17, a dehydrin from Z. mayz), LEA3, (anti MIg3 Z. mays and anti AZM5 Z. mays) and LEA1 (anti EMB564 maíz). Plant photosynthetic performance was slightly disturbed by low temperature but a more significant effect was seen under PEG treatment. LEAs were undetected in crowns. Several LEA proteins were detected in leaves by three out of four tested antisera. R17 (LEA2) and Anti-MIg3 (LEA3) strongly recognized bands under osmotic and cold stress exposure. Therefore, this concerted LEA accumulation may help the plant to cope with multiple stresses from the Maritime Antarctic.

Supported by: CSIC/CONICYT, INACH G-02-08.

Differentially expressed genes induced by cold and UV-B in *Deschampsia antarctica*.

Jorge Dinamarca [1*], Ana Gutierrez [1*], **Alejandra Sandoval[1]**, Raimundo Villarroel[2], Alfredo Herrera Estrella[3] and Manuel Gidekel [1,4].

[1]Laboratorio de Biología Molecular Aplicada, Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera, Temuco, Chile. [2]VIB Department of Plant Systems Biology, Ghent University, Belgium. [3]LANGENBIO, Centro de Investigacion y Estudios Avanzados, Irapuato, Mexico. [4]VentureL@b-Knowledge Center for Business, Universidad Adolfo Ibañez, Santiago, Chile.

Deschampsia antarctica is the only monocot vascular plant that colonizes the Antarctic Peninsula. The survival of this species in a harsh environmental suggests that this plant constitutes a new source of genes associated with cold and UV tolerance. To identify differentially expressed genes during cold and UV irradiance conditions we used suppression subtractive hybridization (SSH). We identified a total of 112 differentially expressed genes from the constructed cDNA libraries. Using similarity search analysis we identified several genes that have not been reported in previous studies. Interestingly, a major part of the isolated genes correspond to unknown or hypothetical proteins. This set of tolerance-related genes can be of relevance to help uncover the mechanisms by which this extremophile to survive in its environment.

Acknowledgments

This work was supported by Fondo de Fomento al Desarrollo Científico y Tecnológico (FONDEF project D03I-1079), the Antarctic Chilean Institute (INACH project 01- 03-Part II), INNOVA BIOBIO project 04-B1-283 L1, Consorcio de Tecnologia e Innovacion para la Salud (PBCT CTE-06). JD was also supported by the Programa Bicentenario-Banco Mundial, Conicyt, Chile PBCT CTE-06 and FONDEF D03I-1079.

ORAL SESSION 5

INVITED SPEAKER

Carbohydrate metabolism in Arabidopsis thaliana

Michael Handford

Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias,
Universidad de Chile, Santiago

Carbohydrates, and their component sugars, perform a variety of fundamental roles in the growth and development of plants, and are involved in metabolism, storage, structure and mobilisation. Specifically, three aspects of carbohydrate metabolism will be discussed. Firstly, although sucrose is the main phloem translocated carbon form in *Arabidopsis*, several species mobilise sorbitol. Once in sink organs, sorbitol may be oxidised to fructose by sorbitol dehydrogenase (SDH) for entry into the glycolytic pathway. Nevertheless, *Arabidopsis* possesses low basal levels of sorbitol and other sugar alcohols, and we have identified a putative SDH (AtSDL) in this species. AtSDL is widely-expressed, is cytosolically-localised, and biochemical and *in silico* analyses show that it is indeed capable of specifically oxidising sorbitol, and not other sugar alcohols. Secondly, plant glycoproteins and non-cellulosic polysaccharides are synthesised and/or modified in the Golgi apparatus and nucleotide-sugar transporters (NST) are required to import cytosolically-synthesised nucleotide-sugars into the lumen of this organelle. Using a reverse genetics approach, the *Arabidopsis* proteins GONST3 and 4 were identified. Assays show that they are indeed localised in the Golgi apparatus and are involved in the import of specific GDP-sugars, including GDP-L-galactose which is only required for the decoration of certain pectin polysaccharides. Finally, the acquisition of photosynthesis by eukaryotic cells through enslavement of a cyanobacterial ancestor represents one of the most remarkable turning points in the history of life on Earth. However, the precise mechanism of how the photosynthate was initially exported from endosymbiont to host cell remains unknown. Evidence will be presented that these same NST proteins have an innate ability for transporting the bacterial specific metabolite ADP-glucose, a molecule that was previously suggested as a candidate for carbon efflux from the endosymbiont.

Funding: Fondecyt Iniciación 11060470 and Innova 07-CN13PBD-19

Citokinin and sucrose modulate ε Adaptin role in plant cell division.

Daniela C. Urbina (daniurbina@gmail.com) y Lee Meisel.

Núcleo Milenio en Biotecnología Celular Vegetal, Laboratorio de Genética Molecular Vegetal del Centro de Biotecnología Vegetal, Universidad Andrés Bello.

The secretory pathway is a principal player during plants cell division. In mammalian polarized cells, AP-4 is a heterotetrameric complex (ε, β4, μ4 and σ4 Adaptin proteins) which is necessary for apical protein sorting. In this work, we are investigating role of ε adaptin, during plant cell division in *Arabidopsis thaliana*. Using polyclonal antibodies against ε adaptin of *Arabidopsis thaliana*, we have determined this protein is soluble and associated to crude microsomal extract. The ε adaptin protein and its transcript are highly accumulated in tissue undergoing cells division such as siliques and embryos. Additionally, the ε adaptin transcript abundance is up-regulated by exogenous applied cytokinin and sucrose. Using whole mount plant immunolocalization, we have observed that this protein is accumulated in early stages of mitosis. These results suggest a role for ε adaptin in plant cell division. Beca de Postgrado CONICYT y Núcleo Milenio en Biología Celular Vegetal, PCB-P06-065-F

Acemannan and fructan from *Aloe barbadensis* miller (aloe vera) undergo structural modification during water stress.

Salinas, C., Cardemil, L.

Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

Aloe vera, a CAM plant, is adapted to live in arid environments. The gel from Aloe vera leaves accumulates water and has commercial value in medicine and cosmetology. Aloe was introduced into the III and IV Regions and irrigated with abundant water even though water use efficiency increases with moderate water deficit. Therefore, it is necessary to know if moderate water treatments affect the quantity and quality of the gel, especially the structure of galactoglucomannan (acemannan), which is the main component of the gel and is probably responsible for the medicinal and cosmetological properties. We wanted to know, in addition, if reduced water treatments affect other osmotically-adjusting polysaccharides. The objective of this work was to determine the changes in structure of oligo- and polyfructans and acemannans in leaves of plants subjected to different watering regimes. Plants were irrigated with water treatments T1, T2, T3 and T4 corresponding to 100%, 75%, 50% and 25% of Soil Field Capacity, respectively. Oligo- and polyfructans and acemannans were extracted and purified. The sugar analyses of the purified polysaccharides were performed by gas chromatography (GC) of the alditol acetates and the glycosidic linkages determined by GC-mass spectrometry (GC-MS)of the permetylated alditol acetates sugars. Both, oligo-and polyfructans increased in quantity with increasing water deficit, and the degree of polymerization also increased with water restrictions. The trisaccharide neokestose, terminal-fructose and 6-glucose, all characteristics of the neo-levan type of fructans, appeared in T4 plants. The results from GC-MS analysis of the acemannan showed higher concentrations of galactose in T3 and T4. Additionally, in the acemannan the α-(4→3) and the α-(4→6) mannoses increased with water deficit indicating that under water stress the polysaccharide became more branched. Funding: FONDECYT 1070899 and MULT 05/30-2 de la Dirección de Investigación, Universidad de Chile.

Heterologous expression of the gene *Egbor1* of *Eucalyptus globulus* in *Saccharomyces cereviseae*.

Hans Pieringer [1], Susana Rasmussen-Poblete [1,2], Erwin Krauskopf [1,2]

[1] *Departamento de Cs. Biológicas, Universidad Andrés Bello, MIFAB, Santiago.*
[2] *Fundación Ciencia para la vida, Zañartu 1482, Santiago.*

Email: ekrauskopf@unab.cl

Boron is a micronutrient that plays a fundamental role in plant cell wall production. Nevertheless, an excess of boron in soil generates severe damage to the respiratory tissue of plants. The *Atbor1* gene encodes a boron transporter which functions by distributing this micronutrient in plants. We isolated from a *Eucalyptus globulus* cDNA library the *Egbor1* cDNA. However, its nucleotide sequence exhibited several stops codons within the coding region. Bioinformatics analysis suggests that this interruption corresponds to an intron, which does not allow the correct expression of the complete protein. We over expressed the *Egbor1* gene in *Saccharomyces cereviseae* to test whether it was capable of restoring the phenotype of a yeast mutant that lacked boron transporter. At the same time, we over expressed *Egbor1* in wild type yeast. In both cases we observed a significant increase of boron tolerance suggesting the encoded transporter was fully functional. Currently we are trying to establish if the expressed boron transporter is the product of the full-length or the truncated protein.

Funded by PFB-016, MIFAB and DI/UNAB

Characterization of voltage regulation, pH regulation and role under abiotic stress conditions of KAT1 and SKOR potassium channels from *Arabidopsis thaliana*.

Wendy González[1], Samuel Morales[1], Fernando Danilo González-Nilo[1], Simón Ruiz-Lara[1], Ingo Dréyer[2].

[1]*Universidad de Talca,* [2]*Universität Potsdam*

Shaker channels are transmembrane proteins that transport potassium ions (K+) in a fast way in plant tissues. They are divided in two groups according to their voltage-dependent activation properties: I) Inward rectification channels (Kin) are activated by membrane hyperpolarization transporting K+ into the cell and; II) Outward rectification channels (Kout) are activated by a membrane depolarization transporting K+ to the apoplasm. KAT1 channel transports K+ to the cytoplasm of guard cells in *Arabidopsis thaliana* belongs to group I. SKOR channel releases K+ to the xylem in *A. thaliana* belongs to group II. Using structural bioinformatics and electrophysiology we compared both channel structures in order to identify the amino acids that confer diversity between Kin and Kout channels. We found residues placed in S5 and S6 transmembrane segments related to voltage-dependent activation differences between Kin and Kout channels. These differences are reflected also in the activation mediated by pH of environment. SKOR is blocked by acidification meanwhile KAT1 is activated in same conditions. Using quantum mechanics and electrophysiology we found a consensus histidine reported as extracellular pH sensor in KAT1 homolog channel in potato, KST1, cannot be the extracellular pH sensor in KAT1 because it is establishing an interaction with a phenylalanine in its vicinity. Functional diversity of Kin and Kout channels determines their role in plants adaptation to the environment. KAT1 plays a key role in the adaptation of A. thaliana to abiotic stressed environments. Trough phenotypic analysis of transgenic plants for SKOR we found that this channel is essential for seed germination under hydric stress conditions.

Growth patterns of Near Isogenic Lines for the aluminum tolerance *TaALMT1* gene in wheat grown in phosphorus-deficient and high aluminum content soils.

Mónica Mathias[1], Adolfo Montenegro[1], Enrique Peñaloza[1], Braulio Soto[1], and Javier Zúñiga[1,2].

1 Instituto de Investigaciones Agropecuarias INIA-Carillanca, Casilla 58-D,
2 Temuco, Chile.

Corresponding author, E-mail: jzuniga@inia.cl.

Aluminum (Al^{+3}) toxicity is one of the major soil constraints affecting crop production in acid soils worldwide. This stress is frequently associated with phosphorus deficiency in most soils of southern Chile where wheat (*Triticum aestivum L.)* is broadly cultivated. To improve Al^{+3} tolerance in this species, a marker assisted backcrossing strategy was used to introgress different *TaALMT1* gene variants into a high-yielding but Al^{+3} sensitive Chilean cultivar. As result, a series of Near Isogenic Lines for the *TaALMT-1* alleles I, V and VII was developed. To know whether different alleles for this gene affect the plant performance, these lines were grown in two phosphorus-deficient acid soils contrasting in Al^{+3} saturation. The P-Olsen in both soils was about 8 $mg*kg^{-1}$, while the aluminum saturation was 1.2 and 20%. Plants were grown in potted soil under greenhouse, which were arranged in a split plot design. Above and underground plant biomass was harvested every 15 days, on which fresh weight, dry weight, leaf area and grain yield were measured.. Compared to the Al-tolerant genotypes (alleles V and VII), Al-stress significantly reduced plant biomass and leaf area in the Al-sensitive genotype (allele I). Consequently, grain yield of sensitive lines was reduced by over 90%. Though both tolerant genotypes showed similar growth pattern in each soil, there was a trend towards a better performance in the soil having a 20% Al saturation. Although further studies are required to fully explain the observed behavior, these results emphasize the importance the *TaALMT1* gene might have in developing wheat cultivars adapted not only to phytotoxic aluminum but also to phosphorus deficient soils.

Keywords: Wheat breeding - *TaALMT1* - Aluminum toxicity - Acid soils.

Poster Session

1.

Functional analysis of GONST3 and 4, nucleotide-sugar transporters of *Arabidopsis thaliana.*

Mariela Huichalaf, José Patricio Miranda, Diego Ampuero and Michael Handford

Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias,

Universidad de Chile, Santiago

Plant glycoproteins and non-cellulosic polysaccharides are synthesised and/or modified in the Golgi apparatus and transporter proteins are required to import cytosolicallysynthesised nucleotide-sugars into the lumen of this organelle. Using a reverse genetics approach, GONST3 and 4 from *Arabidopsis thaliana* were identified. Both possess the molecular characteristics of previously-identified nucleotide-sugar transporters (NSTs) transporting GDP-, but not UDP-sugars. Phylogenetic analysis suggests that GONST3 and GONST4 arose early in the evolution of NSTs. Our work is focussed on determining their substrate specificity and on analysing their role in planta. To achieve these aims, GONST3 and 4 were fused to epitope tags; GONST4-His and GONST4-GFP were localised to the Golgi apparatus in agro-infiltrated tobacco leaves. We synthesised GDP-L-galactose, a potential substrate of GONST3 and 4, from GDP-Dmannose for use in transport assays. Both GONST4-His and GONST4-GFP transported GDP-L-galactose and GDP-L-fucose, but not GDP-D-mannose or UDP-D-glucose, into the lumen of a Golgi-enriched fraction extracted from agro-infiltrated tobacco leaves. Experiments with GONST3 are in progress. In addition, to determine their function in vivo, GONST3 and 4 expression levels were reduced by post-transcriptional gene silencing. Preliminary cell wall analysis by HPLC of transformants with reduced GONST3 or GONST4 expression did not reveal any differences in sugar composition; analyses of specific glycoconjugates by GC-MS are currently underway. Using *Arabidopsis* lines transformed with promoter-GUS fusion constructs, both NSTs are highly expressed in specific floral organs and roots and exhibit differential expression profiles during early stages of development. GONST4 is thus the first known NST capable of transporting GDP-L-galactose and the only known polysaccharide requiring this substrate is the pectin, rhamnogalacturonan II.

Funding: Fondecyt Iniciación 11060470

2.

Neighborhood analisys in gene coexpression networks to predict new *Arabidopsis thaliana* cell wall genes.

Alexander Vergara[1-2-3], Ariel Orellana[2], Rodrigo A. Gutiérrez[3].

1.Doctorado BMCN, Facultad de Ciencias, Universidad de Chile.

2.Centro de Biotecnología Vegetal, Universidad Andrés Bello. Núcleo Milenio en Biología Celular Vegetal.

3.Departamento de Genética Molecular y Microbiología. Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. Núcleo Milenio en Genómica Funcional de Plantas.

Arabidopsis thaliana has the best characterized genome among plants. However, around 50% of its genes still do not have a biological process assigned and their annotation is a key challenge in functional genomics. The objective of this work is to identify new andidate genes involved in cell wall metabolism. The DNA microarray technology is currently the most widely used approach for monitoring genome-wide gene expression changes. In this work, using publicly available microarray data we performed a global pair-wise linear correlation analysis of expression profiles using 1.701 quality-screened AffymetrixTM ATH1 microarray chips and constructed gene co-expression networks. In order to find new cell wall genes, we tested three neighbourhood based methods using Gene Ontology (GO) terms to spread the annotations in the networks. We obtained the best precision of annotations using a counting based method that consider the correlation values between the gene query and its neighbours in the network. Using this method, we proposed 43 new genes involved in the cell wall metabolism, spreading the GO term GO:0007047 "cell wall organization and biogenesis" , with a precision of 48.92%. Of the proposed genes, 7 still not have a biological process or molecular functions assigned in its annotations ("unknown proteins"), and they are very interesting if the focus is to find new cell wall genes.

3.

Cytokinin signaling regulates cambial activity

Juha Immanen and Yrjö Helariutta

University of Helsinki, Finland

Although a substantial amount of plant biomass originates from the activity of vascular cambium, molecular basis of radial plant growth is still largely unknown. To address whether cytokinins are required for normal cambial activity, we studied cambial cytokinin signaling in two hardwood tree species; poplar and birch. We observed a peak in the expression of putative cytokinin receptor genes in the cambial cells. For functional studies we engineered transgenic poplar trees expressing a cytokinin catabolic gene from *Arabidopsis,* CYTOKININ OXIDASE 2, under the promoter of a birch cytokinin receptor. Reduced cytokinin signaling correlated with decreased radial growth and low cambial activity. To enhance cambial cytokinin signaling, we have recently constructed transgenic trees overexpressing cytokinin signalling components.

4.

Sorbitol metabolism in plants

María Francisca Aguayo, Yu-Wen Tang, María Sofía Zamudio and Michael Handford
Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile.

In members of the Rosaceae family, which includes peaches, pears and apples, sorbitol is the main product of photosynthesis, synthesised from glucose 6-phosphate by the action of sorbitol 6-phosphate dehydrogenase and sorbitol 6-phosphate phosphatase (S6PDH and S6PP, respectively). Sorbitol is the main form of carbon translocated around these species via the phloem. Once in the carbon sink organs, and in a species-specific manner, a proportion of this sugar alcohol is metabolised to fructose via sorbitol dehydrogenase (SDH) or to glucose by sorbitol oxidase. These sugars are then metabolised or are stored, providing enhanced sweetness to the organ, especially in the case of fruits. We have identified two plant enzymes described as SDHs in the literature. The cDNAs that code for these proteins have been cloned into plant binary vectors under the control of different promoters, including a fruit-specific promoter. In order to test the functionality of these constructs, tomato plants have been transiently and stably transformed using *Agrobacterium tumefaciens.* Advances in the progress of these experiments will be discussed. Even though the main phloem-translocated carbon compound in non-Rosaceae species is sucrose, sorbitol and other sugar alcohols are present, particularly under drought and cold stress conditions when they act as compatible solutes. Using a reverse genetics approach, we have identified two genes potentially involved in sorbitol metabolism in such species. AtS6PDH is putatively involved in sorbitol synthesis in *Arabidopsis thaliana,* whereas VvSDH is a potential sorbitol dehydrogenase from grapevine (*Vitis vinifera*). Progress in the molecular cloning and characterisation of these two genes will be presented.

5.

Screenings for substrates of AIP2, an E3 ubiquitin ligase from *Arabidopsis thaliana*.

Himanen, K [1]., Farías, D[2]., Pavicic, M[2]., Salinas, P.[2], Ehrenfeld, N[2]. & Garretón, V.[2]

1. *Department of Plant Systems Biology, Ghent University VIB Belgium.*
2. *Universidad Santo Tomás, Escuela de Biotecnología, Santiago, Chile.*

AIP2 is an E3 ubiquitin ligase from *Arabidopsis thaliana* involved in the germination process, by regulating the stability of the transcription factor ABI3. However, the expression pattern of AIP2 in other tissues and stages of development, suggests its involvement in other biological processes such as development of the root system. Since AIP2 described role is as post-translational regulator, interaction with other proteins in a specific manner is expected. In this work, we look forward for other proteins that may interact with AIP2, carrying out two massive screening technologies: double hybrid screening and Tandem affinity purification (TAP). With double hybrid experiment, we used as bait an AIP2 fragment without the first 55aa to avoid auto-activation of the reporter gene, and as prey, a gene library with cDNAs from seedlings with five days of germination. For TAP assays we also used AIP2 as bait and as prey we used cell cultures transformed with a construction including an AIP2 fusion protein with two Tags. From the TAP Assay, forty eight proteins were selected, which would bind to AIP2. From double hybrid screening, five positive clones were selected as the most probably interaction with AIP2, from which three candidates were chosen for intensive analysis: At5g60410 a SUMO E3 ligase, at3g20120, a putative transcription factor of the MYB family and At2g21240, a transcription factor with not further references. In order to verify these results and to determine if these candidates are involved in the development of roots as substrates from AIP2, we are performing BIFC assays and pull-down assays.

Fondecyt Nº 11060072.

6.

Rol of AIP2, an E3 ligase in the root development of *Arabidopsis thaliana*.

Pavicic, M.[1], Farías D.[1] Ehrenfeld, N.[2] & Garretón, V[1,2.]

1. Universidad Santo Tomás, Escuela de Biotecnología, Ejército 146, Santiago, Chile.

2. Laboratorio de AustralBiotech S.A., Ejército 146, Santiago, Chile.

The development of the root system in plants is a complex process widely studied but not fully elucidated. AIP2 is an E3 ubiquitin ligase from *Arabidopsis thaliana*, which regulates the stability of the transcription factor ABI3 during germination. However, the expression patterns of AIP2 in other tissues suppose also its participation in other biological processes. To characterize the expression pattern of AIP2 during development of root systems, we used transgenic *A. thaliana* plants expressing the gene for the enzyme β-glucoronidase (GUS) under the control of AIP2 promoter. In parallel, to characterize the phenotypic influence of AIP2 in the development of roots, we used transgenic plants over expressing AIP2 and also a AIP2 knockout line. The results of GUS assay indicate that expression of AIP2 appears from 2 to 3 days after germination, in a group of cells located in the transition tissues between stem and root. In later days, the expression varies in location and extent, reaching a peak around the day 4 when the expression is observed located in all tissue except in the root tip. In the subsequent days, the expression found in the emerging lateral roots, repeats the same expression pattern of the primary root. In the case of phenotypic characterization, the over expressing AIP2 line showed more number of lateral root, but not difference is detected in the development rate between the control and knockout aip2.

Fondecyt Nº 11060072.

7.

The Novel Nitrate Responsive miR393:AFB3 Regulatory Module Controls Root System Architecture in *Arabidopsis thaliana*

Elena A. Vidal, Viviana Araus, Cheng Lu, Pamela J. Green, Gloria M. Coruzzi and Rodrigo A. Gutiérrez.

Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile.

One of the most striking examples of plant developmental plasticity to changing environmental conditions is the modulation of root system architecture (RSA) in response to nitrate supply. Despite the fundamental and applied significance of understanding this process, the molecular mechanisms behind nitrate regulated changes in developmental programs are still largely unknown. Small RNAs (sRNAs) have emerged as master regulators of gene expression in plants and other organisms. To evaluate the role of sRNAs in the nitrate response, we sequenced sRNAs from control and nitrate-treated *Arabidopsis* seedlings using the 454 sequencing technology. We identified miR393, a nitrate induced microRNA that targets transcripts that code for a bHLH transcription factor and for the auxin receptors TIR1, AFB1, AFB2 and AFB3. However, only AFB3 was regulated by nitrate in roots under our experimental conditions. Analysis of the expression of this miR393:AFB3 module, revealed an incoherent feed-forward mechanism that is induced by nitrate and repressed by N metabolites generated by nitrate reduction and assimilation. To understand the functional role of this N-regulatory module for root development, we analyzed the RSA response to nitrate in AFB3 insertional mutant plants and in miR393 overexpressors. RSA analysis in these plants revealed that both primary and lateral root growth responses to nitrate were altered indicating that miR393:AFB3 is a novel N-responsive module that controls root system architecture in response to external and internal N availability in *Arabidopsis*.

Acknowledgements: FONDECYT_1060457, ICGEB_CRPCHI0501 NIH-FIRCA_ and Millennium Nucleus PFG_P06-009-F and PhD fellowship from CONICYT_AT-24080114.

8.

ROLE OF TGA1 AND TGA4 IN THE NITRATE RESPONSE OF *ARABIDOPSIS THALIANA* ROOT

Álvarez, J.M., Flores, F., Tamayo, K., Gutiérrez, R.A.

Plant System Biology Lab, Pontificia Universidad Católica de Chile.

In agricultural soils, nitrate is the most important source of nitrogen (N). Nitrate regulates plant root morphology and serves as a potent signal to control gene expression in *Arabidopsis.* The mechanism involved in regulating gene expression in response to nitrate in plants are mainly unknown. To indentify regulators of the nitrate response in *Arabidopsis,* we performed a bioinformatic approach. We used the publicly available microarray data of nitrate treatments and employed diferents criterions to select regulatory genes. This strategy led us to the identification of genes with nitrate regulatory potential and a ranking of candidates was generated. The top candidate of our bioinformatic analysis is TGA1, a bZIP transcription factor. TGA4, is a closely related member of the bZIP family that was found in the ranking and was also chosen for functional studies. Both TGA1 and TGA4 mRNA accumulated strongly and quickly after nitrate treatments. To evaluate the function of these transcription factors, we analyzed the tga1/tga4 double mutant phenotype under different nitrate conditions. The *tga1/tga4* double mutant plants grown in a medium containing a sufficient amount of nitrate showed a shorter primary root than wild-type plants. However, this phenotype was not observed under nitrate limiting conditions. To understand the molecular basis of this phenotype, the effect of nitrate on the expression of selected putatives target genes of these transcription factors was evaluated in wild-type and *tga1/tga4* double mutants plants. The results indicates that TGA1 and TGA4 are necessary for nitrate dependent regulation of such target genes. This results suggest that TGA1 and TGA4 could be importants regulators in the nitrate response of *Arabidopsis* root.

Acknowledgment:Núcleo-Milenio-P06-009-F,NIH-FIRCA-1R03PW007823-01A1 F614-01,FONDECYT-1060457,ICGEB-CRPCHI0501 and CONICYT grant.

9.

IDENTIFICATION OF TGA1 AND TGA4 TRANSCRIPTION FACTOR TARGET GENES IN THE NITRATE RESPONSE OF *ARABIDOPSIS THALIANA*.

Riveras, E.J., Álvarez, J.M., Gutiérrez, R.A.

Plant Systems Biology Lab, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. Núcleo Milenio en Genómica Funcional de Plantas.

Nitrogen is an essential macronutrient for plants. In agricultural soils, nitrate is the most important source of nitrogen. Microarray experiments in *Arabidopsis thaliana* has revealed that nitrate controls the expression of hundreds of genes that are involved in a wide variety of processes. However, the molecular mechanisms of N perception and signal transduction are not yet fully understood. We identified TGA1 and TGA4 bZIP transcription factors as important regulatory factors mediating nitrate responses in *Arabidopsis.* TGA1 and TGA4 possess a high degree of sequence identity and are functionally redundant. We used bioinformatics approaches to identify putative targets of these transcription factors. As a first approach to validate the predictions, we analyzed expression of the target genes in *tga1/tga4* mutant lines. Consistent with our predictions, we demonstrated that TGA1 and TGA4 are required for nitrate dependent regulation of the selected target genes.

10.

Role of cytokinin perception and biosynthesis in the nitrate response of *Arabidopsis thaliana.*

Tamayo, K.P., De la Cruz J., Naulin, P., and Gutiérrez, R.A.

Departamento de Genética Molecular y Microbiología. P. Universidad Católica de Chile.

Plants have evolved sophisticated strategies to cope with environmental changes, such as the heterogeneous Nitrogen availability in soils. Nitrogen is an essential macronutrient for plants. In agricultural soils, nitrogen is available mainly as nitrate. It is known that nitrate can act as a signal to modulate global gene expression in *Arabidopsis thaliana*. However, the molecular mechanisms involved in the plant response to nitrate are mostly unknown. Previous studies have shown that cytokinins play an important role in the nitrogen response in *Arabidopis* and other plants. To understand the nitrate:cytokinin interaction, we evaluated the growth response of cytokinin receptor cytokinin biosynthesis mutant plants under different growth regimes. Phenotypic analysis of the mutants indicated that cytokinin signal transduction and biosynthesis is required for primary root growth in the presence of nitrate. Both types of mutants exhibit significantly shorter roots as compared to the roots of wild-type plants. This growth phenotype is not a general root growth defect of the mutants, but it is manifested specifically in the presence of nitrate. Re-supplementation of biosynthesis mutants with exogenous cytokinin reversed the observed growth phenotype in a dosedependent manner. These results suggest that adequate internal cytokinin levels are essential for proper root growth in nitrate as the sole nitrogen source. Further histological analysis is under way to understand the phenotype of the mutants in presence of nitrate.

Acknowledgements: FONDECYT (1060457), Núcleo Milenio en Genómica Funcional de Plantas P06-009-F, ICGEB CRPCHI0501, NIH-FIRCA 1R03PW007823-01A1 F614-01

11.

Microarray analysis of the nitrate response in the auxin receptor mutant *afb3-1*

Elena A. Vidal, Eleodoro J. Riveras y Rodrigo A. Gutiérrez.

Millennium Nucleus for Plant Functional Genomics. Departamento de Genética Molecular y Microbiología,

Pontificia Universidad Católica de Chile.

Nitrogen (N) is an essential macronutrient available to plants mainly as nitrate in agricultural soils. Besides its role as a nutrient, inorganic and organic N sources play key roles as signals that control genome-wide gene expression. Despite the fundamental and applied significance of understanding these processes, the molecular mechanisms underlying N regulated changes in gene expression are still largely unknown. We have previously identified a root N-responsive regulatory module consisting of microRNA miR393 and one of the known auxin receptors, *AFB3. AFB3* is directly induced by nitrate and is downregulated by N metabolites downstream of nitrate reduction and assimilation by a pathway mediated by miR393. We have also shown that both *AFB3* mutants and *miR393* overexpressor plants show altered lateral and primary root growth responses to nitrate. To identify the molecular mechanisms acting downstream miR393:*AFB3,* we analyzed the genome-wide nitrate response in roots of wild-type plants and *afb3-1* mutants using the Affymetrix ATH1 chips. Two-way ANOVA and FDR analysis of microarray data revealed that 56 genes were differentially regulated between wild-type and *afb3-1* plants. Among the differentially regulated genes, we identified a NAM transcription factor. This gene is induced by nitrate in wild-type plants but is not differentially regulated in *afb3*-1 plants. NAM has also been independently identified in our laboratory as a putative key regulatory gene mediating nitrate responses in *Arabidopsis*. However its role in Arabidopsis development has not yet been analyzed. We are currently studying the root system architecture response to nitrate in NAM mutants to unravel its role in the miR393:AFB3 pathway.

Acknowledgments: Millenium_Nucleus_PFG_P06-009-F, NIH-FIRCA

1R03PW007823-01A1_F614-01, FONDECYT_1060457, ICGEB_CRPCHI0501,

CONICYT_scholarship_AT-24080114.

12.

Identification of novel nitrate responsive small RNAs by Illumina high-throughput sequencing technology.

Tomás C Moyano[1], Elena A Vidal[1], Gabriel Krouk[2], Milos Tanurdzic[3], Gloria M Coruzzi[2] y Rodrigo A Gutiérrez[1,2]

1 Millennium Nucleus for Plant Functional Genomics. Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile. 2 Department of Biology, New York University. 3 Cold Spring Harbor Laboratory.

Nitrogen (N) is an essential macronutrient for plant growth and development. Nitrate is the most important N source for plants in agricultural soils. Nitrate can act as a potent signal to regulate genome-wide gene expression in plants. Previous studies showed that post-transcriptional regulation by small RNAs (sRNAs) is an important mechanism in the nitrate response in *Arabidopsis* roots. sRNAs are 21-24 nt non-coding RNAs that have emerged as key regulators of gene networks in plants and other organisms. The development of new technologies for high-throughput sequencing, such as 454 and Illumina, facilitated the discovery of many sRNAs in *Arabidopsis* and in other species. In this study we focused in the identification of novel nitrate responsive miRNAs in *Arabidopsis* roots. We sequenced sRNAs from nitrate- and control-treated *Arabidopsis* roots using Illumina technology. We obtained approximately 6 million reads from each sample and analyzed the raw sequence data using public and custom made bioinformatics tools. We identified several known miRNAs in our samples, some of which were nitrate regulated. Using algorithms for predicting new miRNAs, we also identified small RNA molecules corresponding to potentially novel miRNAs. We were able to validate the expression of some of these novel miRNAs using independent experiments. Our results highlight the importance of post-transcriptional regulatory networks in the N response of *Arabidopsis thaliana* roots.

Acknowledgments: Millennium Nucleus in Plant Functional Genomics P06-009-F, NIH-FIRCA 1R03PW007823-01A1 F614-01, FONDECYT(1060457), ICGEB_CRPCHI0501.

13.

Arabidopsis thaliana associates with nitrogen fixing bacteria which promote its growth under nitrogen deprivation condition.

Kraiser, T.[1]; González, B.[2]; Gutiérrez, R.[1]

Depto. Genética Molecular y Microbiología. Facultad de Ciencias Biológicas. P. Universidad Católica de Chile. Santiago, Chile1. Facultad de Ingeniería y Ciencias. Universidad Adolfo Ibáñez2. tdkraise@uc.cl.

Nitrogen (N) is an essential macronutrient for plant growth and development. In natural ecosystems and agricultural systems, N fixing (fixN) bacteria are important as they provide N to plants. Previous studies described the association between fixN bacteria and non-leguminous plants. However, the importance of this association to plant nutrition and the molecular mechanisms involved in regulating the fixN bacteria: non-leguminous plant interaction remain largely unknown. Arabidopsis thaliana is a non-leguminous plant and one the most important model systems for plant biology. In this study, we evaluated whether Arabidopsis can associate with fixN bacteria and the effect of these association for plant growth under N-limiting conditions. We found many different endophytic bacteria that are naturally associated with Arabidopsis in an organ specific manner. We were able to isolate a number of different endophytic bacteria, some of which have the capacity to fix atmospheric nitrogen. Our results indicate that Arabidopsis and fixN bacteria associate functionally. This association is beneficial for plant growth under N limiting conditions. These results suggest that fixN bacteria: non-leguminous plant interactions may be of greater importance for plant N nutrition than previously thought.

Acknowledgements: FONDECYT (1060457), Núcleo Milenio P06-009-F y Beca de estudio doctorado CONICYT.

14.

Cytokinins are important for root development in nitrate grown *Arabidopsis thaliana.*

Naulin PA, Tamayo KP, De la Cruz PA, Gutiérrez RA.

Departamento de Genética

Molecular y Microbiología. P. Universidad Católica de Chile

Nitrate is the main source of nitrogen available in agricultural soils and acts as a signal to modulate global gene expression in *Arabidopsis thaliana*. Despite its fundamental and applied importance, the molecular mechanisms involved in nitrate regulation of gene expression are not well understood. Previous studies in our group using mutants in the perception and biosynthesis of cytokinins indicate that cytokinins are necessary for nitrate induced primary root growth. Both perception and biosynthetic mutants show shorter roots when grown with nitrate as the only nitrogen source. To explain the observed phenotypes, we performed histological analysis of the root tip in cytokinin signalling mutants. Our results indicate that the observed phenotype is due to an alteration in the cellular characteristics and developmental pattern at the root tip. The root tip of mutant plants showed a decrease in cell division and elongation as compared to wild type plants grown in nitrate. These plants did not show the characteristic cellular patterns in the root tip. These results suggest that cytokinins are important for the proper cellular patterning of the root tip when developing in the presence of nitrate as the sole N source.

Acknowledgements: FONDECYT (1060457), Núcleo Milenio en Genómica Funcional de Plantas P06-009-F, ICGEB CRPCHI0501, NIH-FIRCA 1R03PW007823-01A1 F614-01.

15.

FUNCTIONAL ANALYSIS OF LLP (LECTIN LIKE PROTEIN) AN *ARABIDOPSIS* LECTIN INDUCED BY SALICYLIC ACID AND INVOLVED IN THE DEFENSE RESPONSE.

Armijo, G., Salinas, P., Leiva D. y Holuigue, L.

Departamento de Genética Molecular y Microbiología,

Facultad Ciencias Biológicas, P. Universidad Católica de Chile.

Salicylic acid (SA) is a crucial hormone for the establishment of defense responses to biotrophic pathogens that are specifically recognized by the plant. Gene activation mediated by this hormone is essential for the local response of the plant and the subsequent systemic immunization. Previously in our laboratory we identified a group of early SA-inducible genes in *Arabidopsis thaliana*. Within this group, *LLP* (lectin-like-protein) has the highest level of activation. This gene codes for a protein with similarity to proteins of the legume lectin family and has not been associated to any biological function. Also, we previously showed that *LLP* is transcriptionally activated by SA and by inoculation with *Pseudomonas syringae* pv tomato (Avr Rpm1). The purpose of this work is to evaluate the role of LLP in the defense response to pathogens in *Arabidopsis*. For this, an homozygous mutant line null for *LLP* was isolated and characterized. In parallel, we developed transgenic lines overexpressing *LLP* fused to c-Myc epitope or to GFP protein. Our results of subcellular localization, by using confocal microscopy, indicate that LLP-GFP is located in the plasma membrane of the plant cell. Then we made a loss or gain of function analysis, by evaluating the proliferation of Pseudomonas in the null and overexpressor lines. We determined that LLP participates in the defense response to Pseudomonas syringae AvrRpm1, reducing bacterial proliferation. Currently we are investigating the specific role of this gene in the defense response.

Financed by FONDECYT-CONICYT (1060494) and Millennium Nucleus for Plant Functional Genomics (P06-009-F).

16.

GrxS13 is induced by high light stress and by salicylic acid signaling in *Pseudomonas syringae* pv. tomato infection (AvrRpm1)

Olate E., Laporte D., Salazar M., Blanco F. and Holuigue L. Departamento de Genética
Molecular y Microbiología, Facultad de Ciencias Biológicas,
Pontificia Universidad Católica de Chile.

Salicylic acid (SA) and reactive oxygen species (ROS) play a key role in cellular responses to different stress conditions. Our group has previously reported the identification of a set of early SA-induced genes (SAIGs); one of these genes codes for the glutaredoxin GRXS13. Glutaredoxins are small oxidoreductases involved in the reduction of disulphide bridges or glutathionylated cysteines from proteins, being crucial for proteins protection under oxidative stress conditions. To obtain a deeper understanding of the role of GRXS13 in protection to stress, we studied the expression profiles of AtGRXS13 gene in response to SA, high light (HL) treatments and *Pseudomonas syringae* pv. tomato (AvrRpm1) inoculation, in wild type and *sid2* mutant (impaired in SA biosynthesis) *Arabidopsis* plants. To determine the role of *GRXS13* in oxidative stress, we have obtained different transgenic lines that silence and over-express this gene. Analysis of the tolerance of these lines to high light stress indicates that GRXS13 play a crucial role in controlling redox homeostasis under stressful conditions. On another hand, we studied the subcellular localization of this protein, by expressing AtGRXS13 fused to GFP in stable transformation assays. Under basal conditions GRXS13-GFP fusion protein is located in nucleus and cytoplasm. Supported by FONDECYT-CONICYT (grant Nº1060494) and Millennium Nucleus for Plant Functional Genomics (P06-009-F).

17.

Detection and Variation of Aphid Borne Viruses in the Chilean Native Strawberry *Fragaria chiloensis* (L.) Duch

[1*]**Rojas, P.,** [2]Martin, R., [3]Sandoval, C. & [1]Caligari, P. D. S.

1Instituto de Biología Vegetal y Biotecnología, Universidad de Talca. 3 Departamento de Producción Agrícola, Facultad de Ciencias Agrarias, Universidad de Talca. USDA-ARS

*2 Horticultural Crops Research Laboratory, Corvallis, OR. USA. *email: projasb@utalca.cl*

The native strawberry, *Fragaria chiloensis ssp chiloensis* (L.) Duch, is distributed naturally in Chile. Two botanical forms have been described, the white-fruited chiloensis form, which is cultivated in Chile coastal areas, and the red fruited patagonica form which grows in Chile and Argentina. Because *F. chiloensis* fruits have good organoleptic quality, there is an increasingly interest in including their germplasm in *Fragaria* x *Ananassa* Duch. breeding programs. Nevertheless, F. chiloensis shows several problems affecting their fruit yields like virus diseases. More than 30 viruses were found in *F x ananassa*, and probably a similar situation occurs in *F. chiloensis*. The objective of this work was study the presence and phylogenetic relationships of aphid borne viruses in *F. chiloensis*. 17 ecotypes of *F. chiloensis* between latitudes 35° and 42° were collected. The techniques ELISA and RTPCR were used for detect *Strawberry mild yellow edge* (SMYEV), *Strawberry mottle* (SMoV), *Strawberry crinkle* (SCV), *Strawberry vein banding* (SVBV), *Strawberry latent ringspot* (SLRSV), *Tobacco necrosis* (TNV), *Tomato ringspot* (ToRSV) and *Tomato black ring* (TBRV) in the strawberry populations analyzed. The partial conserved sequences obtained for SMYEV, SCV, SMoV and SVBV were compared with online available virus's databases for study the phylogenetic structure of the Chilean races. Finally an in vitro tissue culture system was improved to provide virus free material to study plant-virus interactions in *F. chiloensis.*

Acknowledgments: P. R. was supported by a Universidad de Talca and CONICYT fellowships.

18.

Characterization of the emerging begomovirus-whitefly complex infecting tomatoes in the north of Chile

Claudia Medina[1], Paulina Sepúlveda[1], Roxana Mora[1], Mónica Madariaga[1], Germán Sepúlveda[2] and Marlene Rosales[1]

1.*Departamento de Mejoramiento y Biotecnología, Instituto de Investigaciones Agropecuarias, Centro Regional La Platina. Casilla 439-3, Santiago, Chile.*

2.Departamento de Recursos Ambientales, Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Arica.

Whitefly-transmitted geminiviruses (genus Begomoviruses) are one of the most important groups of plant viruses, due to their high incidence and disease severity in vegetable and field crops in tropical and subtropical areas of the world. They affect many important food and industrial crops in Latin America, but until last year, they had not being described in the Chilean territory. Recently symptoms of yellowing, chlorotic curled leaves and stunted plants associated with high levels of whitefly populations were observed in tomato fields in the Region of Arica and Parinacota, in the north of Chile. Symptomatic plants and whiteflies have been collected since 2008 in open fields and greenhouse tomatoes from different locations in the Azapa Valley, with the objective of studying disease incidence, and the characterization of the virus and whiteflies. Over 500 samples have been analyzed for the presence of begomovirus by using genomic markers that allow the universal detection of viruses belonging to this genus. The results confirm the presence of these emerging viral pathogens in the region, with an estimated prevalence of over 30% of the analyzed samples. In all cases, nucleotide sequence analysis revealed the presence of a bipartite begomovirus that share over 92% identity with isolates of Tomato yellow vein streak virus (ToYVSV), a virus that has been described infecting solanaceous crops in Brazil and Argentina. By the other hand, biotype characterization of the whitefly populations, by means of the amplification and sequencing of an 879 bp fragment of mitochondrial cytochrome oxidase I, along with SSR markers, indicated that the B biotype is widespread in the area colonizing different hosts of economic importance.

19.

Molecular analysis of differentially expressed genes in response to big-vein disease of lettuce

Elizabeth Peña, Claudia Medina and Marlene Rosales.

Departamento de Mejoramiento y Biotecnología, Instituto de Investigaciones Agropecuarias, Centro Regional La Platina. Casilla 439-3, Santiago, Chile.

Big vein (BVD) is an economically damaging disease complex of lettuce (*Lactuca sativa L.)* that occurs in all lettuce-producing areas. This disease involves two different viruses: Mirafiori lettuce big-vein virus (MLBVV) and Lettuce big-vein associated virus (LBVaV), both transmitted by the soil-borne fungus *Olpidium brassicae*. The disease is difficult to control since the resting spores of the fungus can persist for over 20 years in soil and because there are no major resistance genes available in commercial varieties of lettuce. Typical symptoms associated to BVD are chlorotic clearing around the leaf veins, leaf distortion and reduction of the head size. In this work we investigated the changes in the expression profiles upon virus infection occurring in the susceptible lettuce variety Sharpshooter. Using cDNA-amplification fragment length polymorphism (AFLP), we selected and analyzed over 100 transcript-derived fragments (TDF) that were differentially expressed between healthy and diseased plants. These TDFs were assigned to one of the following functional categories: metabolism, photosynthesis and energy, signal transduction, responses to stress and defense and genes with unknown function. Genes involved in photosynthetic processes showed a suppressed expression over time, while those related to defense and stress, in general, were expressed in a transient way. In addition, there were some transcription factors induced during the infection. We are currently analyzing the expression patterns of some of these TDFs in lettuces varieties with different susceptibility to this disease, in order to select some candidate genes that may play a relevant role in this plant-virus interaction.

Research funded by Proyecto Fondecyt de Iniciación Nº11060173

20.

Germplasm evaluation for bean virus resistance and use of SSR markers for molecular assisted selection

Elizabeth Peña, Gabriel Bascur and Marlene Rosales

Departamento de Mejoramiento y Biotecnología, Instituto de Investigaciones Agropecuarias, Centro Regional La Platina. Casilla 439-3, Santiago, Chile.

Common bean (*Phaseolus vulgaris L.*) is one of the most important grain legumes and the leading source of low cost quality proteins. INIA maintains an active bean-breading program whose main objectives are to breed cultivars with special plant habits such as high yields, resistance to diseases, adaptability to mechanical harvesting and other characteristics for agroindustry. Among them, the principal limiting factor for its production are viral diseases. Since the most effective way to control viral diseases are the use of resistant material, the objective of this work was to search for sources of resistance against the most important viral diseases affecting this crop in Chile: Bean common mosaic virus (BCMV), Bean yellow mosaic virus (BYMV), Alfalfa mosaic virus (AMV) and Cucumber mosaic virus (CMV). Sixteen accessions of runner bean (Phaseolus coccineus L.) were mechanically and naturally infected with each one of the four viruses under study. The plants were regularly evaluated for viral infection by using diagnostic techniques specifically developed for the identification of the pathogens, such as RT-PCR and immunotissue blot. The results indicated that four accessions were immune to all viruses studied, and 12 of them show resistance to the new and emerging viral complex affecting bean production, which is caused by CMV and AMV. Additionally, several SSR-markers were evaluated for pedigree analysis and used to follow interspecific crosses between *P. vulgaris* and *P. coccineus*. With these tools, now we are able to accelerate the breading process and to introgress new sources of virus resistance into the new varieties produced by our breeding program.

21.

Molecular evaluation of the *D4E1* gene in transgenic plants of *Arabidopsis thaliana* and its effect on *Rhizoctonia solani.*

Alejandra Fuentes[1], Alejandra Sandoval[1*], Graciela Berríos[1*], Manuel Gidekel[2] and Ana Gutiérrez Moraga [**1,2]

1.Laboratorio Biología Molecular Aplicada, Facultad de Ciencias Agropecuarias y Forestales, Departamento de Producción Agropecuaria. Universidad de La Frontera, Temuco-Chile.

2 .Venturel@b, Escuela de Negocios. Facultad de Ciencias e Ingeniería, Universidad Adolfo Ibáñez, Santiago-Chile.

Using the Floral Dip transformation method, 10 transgenic lines of *Arabidopsis thaliana* containing the *D4E1* gene in its constitutive form were obtained. This gene is characterized as codifying a synthetic peptide with antimicrobial activity. Once obtained from these plants, the insertion, expression and tolerance to pathogens provided by the gene were studied. The analysis by PCR confirmed the transgenic nature of the lines obtained. In the RTPCR, the transcribed *D4E1* was detected in all the transgenic lines. The Southern blot analysis determined a copy of the *D4E1* gene in the genome of each of the *A. thaliana* lines. The Northern blot analysis revealed mRNA in all the lines studied with the exception of lines 8 and 9 and the wild plant. The results of the densitometry analysis on hybrid gels with the *D4E1* probe and the α-tubulin probe (Tukey $p \leq 0.05$) established significant differences in the expression of the constitutive *D4E1* gene. The results of the resistance assays (Tukey $p < 0.05$) agree with Northern blot hybridizations. The lines that showed greater band intensity (pixels/area) obtained a higher survival percentage against *Rhizoctonia solani.* Lines 8 and 9 showed a survival percentage similar to that of the wild plant, probably because these possess a low level of transcription which is not detectable by the Northern technique. The constitutive expression of the *D4E1* gene in transgenic *A. thaliana* plants has resulted in increased tolerance to infection by *R. solani*. This establishes that the *D4E1* confers antimicrobial activity, improving the plants' defense system.

Supported: *CONICYT Doctorate Scholarship, Programa Doctorado en Ciencias m/Biología Celular y Molecular Aplicada and Uxmal S.A.Venturel@b, Universidad Adolfo Ibañez.

**Corresponding author: hilda.gutierrez@uai.cl

22.

Characterization of an ankyrin-like protein up-stream region from *Vitis vinifera* is highly induced by *Botrytis cinerea* infection.

Miccono, M.A.[1], Ortega, M.,[2] Montes, C.,[1] Barba, P.,[1] Rubio, J.,[1] Peña-Cortés, H.[3] and Prieto, H.[1]

1 Biotechnology Laboratory, INIA-La Platina Station.

2 Doctoral Program in Biotechnology, University of Santiago de Chile.

3 D. Alkalay L. Biotechnology Center, Federico Santa María University

Grey mold, caused by *Botrytis cinerea*, is a main grapevine disease in Chile. *B. cinerea* is a necrotrophic filamentous fungus of a complex agricultural management because of a broad host spectrum and to the different sources of inoculation. In the "Genome I: *Botrytis*-Grape Interaction" project, a macroarray conformed by 4803 ESTs was designed. Gene expression studies using this macroarray allowed the comparison between mRNAs from infected and non-infected grapevine field plants from two contrasting cultivars: Thompson Seedless and Carménère. By this analysis, we established the involvement of a putative ankyrin-like protein highly induced in response to the pathogen infection. Although is not expected to find natural resistance sources to *Botrytis* in breeding grapevine studies using susceptible cultivars, the knowledge of highly responsible sequences to fungal challenge could be of significant relevance from a biotechnological approach. In this way, we propose that the promoter region of this gene may contain important responsive element which may provide information about the specificity of the plant response after fungal attack. A first step in this work was to verify and compare the observed induction of the grapevine ankyrin-like gene under fungal attack by specific real time PCR analysis of infected Thompson Seedless *in vitro* plantlets. Afterwards, in silico analysis was carried out leading to the definition of an 890 base pairs up-stream zone, which was experimentally characterized. GFP fusion constructs were prepared and used in transient expression assays in tobacco leaves. Wounding and *Botrytis* inductions were analyzed on these agroinfiltrated tissues. Results show that both type of stress clearly induce GFP expression in transformed tissues.

Work funded by Programa Genoma en Recursos Naturales Renovables, Iniciativa Genoma Chile, CONICYT, Grant "Scientific-technology platform to develop plant genomics in Chile. Phase II: Grapevine functional genomics" G07I1003.

23.

Development and evaluation of transgenic Thompson Seedless grapevine lines tolerant to Grapevine fanleaf virus (GFLV) using the coat protein gene silencing strategy.

Zúñiga, C.[1], Castro, Á.[2], Álvarez, C.[3], Olmedo[3], B., Muñoz[3], M., and Prieto, H.[3]

1Plant Health Department, Agricultural Sciences Faculty, University of Chile.

2Biotechnology Doctoral Program, University of Santiago de Chile.

3Biotechnology Laboratory, INIA – La Platina Station.

Grapevine fanleaf virus (GFLV) is the most severe viral disease affecting grapevines and causing significant economic loses. It belongs to the Nepovirus genus and is worldwide distributed, transmitted by the ectoparasite nematode *Xiphinema index*. Due to the unknown occurrence of natural tolerance/resistance sources against GFLV in the *Vitis* genetic background, an interesting alternative for the generation of tolerant lines shows up from the generation of transgenic resistant lines by means of the well-known mechanism of post-transcriptional gene silencing (PTGS). PTGS regulates excessive gene expression by generation of small complementary interfering RNAs that target messengers of the deregulated gene, leading to its degradation and in that way avoiding protein expression. In this work, we describe the generation of toleran/resistant GM grapevine lines using A. tumefaciens-mediated genetic transformation and the GFLV coat protein (CP) gene silencing. CP gene sequences were obtained by PCR amplification and cloning using local viral isolates and recombined into donor and expression vectors of the GatewayTM technology. Five different transgenic Thompson Seedless lines were evaluated using micro-grafting techniques on Saint George GFLV positive rootstocks. Interesting differential levels of tolerance/resistance have been detected on the first season of evaluations by ELISA and qPCR, in comparison to wild-type micro-grafted controls. Relevance of these results and massive use in grapevine technology for the design of tolerant rootstock materials will be discussed.

This is a Research Program of Biofrutales Consortium, from PBCT-Chile Initiative.

24.

Effect of deciduous vs. evergreen leaf habit on the transduction of energy in leaves of *Vitis vinifera* and *Citrus sinensis*.

Sebastián Vega[1], Claudio Pastenes[2], Herman Silva [1,2], Francisco Alfaro[1] and Nicolás Franck [1,2]

1 Centro de Estudio de Zonas Áridas & 2 Departamento de Producción Agrícola

Universidad de Chile

The leaf habit of woody species directly affects the way in which solar energy is captured and transduced for assimilating carbon via photosynthesis: deciduous species achieve higher photosynthetic rate per unit leaf area whereas evergreen species have a longer photosynthetic season. In this work we studied the energy transduction in leaves of a deciduous (*Vitis vinifera*) and an evergreen (*Citrus sinensis*) species grown in Cerrillos de Tamaya, in the semi-arid North of Chile at three different stages of fruit development. Energy being used photochemicaly as compared to the proportion destined to dissipation as heat was estimated from the measurement of chlorophyll fluorescence parameters by means of a fluorometer (LI-COR 6400-40) at three different times of the day on East and West row orientations and three different leaf ages. Photochemical (qP) and non photochemical (qN) quenching of absorbed solar energy was mainly affected by row orientation whereas leaf habit resulted in different responses according to time of day and stage of fruit development. The trends of daily and seasonal evolution of qP and qN, as well as other fluorescence parameters for different leaf habits and row orientations are discussed regarding climate, leaf morphology and carbon partitioning.

Acknowledgements: funding by FONDECYT project N°1080450

25.

Effect of aquaporins blockers on water intake in grape berries.

Alejandro Riquelme[1], Alejandra Oyarzún[1], Manuel Pinto[2], Thomas Fichet[1], Patricio Hinrichsen[2].

1/ Facultad de Ciencias Agronómicas. Universidad de Chile. Santa Rosa 11315. La Pintana. Santiago. Chile. (email: alriquel@uchile.cl)
2/ Instituto de Investigaciones Agropecuarias. Casilla 439. Correo 3. Santiago. Chile.

To get a commercial berry size of 'Sultanina' grapevine (*Vitis vinifera L.*) it is necessary to spray gibberellic acid (GA3) that increases water intake in berries after veraison. Since at this stage the pedicel xylem functionality is lost, it is presumed that water transport occurs via phloem. This implies a possible role of water channels in cell membranes, called aquaporins. Our previous work proved the induction of aquaporin genes after GA3 application. The main goal of our present study is to verify the functionality of GA3-induced aquaporins during development of Sultanina berries. During veraison stage, GA3-applied bunches were treated with two aquaporin blockers: TEA (Tetraethyl ammonium) and $HgCl_2$. Both compounds were effective in decreasing berries final volume. Treated berries did not show change in soluble solids (ºBrix), but in water content. These results suggest that aquaporins partipate activately in water intake after veraison in case of GA3-applied grape berries.

This work was supported by Fondef- Genoma G07I-1002.

26.

Differential expression levels of contrasting phenotypes in segregating individuals from table grape (*Vitis vinifera* L.)

Muñoz-Robredo, P.[1], Gudenschwager, O.[2], Casanueva, X.[3] and González-Agüero, M.[2,4]

1 Facultad de Ciencias Físicas y Matemáticas, Departamento de Ingeniería Química y Biotecnología, Universidad de Chile

2 Instituto de Investigaciones Agropecuarias (INIA) – CRI La Platina

3 Universidad Andres Bello, Chile.

4 The Plant Cell Biotechnology Millennium Nucleus (PCB-MN)

Email: maugonzalez@inia.cl

Chile is one of the most important exporters worldwide of table grape (*Vitis vinifera* L.). An absence of seeds (seedless) and large berry are important quality parameters for fresh consumption. Although progress has been made in understanding the molecular basis of stenospermocarpy, the biochemical and molecular changes underlying this process are poorly understood. In this work we characterized and analyzed the expression of two genes, PMEI (Inhibitor of Pectimethylesterase) and SPY (Spindly), which are strongly related to seedless condition. Both genes were obtained by quantitative trait locus (QTL) mapping using a reference population (from a 'Ruby Seedless' and 'Thompson Seedless' crossing), which includes contrasting phenotypes, i.e. seeded and seedless, large and small berries. We identified, cloned and characterized the expression pattern of these key genes in seven different stages of development from samples of four individuals with contrasting phenotypes related to seed and berry development. The expression profile was characterized by real-time PCR. The amplification assays were performed with several isoforms of PMEI and SPY identified in *Vitis*. As berry fruit development progressed, we observed a change of transcript levels for the most of analyzed genes. The significance of the changes in the expression profile measured from table grape is discussed. (Funded by Fondef G07I1002).

27.

Modulation of proanthocyanadin synthesis and polimerization by light in *Vitis vinifera* L, cv. Carmenere.

Betzabé Galaz*, Alvaro Peña-Neira, Claudio Pastenes
Laboratorio Fisiología del estrés en Plantas
Facultad de Ciencias Agronómicas
Universidad de Chile

Condensed tannins also known as proanthocyanidins are flavonoid phenolic compounds localizated in skins, seeds and stems of grapes. They contribute to the astringency and bitternes of grapes and wines and their content in wines also affects color stability; body ability for wine aging. A common practice in viticulture is to exposure of bunches to light by means of leaves removal around bunchesin order to improve berry quality. Such protocol would lead to increasing phenolic concentration and improving the quality of tannins. However, little is known about the effect of light on the synthesis, degradation and evolution of skin tannins. In this study, we have assessed the effect of different light regimes by means of leaf removal on the phenolic composition of skins berries from Carménère vines. In Haras de Pirque vineyard, during the season 2007-2008, four treatments were implemented: bunches in dark (T1), bunches without leaf removal (T2), bunches with leaf removal since pea size (T3) and bunches with leaf removal since véraison (T4). From pea size, total phenols, total anthocyanin, total tannins, monomeric, oligomeric and polymeric concentration, mean degree of polymerization besides low molecular weight phenolics were assessed, as well as the anthocyanin profile at harvest. Light exposure induced an increase in skin fresh weight, leading to higher concentration of total phenols, tannins and anthocyanins compared with the fruit growing in dark conditions. However at harvest there were no significant differences between treatments on those parameters. As for tannins, bunches exposed light since pea size, had higher content of oligomeric and polymeric tannins. In the other hand, shading caused an increase in the gallate percent at harvest, a higher proportion of (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-O-gallate and a lower proportion of (-) epigallocatechin as extension subunits. It seems that a high degree of bunch exposure to light in hot climates stimulates the synthesis of phenolic compounds. The concomitant high berry temperature, however, leads to a reduced synthesis rate and/or increase metabolite degradation. Our findings should support the development of better agronomical protocols for improving quality characteristics of tannins and, consequently, the sensory properties of the wine.

28.

Transcriptional analysis of genes involved in the synthesis and accumulation of tannins during fruit development in grapevine cultivars Carménère, Merlot and Shiraz

Pérez-Díaz, R[1]., Pérez-Díaz J[1]., Peña-Cortes, H[2]., González, E[1]. and Ruiz-Lara, S[1].

1 Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, casilla 747, Talca, Chile

2 Centro de Biotecnología Daniel Alcalay, Universidad Técnica Federico Santa María. Valparaíso, Chile

Proanthocyanidins (PAs) or condensed tannins are flavonoids that play an important role in taste and astringency in red and white wines. These secondary metabolites are increasingly recognized as having beneficial effects on human health. Grapevines synthesize and accumulate PAs in the seeds and skin of the fruit and it occurs during early berry development prior to onset of ripening (veraison). The accumulation begins before flowering but is more active between flowering and fruit setting.

Two enzymes play a critical role in the PAs biosynthesis, anthocyanidin reductase (ANR) and leucoanthocyanidin reductase (LAR), which can produce catechin and epicatechin, the monomers used to assemble PA polymers.The expression of these structural genes of the PAs pathway is regulated by the transcription factor VvMybPA1, which has shown to be specific for this pathway. In this work we performed a comparative study of the gene expression of *VvANR*, *VvLAR* and *VvMYBPA1* in the cultivars Carménère, Merlot and Shiraz during fruit development process. The results reveal significant expression differences between the cultivars analysed and such defferences are likely to be cultivar-dependent. The transcript abundance of the analysed genes shows a correlation with tannin accumulation in grapevine.

Support: Project Fondef G07I1003

29.

Transcriptional analysis of genes involved in polyamine and ethylene production during the grapevine bud development

Cabrera N.E. *; Almada R.D; Peña-Cortez H., González, E. & Ruiz-Lara, S.

*Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, 2 Norte Nº 685, Universidad Técnica Federico Santa María. *email: ncabrera@utalca.cl*

S-AdoMet is the major methyl donor in plants and is used as a substrate for many biochemical pathways, including polyamines and ethylene biosynthesis. The rate-limiting step of ethylene synthesis is the conversion of S-AdoMet to ACC by ACC synthase, in the same way the decarboxylation of S-AdoMet is the limiting step in the production of spermidine and spermine. According to this, it was reported that spermine inhibited ethylene biosynthesis suggesting that temporally concerted changes in the levels of polyamines and ethylene may influence specific physiological processes in plant development. In this study we analyse the expression of SAM descarboxylase, ACC synthase and ACC oxydase genes during the *V. vinifera* cv. Carménère bud development, covering important ontogenetic events as flowering induction, dormancy and flower development. *SAMDC* genes show a high expression in first growing season latent buds during the inflorescence meristem formation and they decrease to a minimun before the entrance in dormacy. Their expression increase again before the bud burst. *ACCSyn* messenger is unvariable detected almost all along the periods sampled and reaches a maximun at the end of the bud dormancy. Finally *ACCO* gene is expressed in latent buds without detectable transcriptional activity in bud at dormancy and breaking stages. The expression profile of the analyzed genes correlates with periods of celular proliferation and differentiation in grapevine bud development.

Acknowledgments: This work was funded by grants from Consorcio Biofrutales S.A. R. A. and N. C. were supported by a Universidad de Talca fellowship.

30.

Effect of exogenous abscisic acid on the phenylpropanoid pathway in grape berries cv. Carménère.

Luis Villalobos*, Freddy Ibáñez, Claudio Pastenes.

Laboratorio Fisiología del estrés en Plantas
Facultad de Ciencias Agronómicas
Universidad de Chile

Abscisic acid (ABA) is a phytohormone, involved in plant stress responses. Also, is known to increase the concentration of flavonoids in Vitis vinifera grape berries, and to trigger secondary metabolism in berry skins. ABA has a peak value few days before the onset of ripening (*veraison*), preceding sugar and colour accumulation and the expression of transcripts regulating the phenylpropanoid pathway. In order to better understand the mechanism for ABA effect on the responses of gene expression for the phenylpropanoid pathway and their corresponding metabolites, applications of exogenous ABA were conducted in the field in cv. Carmenere few days before *veraison*. We observed exogenous ABA treatment increased the (+)-ABA content and the gene expression of all transcripts (*VvMYBA1, VvMYB4A, VvPAL, VvDFR, VvANS, VvUFGT* and *VvLAR2*) analyzed in grape berry deseeded at *veraison*. In relation to the anthocyanin biosynthesis, the transcripts of *VvPAL, VvDFR, VvANS, VvUFGT* and *VvMYBA1* has a similar pattern throughout the season. The greatest differences in anthocyanin concentration were found up to 2 months after *veraison* in exogenous ABA treatment. In transcripts related to flavanol biosynthesis, *VvMYB4A* and *VvLAR2*, despite a decrease from *veraison* until two months after *veraison*, the relative expression was increased in grape berries treated with ABA. In order to determine the different anthocyanins and polyphenols content we have conducted a detailed analysis of these compounds by HPLC analysis.

31.

Genome-wide analysis of the grapevine BURP-domain family identifies three grape *rd22* homologue genes with differential expression patterns during fruit development and in response to stress conditions

Jose Tomás Matus, Felipe Aquea, Andrea Vega, Paola Cañón, Amparo Rodríguez, Jennifer Serrano-Acevedo and Patricio Arce-Johnson.

Pontificia Universidad Católica de Chile.

RD22 (*responsive to dehydration 22*) gene is a molecular link between abscisic acid (ABA) signalling and abiotic stress responses. Although its function remains unclear, its expression is used as a reliable ABA early response reporter gene. In Arabidopsis, the single copy *RD22* gene possesses a BURP domain, located at the C-terminus of several different plant proteins. In grapevine, an *RD22-like* gene has been recently characterized, although previous Affymetrix expression data suggest additional homologues form part of an *RD22*-like subfamily. A genome wide analysis was performed to search for BURP-domain containing proteins in grapevine (*Vitis vinifera* L.). Twenty seven gene models were identified. The three genes most similar to *Atrd22* were studied, including the recently characterized *Vvrd22-a* gene. QPCR and Affymetrix-based gene expression data both revealed that grapevine *Vvrd22-a, b* and *c* genes possess different expression patterns during organ and fruit tissue development and under abiotic (salt, drought) and biotic (virus*, Botrytis cinerea*) stress. *In silico* analysis of the *rd22* promoter regions shows some differences in the presence and location of ABA/drought-related MYB and MYC-binding elements but this does not necessarily explain the different expression of these genes. Genome wide analysis of BURP domain-containing genes revealed an expanded cluster of *rd22* genes in grapes when compared to Arabidopsis. In addition, these genes differ in their expression throughout organ development and in response to stress, suggesting they could possess complementary opposing roles. Acknowledgements: Chilean Wine Consortium 05CTE01-03, the Fruit Consortium, 07Genoma01 and Millennium Nucleus for Plant Functional Genomics (P06-009-F).

32.

Bioinformatic identification of SNPs and EST-SSRs polymorphism in *Prunus persica* varieties

Ninoska Delgado, Paula Vizoso, Camilo Avendaño and Lee A Meisel
Millennium Nucleus in Plant Cell Biotechnology; Plant Molecular Genetics Lab of the Center of Plant Biotechnology, Andres Bello University, Av. República 217, 837-0146 Santiago, Chile (ninoskadelgado@gmail.com)

We have previously identified a number of candidate genes associated with peach fruit quality. We are taking a bioinformatic approach towards identifying polymorphic markers (EST-SSRs and SNPs) in these candidate genes, such that they may be used in Marker Assisted Breeding program to identify peaches with improved fruit quality. Using public and private peach EST sequences from 11 different peach varieties, we have detected previously described SSRs in 32 unigenes, of which 8 correspond to the candidate genes mentioned earlier. These 32 EST-SSRs have been used to genotype 19 different peach varieties and to perform phylogenetic analyses of these varieties. 21 of these EST-SSRs are positioned on the genetic map TxE almond x peach F2 2004. Additionally, we have established a bioinformatic pipeline to identify SNPs from EST sequences. These bioinformatic analyses have enabled us to identify a number of putative SNPs in these EST sequences. These putative SNPs are being validated by HRM (High Resolution Melting) analyses as well as sequencing of PCR products.

Supported by ICM P06-065-F, Proyect Genoma GO7I1001 and Proyect Consorcio Biofrutales SA

33.

Proteomics in peaches: Optimizing conditions to analyze the protein profile of *Prunus persica* leaves.

Severin, D.[1,2], Handford, M[1]. and Orellana, A.[2]

1Laboratorio de Biología Molecular Vegetal, Departamento de Biología,
Facultad de Ciencias, Universidad de Chile.
2 Núcleo Milenio en Biotecnología Celular Vegetal,
Centro de Biotecnología Vegetal, Universidad Andrés Bello.

Chile is the largest exporter of peaches and nectarines (*Prunus persica*) in the southern hemisphere. However, the export value of peaches has decreased in recent years, mainly due to the chilling injury that the fruits suffer whilst being transported at low temperatures, with mealiness being one of the most obvious symptoms. Therefore, in breeding programmes, there is a need to develop new varieties which do not suffer from this physiological disorder. To assist in their development, proteomics has emerged as a powerful tool to search for proteins which act as phenotypic markers. Two dimensional gels for the separation of protein extracts from peach leaves were generated, with the aim of analysing the differences in the protein profiles in leaves of a population segregating for the susceptibility of fruit mealiness. Given that lipids, pigments and polyphenols interfere with protein extraction and separation, a new method was successfully adapted to remove these compounds from the leaf samples. The separation of proteins in 2-D gels was highly reproducible, as determined by hierarchical clustering of the technical replicates. Five significantly-more abundant spots were found in samples extracted from leaves of trees producing fruits susceptible to mealiness compared to trees producing fruits less susceptible to this disorder. These spots could be sequenced and the identity of proteins may provide key insights into the molecular mechanisms involved in chilling-induced mealiness of peaches. Additionally, in future breeding programmes, seedlings that express these 'mealiness' markers could be discarded at an early stage.
Funding: Fondef G07I1001, PCB-MN ICM P06-065-F, PFB-16

34.

Prunus persica gene expression and regulation in response to low temperature

Alejandra Cifuentes and Herman Silva

Plant Functional Genomics & Bioinformatics Lab and
Millennium Nucleus in Plant Cell
Biotechnology (PCB).Universidad Andrés Bello República 217, 837-0146, Santiago, Chile.

Peaches and nectarines are stone fruits that belong to the *Prunus* genus,specifically to the Rosaceae family. The world production of peaches is about 11 million tons. The major producers of the north hemisphere are China, Italy and United States and in the south hemisphere Chile is one of the main exporters of this fruit. The fruit ripens and deteriorate quickly at room temperature. Therefore, cold storage is
used to slow these processes and is necessary when the fruit is exported to farther markets. However, extended storage of peach and other stone fruit can negatively affect fruit quality due to physiological disorders such as chilling injury characterized by woolliness, browning and red pigment accumulation. These disorders cause important commercial losses. The cold-storage of peaches affects the expression level of different genes that might unleash the physiological effects mentioned above. Hence we propose the existence of fruit-specific promoters in peach fruit that will regulates the genes involved in the physiopathology of damage by cold in post-harvest conditions. Based in a ESTs transcripcional profile study established for different post-harvest treatments of peaches we identified several genes differentially regulated by low temperatures treatments (4-5ºC). Within these genes were found pathogenic related genes, phenylpropanoid pathway (lignin and of the anthocyanins) genes and transcription factors. By RT-PCR we established that these genes are fine regulated by cold under different time's treatment, being the storage period an important factor in the gene regulation in response to cold. In order to understand the cold regulation in peach, we are isolating and characterizing the promoter region of these genes using GUS fusions. This research was supported by Millennium Nucleus in Plant Cell Biotechnology (PCB) ICM P06-065-F, PBCT R-11 and Consorcio Biofrutales SA.

35.

Cytokinins regulate the expression of putative orthologs genes involved in this hormone mediated response in different development stages of *Prunus Persica* fruits.

Fernanda Rodríguez[1], Sebastián Troncoso[1], Carolina Klagges[1], Ninoska Delgado[1], Daniela Urbina[1], Juha Immanen[2], Yrjö Helariutta[2] and Lee A. Meisel[1]
[1]Millennium Nucleus in Plant Cell Biotechnology, Plant Molecular Genetics Laboratory, Plant Biotechnology Center, Biological Science Faculty, Andrés Bello University, Santiago, Chile. fda_ro@hotmail.com
[2]Department of Biological and Environmental Sciences/Institute of Biotechnology, University of Helsinki, 00790 Helsinki, Finland.

During the past decade research related with plant genomics has provided new tools to better define the molecular mechanisms associated with plant growth and development. In a collaborative project between Chile and Finland, we are using a comparative functional genomics approach towards identifying key regulatory factors responsible for growth related to tree biomass. Although peach trees and poplar trees appear different, they actually share a very high level of similarity at the genomic level. This suggests that there may be a conservation of gene function in these tree species.
It has been demonstrated previously that cytokinins play a key role in biomass accumulation in procambial vascular tissue in Arabidopsis and Poplar. In order to better understand the effects of cytokinin on peach fruit growth and development, we have bioinformatically identified putative peach orthologs genes of cytokinin responsive gene. Exogenous cytokinin treatment of fruit samples, at different stages of development, revealed an increased expression of putative peach orthologs in response to cytokinin at earlier stages of development. Additionally, transient overexpression of the Arabidopsis *CKI-1* in peach fruits increases the expression of several of the putative orthologs identified. These results set the stage to better understand the cytokinin response pathway in peach fruits, such that the relationship between this pathway and fruit development may be further analyzed.

Funded by ICM P06-065-F and AKA/Conicyt CCF 01

36.

Identification of clusters of co-regulated genes involved in *Prunus persica* postharvest fruit physiology

Paula Vizoso and Lee Meisel

Millennium Nucleus in Plant Cell Biotechnology; Plant Molecular Genetics Lab of the Center of Plant Biotechnology, Andres Bello University, Av. República 217, 837-0146 Santiago, Chile

To facilitate the identification of candidate genes that may be incorporated into a peach marker assisted breeding program to improved fruit post-harvest quality, we have performed comparative analyses of the abundance of expressed sequence tags from peaches under four different post-harvest conditions: 1) non-ripe fruits, 2) ripened fruits, 3) non-ripe cold-treated fruits, 4) ripened cold-treated fruits. These analyses have revealed 13 clusters of co-expressed or co-regulated genes under different post-harvest conditions. These clusters of co-regulated genes consist of clusters of genes that show induction/repression in ripe fruits, induction/repression during cold-storage, or induction/repression in woolly fruits. Additional characterizations of these co-regulated genes provide insight into the transcriptional regulatory networks that lead to the characteristics present in fruits under different post-harvest conditions. We present validation of these results by real-time PCR and present new clues towards understanding process related with ripening and senescence in fruits.

Funded by DI-20-09/I , ICM P06-065-F and Proyecto Consoricio BIOFRUTALES S.A.

37.

Genotypification of Chilean Sweet Cherry Varieties by S-allele-specific PCR detection

Klagges, C., Avendaño, C. and Meisel, L.

Millennium Nucleus in Plant Cell Biology and Biotechnology; Plant Molecular Genetics Laboratory, Center of Plant Biotechnology, Andrés Bello University, Av. República 217, 837-0146 Santiago, Chile.
e-mail: carolako@gmail.com

In sweet cherries (*Prunus avium*), gametophytic self-incompatibility is determined by a locus S with multiple alleles. In the style, the S-locus codifies for an allele-specific ribonuclease (S-RNase) that is involved in the rejection of pollen that carries the same S-allele. The knowledge of the S-genotype of sweet cherry cultivars is therefore essential to establish productive orchards by defining compatible combinations. The isolation of the genomic DNA sequences of the sweet cherry S-RNases (*Tao et al., 1999; Yamane et al., 2000; Wiersma et al., 2001; Wûnsch and Hormaza, 2004*) revealed that the two introns found in sweet cherry S-RNases vary in size for each S-allele; this intron variability is the basis of S-allele identification by PCR analysis using conserved PCR primers and/or allele-specific primers. In this work, PCR analysis with both primers has been used to characterize the S-genotype of 52 sweet cherry varieties, including 10 varieties whose S-allele constitution had not been previously described.

Funded by ICM P06-065-F and INNOVA 07CN13PBT-167

38.

Analysis of factors involved in the cracking susceptibility of sweet cherry fruits

Juan Carlos Rios[1], Erika Lang[2] and Herman Silva[1]
[1]Plant Functional Genomics & Bioinformatics Lab and Millennium Nucleus in Plant Cell Biotechnology. Universidad Andrés Bello República 217, 837-0146, Santiago. [2]Center of Major Equipment, University of Chile, Las Palmeras 3425, Santiago.

Chile is the main exporter of sweet cherries (*Prunus avium*) from the south hemisphere. The quality indexes occupied for selection are: color, flavor, solid soluble content and absence of physical damages. Unfortunately, these fruits suffer a series of superficial problems such as compressions, wounding and cracking. The latter is one of major reason of losses in the worldwide production. The damage is produced when the cherry-tree became in contact with water rain for long periods of time. Analyses of the cuticular wax of cherries and tomatoes showed that their components and ratios are very similar and that the n-alkanes could play an important role in the impermeability of the cuticle. This information allows us to hypothesize that the susceptibility to the cracking in fruits of *Prunus avium* is linked to structural components of the exocarp. Three varieties of cherry were analyzed (Bing, Lapins and Rainier) using cracking assays in vitro. These assays indicate that Bing and Rainier are more susceptible to the cracking, than Lapins. Studies of ^{1}H in NMR (one and two dimensions) show the presence of an alkane that is in a high concentration compared to other components. Lapins contain the lower concentration for this alkane and Rainer the higher. Nevertheless, Bing presents a major number of different types of hidrocarbures (2D-NMR) with regard to the others two varieties.

This research was supported by Innova CORFO (07CN13 PBT-167), PBCT R-11, Millennium Nucleus in Plant Cell Biotechnology (PCB) ICM P06-065-F and TA Fellowship UNAB to JCR

39.

Chloroplast DNA diversity in *Fragaria chilensis* spp *chiloensis* populations

San Martín L., Valdés J., Carrasco B., Caligari PDS.

Laboratory of Plant Physiology and Molecular Genetic, IBVB,
Universidad de Talca.

Wild species of *Fragaria* generally have been grouped according to their chromosome numbers. The octoploids, which include *Fragaria chiloensis* are restricted to North America, South America, and Hawaii. The North American and Hawaii plants of *F. chiloensis* have been designated as subspecies, *lucida*, *pacifica*, and *sandwicensis*, whereas the South American plants have been referred to as ssp. *chiloensis* but with two botanical forms: *chiloensis* and *patagonica*. The origin and evolution of Chilean strawberry is obscure, but it is assumed that they were introduced from North America, possibly via bird migrations. Therefore it is necessary to obtain molecular genetic information in order to try to determine *i)* the migration routes to South America and *ii)* the possible origin of the Chilean population of *F. chiloenisis*. In an attempt to evaluate the relationship between the four subspecies of *Fragaria chiloensis* and the other octoploid species, *F. iturupensis,* we used PCR-RFLPs. Six pairs of primers were used to amplify regions of chloroplast DNA, using, as an outgroup *Vasconcellea pubescens*. PCR products were digested with three restriction enzymes: Hae III, HinfI, and Taq I. The results revealed no polymorphism between the North American and Chilean subspecies. In addition, polymorphism was also not observed between these subspecies and the species *F. virginia*. Further, the results reveal an absence of polymorphism within and between the Chilean populations of *F. chiloensis* indicating high level of conservation of chloroplast DNA into *Fragaria* genus.This suggests that there has been no selected mutation in the chloroplast, at least within the area of the chloroplast genome defined by the probes used. This would tend to imply that the speciation is a relatively recent event. Clearly it is important and necessary to evaluate other PCR-RFLP markers and/or other more powerful molecular markers, that might reveal polymorphic sites and allow us to reconstruct the migration history of the Chilean strawberry populations. Such information would be valuable both for strategies of conservation and for genetic breeding.

Acknowledgements: SM.L thanks the University of Talca for financial support of a studentship. The work was carried out with support of the Project on *Fragaria* Integral.

40.

Involvement of xyloglucan endotransglycosilase/hydrolase (XTH) during ripening of *Fragaria chiloensis* and *F. x ananassa* fruit.

Opazo, M.C.[1], Figueroa, C.R.[1], Henriquez, M.J.[1], Bruno, C.[2], Valenzuela, P.D.T.[2], Herrera, R.[1], Moya-León, M.A.[1]
1Laboratorio Fisiología Vegetal y Genética Molecular, Instituto Biología Vegetal y Biotecnología, Universidad de Talca, Talca, Chile.
2Fundación Ciencia para la Vida.

Strawberry fruit has a very short shelf life due to its fast softening rate. Fruit softening has been shown to be related to cell wall degradation. As changes in the hemicellulosic fraction have been observed in *Fragaria chiloensis* fruit, the participation of xyloglucan endotransglycosilase/hydrolase (XTH) was studied. XTH isoforms have been described in kiwi, pear and apple fruit, but not in strawberry. Two XTH genes were identified in *F. chiloensis* fruit with high homology to other plant XTHs. Full-length sequences were obtained in *F. chiloensis* (*Fc-XTH1*, *Fc-XTH2*) and *F. ×ananassa* (*Fa-XTH1*), and the partiallength of *Fa-XTH2*. Phylogenetic analysis suggests that both strawberry *XTH1* and *XTH2* genes belong to distant phylogenetic groups of XTHs. DNA-gel blot analysis indicates different genomic organization between the two genes. RNAgel blot analyses were used to detect XTH transcripts during strawberry development. Gene expression profiles show a decrease in XTH1 transcript levels from large green (LG) stage to ripe fruit stage in both species, coincident with the reduction in fruit firmness. In contrast, XTH2 has an oscillating expression pattern reaching a maximum level at LG stage in both species. Bioinformatic analysis allowed us to predict the secondary structure through homology modeling. We use an immunoassay to quantify the XTH protein levels during fruit ripening, showing an increase in the protein content at the transition stage, the initial step of strawberry ripening. The data is congruent with a probable role of XTH during fruit growth and the initial steps of strawberry ripening.
Acknowledgments: Proyecto PBCT-Anillo ACT-41.

41.

Changes in the structure of Pectins of *Fragaria chiloensis* and *Fragaria* x *ananassa*.

González-Fernández-Niño S.[1], Pauly M.[2] and Orellana A.[1]

[1]*Center of Plant Biotechnology, Universidad Andres Bello, Santiago, Chile*
[2] *DOE-Plant Research Lab. Michigan State University, East Lansing, MI, USA*
Contact: ushisu77@gmail.com , aorellana@unab.cl

The softening of fruits during ripening occurs primarily by loss of the structure and changes in the composition of the cell wall. Major changes in the texture of the fruit flesh have been attributed to the partial or complete solubilization of polysaccharides that constitute it. The cell wall is composed of protein, carbohydrate polymers, inorganic ions and phenolic compounds, which were shown to be modified during fruit development and ripening (Albert *et al.*, 1994; Hayashi, 2006). In the degradation of the cell wall of *F. x ananassa* (Fa) and *F. chiloensis* (Fc), like in other fruits such as tomato (Brummell *et al*., 1999), melon (Rose *et al*., 1998), apples (Peña and Carpita, 2004), peach (Brummell *et al*., 2004) and grapes (Vidal *et al*., 2001), the pectins were shown as one of the major polysaccharides involved in the change in texture during fruit ripening. The solubilization of pectins would be the process that contributes the most to softening of the pulp (Perkin-Veazie, 1995; Rosli *et al*., 2004), without discarding the involvement of hemicelluloses in the process of disassembly of the cell wall (Perkins-Veazie, 1995, Jiménez-Bermúdez *et al*., 2002, Nishizawa *et al*., 2002). Our results indicate that rhamnogalacturonan I is the polysaccharide that changes the most during development and maturation of *F. chiloensis* and *F. x ananassa* cv Camarosa. Additionally we observed significant differences in the amount of the wall monosaccharide galactose between both investigated species of strawberry, which may indicate differences in pectic polymers such as galactans.

Support by Project Basal PFB-16, Project Mecesup UAB602, Internal Project UAB, Research Fellowship UAB.

42.

Generation and evaluation of transgenic *Solanum Lycopersicon* lines silenced in the Gibberellin 2- β-hydroxylase gene.

Gerardo Contreras[1], Christian Montes[4], Alvaro Castro[2], Eduardo Tapia[3], Catalina Alvarez[4], and Humberto Prieto[4]

[1] *Biotechnological Engineering Department, Universidad Tecnologica de Chile INACAP*
[2] *Doctoral Program in Biotechnology, Universidad de Santiago de Chile*
[3] *Doctoral Program in Biotechnology, Universidad Santa Maria de Valparaiso*
[4] *Biotechnology Laboratory, INIA – La Platina Station*

Recently, gibberellins (GAs) have been proposed to be involved in important physiological processes such as berry size and/or seed development. Although there is not enough experimental evidence for these suggestions, several efforts are being conducted in order to demonstrate some of the multiple roles in which GAs can be participating. In the present work, we hypothesized that reduction in GAs catabolism should increase the endogenous levels of this hormone. In that way, this work describes the cloning, characterization, development of a silencing construct and *in vivo* evaluation of the tomato gibberellin 2-β-hidroxilase (GA-2-ox) gene, which generates a gene product that deactivates the endogenous forms of GAs (like GA_1, GA_4). 12 different transgenic lines were generated and screened for GA-2-ox gene silencing of the five possible gene isoforms. Results showed the production of a unique silenced line of the expected 4 and 5 isoforms of the gene whose additional physiological, morphological, metabolic and molecular characterization will be presented.

This is a Research Program of Biofrutales Consortium, from PBCT-Chile Initiative

43.

Organic acid and sugar content changes in tomato fruits that overexpress an ABA-regulated transcription factor

Bastías, A.[1], Gómez-Cadenas, A.[2] y Casaretto, J.A.[1]

1-Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Chile.
2-Departamento de Ciencias Agrarias y del Medio Natural, Universidad Jaime I, Castellón, España.

Fruit development is a complex process regulated by plant hormones and involves several coordinated metabolic and physiological changes. During these events, the phytohormone abscisic acid (ABA) is known to regulate the development and maturation of seeds. The AREB bZIP transcription factors mediate ABA-regulated gene expression involved in desiccation tolerance and are expressed mainly in seeds and in vegetative tissues under stress; however, they are also expressed in some fruits such as tomato. In order to understand the role of ABA signaling in fruit development, the expression of two AREB-like factors were investigated during different developmental stages. Moreover, tomato transgenic lines that over-express and down-regulate one AREB-like transcription factor, *SlAREB1*, were generated to determine its effects on the levels of some metabolites determining fruit quality. No significant changes were found in ethylene content in tomato fruits when analyzed by gas chromatography, which agrees with the normal ripening phenotype observed in transgenic fruits. Content of some organic acids and sugars was analyzed by capillary electrophoresis. Higher levels of citric acid, malic acid, glucose and fructose were observed in *SlAREB1* over-expressing lines compared to those in antisense suppression lines in red-mature fruit pericarp. The higher hexose content correlated with increased expression of genes encoding a vacuolar invertase and a sucrose synthase. These results suggest that ABA affects the metabolism of these compounds during the fruit developmental program.

Support: FONDECYT 1090426; CONICYT International research stay fellowship to A.B.

44.

MOLECULARS MARKERS IN ECOTYPES OF *Triticum sp.* COLLECTED IN THE EIGHTH AND NINTH REGION OF CHILE: PRELIMINARY INFERENCES FROM MICROSATELLITES AND GLIADINES MARKERS.

Alejandra Fuentes; Giovanni Larama; Aliro Contreras; Javier Zúñiga; Ana Gutiérrez y José Sierra.

Laboratorio de Biología Molecular, Facultad de Cs. Agropecuarias y Forestales, Universidad de La Frontera (UFRO), Temuco, Chile y Unidad de Biotecnología de Plantas, INIA Carillanca-CGNA, Carillanca, Chile

Wheat is the more cultivated cereal; it occupies 17% of cultivars in the world and constitutes the basic food for 35% of the planet population. The genetic improvement has increased the yield potential of wheat; however it is depending on internal factors such as fertilizers, fungicides, herbicides, witch the concomitant increasing of the both production and contamination costs. Morphological and physiological characters, traditionally used, provide practical information to breeders but they cannot be sufficient because of low polymorphism and variation under environment. In this sense, wheat ecotypes has been preserved among small farmers where is possible to find genotypic characteristics of great utility and importance that would diminish the susceptibility to diverse biotic and abiotic factors. The microsatellites are broadly distributed between the genomes of eukaryotes organism, and at molecular level they correspond to sequences from 1 to 5 nucleotides repeated in tandem where the number of repetitions reveals genetic differences among individuals (SSR). In the same way, gliadines are wheat proteins which are extracted from the endosperm of wheat seed and its electrophoretic spectra, have proved to be highly polymorphic for genotype identification in wheat. This work studied the combined use of gliadines and SSRs to analyze variability of a Mapuche collection of wheat's cultivars. The phenotypic data were collected from small farmers in different environments including two regions of the central zone of Chile.

Supported by: Dirección de Investigación UFRO
Correspondig author: jsierra@ufro.cl

45.

Characterization of the Phytoene Synthase (PSY) Gene Family in *Brassica napus*

Cárdenas, PD1,2, Gajardo, HA1, Iñiguez-Luy, FL1, Federico, ML1

Unidad de Biotecnología de Plantas, Centro de Genómica Nutricional Agro-Acuícola (CGNA), INIA-Carillanca1. Programa de Magíster en Bioquímica y Bioinformática, Universidad de Concepción2.

Carotenoids are fat-soluble pigments produced by photosynthetic organisms. They provide health benefits acting as vitamin A precursors and antioxidants, and they are also used as food, feed and cosmetic colorants. In plants, the first committed step of the carotenoid biosynthethic pathway is catalyzed by the enzyme phytoene synthase (PSY). In *Arabidopsis thaliana*, this enzyme is encoded by a single copy gene (*psy*) but the existence of *psy* gene families has been documented in several crop species including tomato, tobacco, maize, rice and sorghum. Our goal is to characterize the *psy* gene family in *Brassica napus*, one of the most important oil-producing crops in the world. Based on synteny information between the closely related genomes of Arabidopsis, *Brassica rapa* and *Brassica oleracea*, we were able to identify 6 chromosomal locations where copies of *psy* may exist in *B. napus*. Simultaneously, we have identified *B. napus* ESTs with high similarity to Arabidopsis *psy* and used this sequence information to design PCR primers and clone *Bnpsy* sequences. To date, we have cloned and identified 4 different *Bnpsy* genes. Using this sequence information, we plan to further investigate the copy number of this *psy* gene family using DNA-SSCP. In addition, we will characterize the gene expression profile of each of the identified copies using RT-PCR and cDNA-SSCP. If a *psy* gene is preferentially expressed in seed, our efforts should be focused on manipulating the expression of that particular copy. This knowledge will aid in the future development of transgenic and conventional *B. napus* cultivars capable of producing carotenoid-enriched oil.

Funded by FONDECYT 1090726.

46.

Evaluation of the functionality of *Daucus carota* phytoene synthase and lycopene β-cyclase genes by means of heterologous complementation.

Juan Camilo Moreno, Romina Carvajal and Claudia Stange.

Carotenoids are isoprenoid compounds found in plants, algae, some yeasts and bacteria. In plants, they are synthesized in plastids. Several enzymes are involved in their biosynthesis such as phytoene synthase (PSY) and lycopene β-cyclase (LCYB). PSY, catalyzes the first step of carotenoid biosynthesis, representing a key step in this pathway, whereas LCYB transforms lycopene into β-carotene, a vitamin A precursor with strong antioxidant characteristics. In carrot (*Daucus carota*), two *psy* genes (*psy1* and *psy2*) and lcyb genes (*lcyb1* and *lcyb2*) have been reported. We determined that during root development, the expression of *psy2* is higher than *psy1 and lcyb1* presents the highest increase in expression levels throughout plant development. Moreover, post-transcriptional gene silencing of *lcyb1* results in a decrease in total carotenoid levels, indicating that it participates in the carotenoid synthesis. In this study, the direct functionality of the genes *psy2* and *lcyb1* from *D. carota* was evaluated through a heterolog expression system. Two constructions containing *psy2* and *lcyb1* genes in an expression vector (pETBlue1/psy2 and pETBlue1/lcyb1), were generated. These constructions were used to complement *E. coli* strain BL-21 pBAD/ΔCrtB and pBAD/ΔcrtY, respectively, which contain the complete *Erwinia uredovora* carotenogenic pathway. The *crtB* and *crtY* genes from *E. uredovora* correspond to *psy2* and *lcyb* from *D. carota* and were mutated in the stains used. Wild type BL-21/pBAD generate yellow colonies due to the production of lutein. The complementation reaction generated yellow colonies, indicating that carotenoids were synthesized. This was also corroborated by HPLC analysis. Therefore, we prove that both genes code for functional PSY2 and LCYB enzymes. These results can be used in biotecnological application to improve vitamin A content in plants.

47.

Molecular strategies to study the function of lycopene β-cyclase genes (*lcyb1* and *lcyb2*) in the biosynthesis of β-carotene in *Daucus carota* (carrot).

Nallatt Ocarez, Carolina Rosas, Juan Camilo Moreno, Lorena Pizarro and Claudia Stange.

Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

Carotenoids are isoprenoid pigments involved in abscisic acid synthesis, photosynthesis and photoprotection in plants. β-carotene, the main carotenoid present in carrots, is precursor for vitamin A. Several enzymes are involved in their biosynthesis such as lycopene β-cyclase (LCYB), which catalyzes the convertion of lycopene into β-carotene. In some plants there is more than one gene coding for LCYB enzyme. In tomato, *lcyb* and *cyc-b* genes are differentially expressed in leaves and fruits. Pepper harbours *lcyb* and *ccs* genes, where the last one encodes an enzyme that also has capsorubin-capsanthin synthase (CCS) activity responsible for the synthesis of capsanthin and capsorubin. In *Daucus carota*, two *lcyb* genes have been described (*lcyb1* and *lcyb2 or ccs*). Post transcriptional gene silencing (PTGS) of *lcyb1* gene in carrot diminished β-carotene levels in leaves and the modified root. Phylogenetic and aminoacidic analysis show that *lcyb2* gene is linked with tomato *cyc-b* and pepper *ccs,* however carrot does not synthesize capsanthine or capsorubine, suggesting that LCYB2 should have LCYB instead of CCS activity. For these reasons**,** we evaluated if *lcyb1* and *lcyb2* genes of *D. carota* have LCYB function. Semicuantitative RT-PCR indicated that both genes are expressed in leaves and the modified root of 12-week old plants. *In vitro* functional analysis of *lcyb1* and *lcyb2* genes made from the results of heterologous complementation of BL-21/ΔCrtY *E.coli* strains, correlated with the expression of those genes. *In vivo* analysis performed by simultaneous PTGS of *lcyb1* and *lcyb2,* showed that reduced levels of both transcripts, are associated with a phenotype characterized by reduced size, weakness and leaf damage in the transgenic plants.

Anknowledgement to Fondecyt 11080066.

48.

Expression of the heat shock genes, *hsp70, hsp100* and *ubiquitin* and accumulation of HS-proteins in plants of *Aloe barbadensis* Miller, under heat stress and induced thermotolerance.

***Huerta C.,** and Cardemil L.

Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

Aloe barbadensis Miller, also known as Aloe vera, is well adapted to arid regions with extreme thermal oscillations. Aloe vera gel is important for cosmetical, pharmaceutical and alimentary properties. For these reasons, the plants are cultivated in the III and IV Regions and is planned to be introduced in the I Region. We have studied the Aloe vera responses to heat stress by determining the level of expression of *hsp70, hsp100* and *ubiquitin genes* by semi quantitative RT-PCR using primers designed against conserved regions of orthologous genes from plants close to Aloe vera, and by determining the level of accumulation of proteins encoded by these genes by western analyses using antibodies raised against other plants HSP. Both mRNA and protein levels increased only when plants were incubated to 40º or to 45ºC without increase in the levels at temperatures between 25-40°C. This seems to indicate that sublethal medium-high temperatures are not stressful for Aloe vera. For induced thermotolerance experiments, plants were incubated to sublethal temperatures of 35º or 40ºC for 2 hours, and then to the lethal temperature of 45ºC for other 2 hours. Semiquantitative RT-PCR analysis for the *hsp70* demonstrated that this gene is expressed more than in control plants which were not subjected to a previous sublethal temperature of 35° or 40°C. The results of our research indicate that Aloe vera plants can tolerate the desert temperatures and that this species could be considered as a model plant for heat shock tolerance studies in a near future, since the Earth temperature is raising. This research was funded by FONDECYT 1070899 and MULT 05/30-2 of Dirección de Investigación, Universidad de Chile.

49.

Effect of temperature of *psbA* gene expression in cyanobacteria with targeted mutation in the D1 protein.

[(1+)] **María José Arismendi,** [(2)]Jorge Dinamarca, [(1,3)] Manuel Gidekel, [(1)]Ana Gutiérrez Moraga*

[1]*Facultad de Ciencias Agropecuarias y Forestales, Departamento de Producción Agropecuaria. Universidad de La Frontera. Temuco, Chile.*
[2]*Weizmann Institute of Science, Rehobot, Israel*
[3]*Venturel@b. Escuela de Negocios, Universidad Adolfo Ibañez, Santiago, Chile*

The constant rate and conversion efficiency of solar energy and biomass production by photosynthetic organisms is restricted to a narrow temperature range defined by its native habitat. Mesophilic organisms show a constant maximum around 20-30°C on the other and thermophilic varieties get constanst arround 60-70°C.
The reaction center of photosystem II has a key role in the acclimation of the energy conversion constants at room temperature. It has been discovered previously a motif of specific sequence GxxxG type and cavities in the D1 protein of PSII RC that shows differences between mesophiles and thermophiles. Plus, a mutation has been identified inside this motif that may adjust the conversion of photosynthetic energy and change the maximum photosynthetic constant to higher temperatures.
In this work we used strains with a mutation to a specific motif in the α-helix D of D1 protein from the mesophilic *Synechocystis sp*. PCC6803 that generate stable organisms at elevated temperatures and significantly broader than in the native strain. By real time PCR was determinated the effect of temperature increase in the expression of the gene *psb*A, proving that the mutation behave just like the thermophilous Cys-Ala motif on D1 209-212 produce stable organisms at high temperatures much larger than in native organisms.

Supported: FONDEF project (D03I1079), [+]CONICYT Scholarship Programa Doctorado en Ciencias mención Biología Celular y Molecular Aplicada, Universidad de La Frontera and Uxmal S.A. in Venturel@b, Universidad Adolfo Ibañez, Santiago, Chile.
***Corresponding author: hgutier@ufro.cl**

50.

Cold acclimation and photoperiod influences on Sucrose and total soluble sugars (TSS) accumulation in *Colobanthus quitensis* under laboratory conditions.

Cynthia Retamal [1], Marely Cuba-Díaz [2], Luisa Bascuñán-Godoy [1] y León Bravo [1].

1. Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas;
2. Departamento Ciencias Básicas, Sede Académica Los Ángeles,
Universidad de Concepción.

Colobanthus quitensis (Kunth) Bartl. (Caryophyllaceae) is one of two unique vascular plant that have naturally colonized the Maritime Antarctic, it has physiological and biochemical properties that determine its survival under harsh natural conditions. Some recent studies have considered *C. quitensis* as a cold-resistant plant which accumulate high levels of sucrose after cold acclimation. We have a wider project where the main objective is to study cold acclimation under long and short day photoperiods on SPS activity and protein expression and their relation with sucrose accumulation in the laboratory. These result are part of this project, the principal objective is to establish sucrose and total soluble sugars (TSS) accumulation along 21 days of cold acclimation and how is it influenced by the photoperiod. Preliminary results indicated that cold acclimation increase sucrose content independently of the photoperiod. During long photoperiod (21/3) cold acclimation, sucrose accumulation was slightly higher than during short photoperiod (8/16) cold acclimation. There was not differences detected in the sucrose accumulation in plant cold acclimated and non acclimated at short day photoperiod. There was not observed significant variation in TSS among different treatments. There was a significantly higher sucrose content in roots (60% higher content than in leaves). These preliminary results are very interesting because *C. quitensis* develop it major vegetative growth during antarctic summer, where temperature and photoperiod conditions are similar to cold acclimation laboratory conditions for cold acclimation under long days.

Acknowledgements: This work was supporter by Proyecto DIUC Nº *208.112.044-1.0, Dirección de Investigación Universidad de Concepción.*

51.

Identification of genes related to physiological disorders during cold storage of 'hass' avocados (*Persea americana* MILL.)

González-Agüero, M.[1,2], Gudenschwager, O.[1], Manriquez D.[1] and Defilippi, B.G.[1,2].

[1]Instituto de Investigaciones Agropecuarias, INIA-La Platina.
Casilla 439-3, Santiago
[2]The Plant Cell Biotechnology Millennium Nucleus (PCB-MN)
Email: maugonzalez@inia.cl

The 'Hass' avocados (*Persea americana* Mill.) is one of the most important fruit crops cultivated worldwide, and is particularly relevant for Chilean producers and exporters. A long-term cold storage transit (20 to 45 days) is necessary to reach the final markets, and is a key issue to reduce fruit deterioration. However, the development of physiological disorders induced by low temperatures can strongly affect fruit quality. In this work we show that "Hass" avocados harvested in September-October (spring time in Chile) and stored for 30-40 days at 5°C show less disorders than fruits harvested in November-January (early summer). As a first approach to understand the biochemical processes or mechanisms affected by both harvest time and low temperature storage, a forward Suppression Subtractive Hybridization (SSH) cDNA library was constructed for identifying differentially expressed genes. Until now, near to 800 differentially expressed clones have been isolated and sequenced. We identified and characterized several genes that codify for key enzymes involved in different mechanisms and process, i.e. sugar and lipid metabolisms. RACE-PCRs were conducted to obtain full length cDNAs and real-time PCR to characterize the gene expression profiles. The significance of the changes measured from 'Hass' avocados of two harvest stages is discussed (Funded by Fondecyt 11080236).

52

Raffinose Family Oligosacharides in lupin: possible role in plant defense against environmental stress and characterization of genes coding for a key enzyme in their regulation.

Mónica Mathias [1], Ivan Maureira [1], Alejandra Zúñiga [2], and Véronique Amiard [1,3].

[1] *Centro de Genómica Nutricional Agro-acuícola, Unidad de Biotecnología de Plantas, Instituto de Investigaciones Agropecuarias INIA-Carillanca, Casilla 58-D, Temuco, Chile.*

[2] *Laboratorio de Fisiología vegetal, Facultad de Ciencias, Universidad Austral de Chile.*

[3] *Corresponding author, E-mail: veroniqueamiard@cgna.cl.*

Raffinose Family Oligosacharides (RFOs) are soluble carbohydrates abundantly accumulated in seeds of legume species, especially in the lupin genus (*Lupinus* sp.) where they reach 5 – 16% of the grain dry matter. The RFOs lead to flatulence, and reduce the assimilation of nutrients in monogastric animals limiting the use of lupin as a protein source. Raffinose, stachyose, verbascose, and ajugose, are RFOs with important functions in the plant fitness of several species. Galactinol synthase (GolS) is regarded as a key enzyme in the regulation of these sugars in the plant. Thus, the determination of physiological roles and genetic regulation of RFOs is needed in order to explore the possibility of reducing their content in lupin seeds. In the present work the concentration and distribution of RFOs was studied under stress conditions in four lupin species: *L. luteus*, *L. mutabilis*, *L. angustifolius*, and *L. albus.* Two experiments, one cold stress and other drought stress, were carried out using a split plot statistical design. Cold stress was imposed for a period of 24 hours at 4°C; while for drought, irrigation was suspended during 14 days. Sequences of *GolS* genes in lupin were isolated by PCR using degenerated primers in base to sequences of other legume species. The results suggest that raffinose and sucrose participate in the stress response of lupin, showing different patterns of evolution under these stresses. The expression study of two different *GolS* genes isolated in lupin will allow us to establish their contribution in the genetic regulation of RFOs during stress.

Key words: Lupinus – RFOs – Galactinol synthase – Environmental stress.

53.

Identification and characterization of a *dreb2* gene from *Eucalyptus globulus* (*Egdreb2).*

Fabiola Baltierra[2], Erwin Krauskopf[1,2].

[1]*Departamento de Cs. Biológicas, Universidad Andrés Bello, MIFAB, Santiago.*
[2]*Fundación Ciencia Para la Vida, Zañartu 1482, Santiago.*
Email: ekrauskopf@unab.cl

Dreb genes belong to a family of transcription factors that interact with a *cis*-acting dehydration-responsive element (DRE), activating the expression of downstream genes involved in abiotic stress response in plants. In this work we characterize a *dreb2* gene involved in osmotic and heat stress response in *Eucalyptus globulus.* A cDNA encoding a DREB2 protein was identified in an *E. globulus* cDNA library. We amplified and sequenced the coding region from genomic DNA, establishing that *Egdreb2* is an intron-less gene of 1011bp in length. The predicted 337aa protein has an ERF/AP2 DNA binding domain that characterizes the DREB protein family, exhibiting high sequence identity with *dreb2* genes from *Gossypium hirsutum, Glycine max* and *Zea mays.*In addition, gene expression was studied by RT-PCR using total RNA extracted from *E. globulus* seedlings subjected to heat stress. Preliminary results show that *Egdreb2* transcripts are present throughout the different time periods of heat stress, not revealing a significant difference with the transcription level found in unstressed seedlings. We are currently working on expression studies under different stress conditions like salinity and drought.

Funded by PFB-016, MIFAB and DI/UNAB

54.

Project progress: Detection of QTL analysis and candidate genes associated with the use of nutrients and resistance to water stress in Solanum tuberosum by associative mapping.

Macarena Barra J [2], Boris Sagredo D [1,3], Julio Kalazich B[1]

1 Centro Regional de Investigación INIA-Remehue. Laboratorio de Biotecnología.
2 Universidad de La Frontera. Programa de Doctorado en
Ciencias Mención Biología Celular y Molecular.
3 Correspondiente autor, E-mail: mbarra@ufro.cl.

Global climate change brings with it a rise in average temperatures, changes in precipitation patterns and an increased frequency of extreme weather events. Along with this, degradation and low soil fertility lead to a drop of water and nutrients, respectively, resulting in a serious constraint on plant growth. Cultivated species of Solanum tuberosum are affected by this problem because they are vulnerable to abiotic stress. However, the genetic potential of chilean potato germplasm associated with the use of nutrients and resistance to water stress, remains unknown. This study focuses on exploring this potential, comparing a population of high selection pressure versus the population of moderate / low pressure. To achieve this, 200 genotypes of potato from the breeding program INIA – Remehue will be evaluated. Physiological analysis will be conducted to determine differences in nutrient use and water stress resistance using in vitro and greenhouse plants. Candidate genes and it´s sequences will be searched screening databases and scientific papers and then the alleles present in the population will be identified by SSCP (Single Strand Conformational Polymorphism). Finally a wide range of markers will be determined, using high-density DNA markers such as DArT (Diversity Array Technology) and microsatellites (SSRs).

Keywords: Climate change, water stress, nutrient use, Solanum tuberosum.
Acknowledgements
We thank INIA-Remehue´s Potato Breeding Program (Osorno, Chile) and Universidad de La Frontera (Temuco, Chile).

55.

Identification and characterization of drought tolerance genes in Quinoa, Potential use in drought-sensitive crops

Andrea Morales[1,2], Andres Zurita[1] and Herman Silva[2]**.**

1. Centro de Estudios Avanzados en Zonas Áridas, CEAZA (Consorcio ULS-UCN-INIA)
2. Laboratorio de Genómica Funcional & Bioinformática Vegetal and Millennium Nucleus in Plant Cell Biology and Biotechnology. Universidad Andres Bello

Chenopodium quinoa is an important grain crop from the Andean region of South America. Recently, quinoa has gained international attention for its high nutritional value and tolerance to several abiotic stresses. Field determinations had shown that quinoa is tolerant to drought. The genes involved in this process might be used as biotechnological tools to modify agronomic traits for cultivar breeding, especially in crops sensitive to drought such as grapevines. We are developing a platform to unravel quinoa genes involved in tolerance to drought. The strategy is based in the generation of quinoa cDNAs subtractive libraries from plants subjected to drought. These cDNAs will be used to transform *Arabidopsis thaliana* and look for drought-tolerant transformants due to the expression of transgenes. Candidate genes that confer drought tolerance in Arabidopsis will be further characterised and inserted into grapevine plants, to demonstrate the potential of novel genes from this ancient crop into economically relevant species. To test the feasibility of this strategy we had cloned a vacuolar Na^+/H^+ antiporter (NHX) from quinoa, which is being transferred to Arabidopsis to test whether this gene is able to confer salinity tolerance in over-expressing transgenic plants. In parallel to the drought screening of Arabidopsis transformants with quinoa genes, a set of 200 differentially expressed transcripts will be sequenced. From their functions, a metabolic pathway assembly will be constructed by comparing these sequences to other reported plant sequences in response to drought stress.

Acknowledgments: Project BioTecZA /Innova 06FC01IBC-71 and CONICYT Fellowship D-21080654 to AM.

56.

Simple and combined effects of salinity and nutrients on germination and vigor of ten ecotypes of quinoa (*Chenopodium quinoa* Willd.)

Cortés, D.[1], Navarro, J. [1], Coulibaly, A.K. [2], Payacan, S. [1], Luna, G.[3], León, P. [4], Zurita, A.[5,] Martínez, E.A.[5].

[1]: Departamento de Agronomía, Universidad de La Serena, La Serena, Chile
[2]: Instituto Politécnico Rural de Katibougou, Mali
[3]: Universidad Católica del Norte
[4]: Banco Base de germoplasma, Instituto de Investigaciones Agropecuarias (INIA, Intihuasi), La Serena, Chile
[5]: Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile

Salinity is a major problem in many zones of the world, affecting in Chile mainly the soils of northern arid lands. One alternative to face the problem is to identify tolerant crop species and ecotypes, such as Quínoa. This crop is intendend to be re-introduced en the arid region of Coquimbo, Chile (30ºS). It seeds have a great nutritive value, for ecotypes with high genetic diversity and rusticity. This allows its cultivation in saline, arid lands, often submitted to drought. Besides to low temperatures and nutrient-limited soils. The objective of this work was to evaluate the behavior of ten ecotypes, from contrasting regions of the country respect to a salinity gradient, combined with two nutrient levels. Germination (until 120 hours) and length of stems and roots (vigor, until day 11) was evaluated. Seeds came from a range of 2 thousand kilometers and salinities were 0 and 0,4 M (NaCl). In a second essay nutrients were added keeping the same saline concentrations. Results show strong differences among ecotypes, with significantly different negative effects of high salinity on germination and vigor. Nutrients addition increased germination speed and vigor. This was observed for all ecotypes. Ecotype R-49 (from high Andes), Palmilla and Javi (from saline lowlands of central Chile) did show the best responses to adverse conditions while BO25 (from more rainy southern Chile) was the most affected for all treatments.
FUNDING: Projects Innova Chile, CORFO 04CR9PAD04, FONDECYT 1060281. TWAS-ICGEB.
Keywords: salinity, germination, vigor, root growth, nutrients, arid region.

57.

Development of salt tolerant rootstock derived from *Vitis champini* segregants

P. Flores[1], D. Prehn[1], J. Pérez-Harvey[1], A. Zurita[2]

[1]Departamento de Fruticultura y Enología, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Avda. Vicuña Mackenna 4860, Macul, Santiago, Chile.
[2] Centro de Estudios Avanzados de Zonas Áridas
Dirigir correspondencia a D. Prehn y P. Flores: dprehn@uc.cl, pflores@ uc.cl

In the regions of Atacama and Coquimbo saline soils represent 30.000 has. In this area vineyards take up 31.000 has and are increasing its surface. The use of salt-tolerant rootstock could expand the area of vineyards in the affected areas. One of the most salt tolerant rootstocks is Ramsey or Salt Creek (*Vitis champini*). To increase the supply of suitable rootstocks for the north of Chile we evaluated salt tolerance in a population derived from Ramsey. A selection was made under different conditions of salt stress both *in vitro* and in the greenhouse in order to identify transgressive segregants. Ramsey and 9 descendants, previously selected for their ability to germinate in saline medium were propagated *in vitro* in modified WPM medium. Explants were rooted before being distributed randomly to treatments of 0, 30, 75 and 200 mM NaCl in WPM medium. The experiment was replicated in the greenhouse with 30 cm tall plants potted in peat moss and watered twice a week with equivalent salt concentrations. Salt tolerance of genotypes was assessed by shoot growth, root growth and damage of leaf area. Responses between treatments and genotypes showed significant differences ($p < 0.01$) *in vitro* when compared to the control. Shoot growth decreased 18, 40 and 48% at 30, 75 and 200 mM NaCl, respectively. Root growth also decreased with increasing salt concentration and the damage of leaf area reached 94% at the highest salt dosage. Genotypes treated in the greenhouse responded in a similar way. 75 mM NaCl was the threshold that allowed to discriminate tolerant genotypes. Four lines exhibited a tolerance to salt equal or superior to Ramsey, nevertheless differences in the response of the replicates as well as in Ramsey were detected in all the treatments.

58.

Development and evaluation of salinity tolerant lines of tomato (*Solanum lycopersicum* L.)

María Fernanda Álvarez Viveros & Patricio Arce-Johnson.
Pontificia Universidad Católica de Chile.

Biotic and abiotic stress has a substantial impact on plant growth and development. The understanding of physiological processes and tolerance mechanisms in plants under environmental stress has an immense importance to agriculture. Intra and inter-specific salinity tolerance differences lead to determine that this characteristic possesses a strong genetic base. Salinity tolerance has a multigenic nature. Nevertheless, in some cases transference of only one gene is enough to obtain tolerant plants. An important case of progress via biotechnology is the use of the glyoxalase system, which is important in methilglyoxal detoxification, a mutagenic and highly citotoxic compound. Overexpression of glyoxalase system genes enhances plant salinity tolerance and allows them to complete their life cycle and produce viable seeds under salt stress conditions without reducing their yield. The aim of this work is to generate transgenic lines of tomato (*Solanum lycopersicum* L.) tolerant to salinity by overexpression of glyoxalase system. Tomato plants were genetically transformed via *Agrobacterium tumefaciens*. Transgenic lines were analyzed and verified by PCR, RT-PCR and Real Time PCR. For further investigation, these plants will be characterized at physiological, morphological and chemical level.

Acknowledgments to Vegetal Science Department's Scholarship. Agronomy and Forest Engineering Faculty, Pontificia Universidad Católica de Chile.

59.

Glutaredoxin GRXS13 Modulates Abiotic Stress Tolerance in *Arabidopsis*

Salazar Marcela, Laporte Daniel , Olate Ema, Montero Trinidad and Holuigue Loreto

Pontificia Universidad Católica de Chile, Santiago, Chile. msalazari@uc.cl

Glutaredoxins (GRXs) are small ubiquitous redox proteins involved in theregulation of numerous target proteins via thiol/disulfide exchanges and therefore play key roles in the maintenance of cellular redox homeostasis. In *Arabidopsis*, 30 genes encoding GRXs and GRX-related proteins have been identified. In addition to their role in reducing oxidized thiols from proteins, GRXs can play a fundamental role in adapting organisms to external stresses. One of the genes activated by oxidative stress corresponds to *AtGrxS13*, coding for the monothiolic glutaredoxin S13. Results from our group indicate that *AtGrxS13* is induced by treatment with methyl viologen (MV) and by salinity stress. To investigate the biological roles of the *Arabidopsis thaliana GRXS13* gene in response to abiotic stress, we have obtained homozygous *Arabidopsis* transgenic lines that overexpress and silence *AtGrxS13* gene and analyzed their responses to salt and MV stress. Interestingly, lines silenced in *AtGrxS13* show a higher susceptibility to stress by MV and high salinity treatments than wild-type plants, which suggest a role for this gene in defense to abiotic stress. To evaluate the mechanism of transcriptional activation of *AtGrxS13* by stress, a 1.5-kb fragment of the gene promoter was isolated and analyzed *in silico*. In this region a considerable number of stress-responsive elements were found, which strengthen our proposition for a function of this glutaredoxin in abiotic stress.

Financed by Fondecyt-Conicyt (grant No. 1060494), Programa Bicentenario de Ciencia y Tecnología (PBCT PSD-74) and Millennium Nucleus for Plant Functional Genomics (P06-009-F).

60.

Analysis of the *DaGrx* gene using *Arabidopsis thaliana* under saline stress conditions.

[(1)]Felipe J. Llancapán, [(1)+] Jennifer P. Osorio, [(1) (2)] Manuel Gidekel and [(1) *]Ana Gutiérrez-Moraga.

[1] *Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera, Temuco, Chile.*

[2] *VentureL@b. Escuela de Negocios, Universidad Adolfo Ibáñez, Santiago, Chile.*

Soil salinity is becoming increasingly more common in agriculture and crop fields are being lost as a result. The aim of this study was to discover if the *DaGrx* gene is able to support saline stress using *Arabiodopsis thaliana* modified with this gene. For growth and development, Murashige and Skoog medium were used and, 7 or 15 days after germination, the plants were transferred to other media with saline solution (NaCl). Salt tolerance, fresh weight, root elongation, ROS (reactive oxygen species) production and the percentage of germination were subsequently measured. The transformed plants were better in the ROS experiment with less species production. The same results were found with root elongation and germination at the initial measurement; however, in the fresh weight experiment at 17 and 20 days, the wild plants fared better than the transgenic plants with significant differences between them. The same results were obtained at the second and third measurements of the elongation experiment. Therefore, this gene may be functional early on under stress conditions but after few days is ineffective under saline conditions. Nevertheless, in the evaluation at 45 days under salt conditions, the plants are no differents and in the results from previous investigations show this gene is effective under cold stress. With these results, it was concluded that this gene was not more tolerant than normal plants over time and is not a good option under this kind of stress.

This work was supported by FONDEF project (D03I1079), [+]CONICYT Scholarship Programa Doctorado en Ciencias mención Biología Celular y Molecular Aplicada, Uxmal S.A., "Convenio de Desempeño I y II" Universidad de La Frontera (DI08-1001, DI08-2002, respectively) and Applied Molecular Biology Laboratory, Universidad de La Frontera, Temuco, Chile.

* Corresponding author: hgutier@ufro.cl

61.

Boron toxicity responses in Quinoa (*Chenopodium quinoa*Willd.) seedlings.

Susana Lizardi[1], Adriana Covarrubias[1], Rodrigo I. Alvarez[1], Karina Ruiz-Carrasco[2], Fabiana Antognoni[2], Stephania Biondi[2], AmadouK. Coulibaly[3], Hernán P. Burrieza[4], Sara Maldonado[4], Viviana R. Ordenes[1], Enrique A. Martínez[1] and Andrés Zurita-Silva[1]*

[1]Laboratorio de Biología Molecular Vegetal, Centro de EstudiosAvanzados en ZonasÁridas CEAZA, Benavente 980, La Serena, Chile.
[2]DipartimentodiBiologiaEvoluzionisticaSperimentaleetFacoltadiAgraria, Universitàdi Bologna, Italy
[3]Universite' du Mali IPR / IFRA Katibougou, Mali.
[4]Facultad de Ciencias Exactas y Naturales- Universidad de Buenos Aires, Argentina
** Corresponding author: andres.zurita@ceaza.cl*

Boron (B) is an essential micronutrient for vascular plant growth and development. The physiology of boron tolerance and boron toxicity is not fully understood. At first glance it seems that the optimum B level for one species could be either toxic or insufficient for other species. Genetic variation in response to high B concentrations has prompted research into the mechanism operating in plants against B excess.
Quinoa is considered one of the few crops naturally adapted to extreme climatic and geographical conditions typical of arid or semi-arid regions. Six different Chilean ecotypes of quinoa collected along a latitudinal gradient of ca. 2700 kilometres were evaluated in their tolerance to boron toxicity.Germination assays were performed at increasing boron concentrations, resulting that only ecotype BO78 (a southern Chile'slandrace) was most sensitive to boron toxicity stress. The other 5 ecotypes displayed no significant effectsat increasing concentrations of H_3BO_3. We also assayed boron-induced modifications both on growth and development, biochemical parameters and geneexpression, resulting that quinoa (*Chenopodiumquinoa*Willd.) plants exhibited tolerance to toxic boron levels.

Funded by ICGEB-TWAS Joint Plant Biotechnology Programme, project CRP.PB/CHI06-01

62.

Deregulation of *ATEXLA3* increases boron tolerance in*Arabidopsis*.

Darwin J. Mutarello-Wuillans[£], Nia Oetiker[*], Mauricio Toro-Nahuelpan[*],RodrigoAlvarez[£],Viviana R. Ordenes[£ς] andAndrés Zurita-Silva[£ς].

(£)Laboratorio de Biología Molecular Vegetal, Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile; (ς) Centro de Biotecnología para el Desarrollo en Zonas Áridas (BioTecZA) La Serena, Chile; (*) Licenciatura en Bioquímica, Facultad de Química y Biología, Universidad de Santiago de Chile.

ATEXLA3 (*Arabidopsis thaliana* expansin-like 3gene), which codifies for a protein implicated in cell wall modification was studied using an insertion mutant line (SALK_146621).We have already demonstrated that mutant line shows overexpression of *ATEXLA3*gene, whose T-DNA insertion was integrated into 3'UTR.To find out differences between wild type (wt) and mutant transcript, we carried out a 3' RACE. Indeed, *atexla3* mRNA carries a DNA fragment that corresponds to T-DNA left border. This sequence is co-transcribed and interestingly, both mutant and wt transcripts have the same length at 3' UTR. Overexpression of *ATEXLA3* leads to an enhanced tolerance to metalloids and transition metals. In order to elucidate how deregulation of *ATEXLA3* expression promotes plant tolerance to these abiotic stresses, we carried out germination and growing assays in high Boron (B) levels (up to 40 mM H_3BO_3 added) and transition metal Cu^{2+} (1.6µMavailable). These assays demonstrated that mutants germinate earliest and grow better than wt plants in all treatments applied. There are two possible explanations about the effect of *ATEXLA3* deregulation. First, it has been demonstrated that expression of expansins are associated with endosperm weakening during seed germination. Then, *ATEXLA3* may participate in this process promoting radicle emergence. Second, borate cross-links two Rhamnogalacturonan-II molecules by ester bonds (dRG-II-B) on cell wall. Divalent metal ions such as Cu^{2+} bound to dRG-II-B complex. The metal binding is speculated to relate with structural stabilization of the dRG-II-B complex.

Acknowledgements: INNOVA-CORFO, 05CR11PAT-19and Centro de Biotecnología en Zonas Áridas – BIOTECZA, 06FC01IBC-71

63.

Physiological and molecular characterization of antarctic bacteria

Graciela Berríos[1+], Leticia Barrientos Díaz[2], Manuel Gidekel[3] and Ana Gutiérrez Moraga[1*]

1Laboratorio de Biología Molecular Aplicada, Facultad de Ciencias Agropecuarias y Forestales, Departamento de Producción Agropecuaria. Universidad de La Frontera.
2Facultad de Medicina, Departamento de Ciencias Básicas, Universidad de La Frontera
3Venturel@b. Escuela de Negocios, Universidad Adolfo Ibañez, Santiago, Chile

Microorganisms represent the largest reservoir of biodiversity yet to be described, and consequently offer a great potential for the discovery of new natural products. Many of the isolated microorganisms from the Antarctic have been derived from maritime environmental studies (sea ice o seawater) or from the semi-marine lakes of Vestfold Hills, McMurdo Dry Valleys and Larsemann Hills. Few studies have isolated bacteria in the soil environment of ice-free areas. *Deschampsia antarctica* is the only gramineae that has been capable of colonizing the Antarctic with its extreme climate and soil environment. In the present research, bacteria colonizing the rhizospheric soil of *Deschampsia antarctica* were isolated and characterized. The studies of the soil showed that it possesses a varied spectrum of psychrotolerant bacteria with an extensive, varied antibiotic resistance as well as a heavy metal tolerance. Based on the strain identification with the partial characterization of the16S rRNA gene, it can be observed that the majority of the isolates correspond to different *Pseudomonas* species, and species of the genus *Flavobacterium* sp. and *Arthrobacter* sp. Considering that less than 1% of soil bacteria can be isolated in pure culture, the isolated strains maintained in collection in this research constitute a unique collection for future, more detailed taxonomic analysis and physiological characterization as part of the search for potential biotechnological uses. This and other findings have a great potential to develop new biotechnological products from Antarctic microorganisms.

Supported: INACH 0301; DIUBB 055209 2/ R 2005; Vitrogen S.A. +CONICYT Doctorate Scholarship, Programa Doctorado en Ciencias m/Biología Celular y Molecular Aplicada.

*** Corresponding author: hgutier@ufro.cl**

64.

Identification of aluminium-regulated genes by cDNA-AFLP in blueberry (*Vaccinium corymbosum* L.)

Inostroza-Blancheteau, C.[1,2], Aquea, F.[2], Reyes-Díaz, M.[3,] Alberdi, M.[4], Mora, M.L.[4], P. Arce-Johnson P[2*].

1Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Casilla 54-D, Temuco, Chile.
2Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile, e-mail: parce@bio.puc.cl.
3Instituto de Agroindustria, Universidad de La Frontera, Temuco, Chile.
4Departamento de Ciencias Químicas, Universidad de La Frontera, Temuco, Chile.

Blueberry (*Vaccinium corymbosum* L.) in Chile is mainly cultivated in acid soils, being the aluminum (Al) toxicity the major limiting factor for crop productivity in these soils. The major symptom of Al toxicity is a rapid inhibition of root growth. Several Al-regulated genes have been identified in the roots of different plant species. To investigate the molecular bases of Al toxicity and Al tolerance of blueberry, cDNA-amplified fragment length polymorphism (cDNA-AFLP) was used for identifying Al-regulated genes in roots of an Al-tolerant genotype, Brigitta, and an Al-sensitive one, Bluegold. One year old seedlings of both genotypes were transferred to hydroponic medium supplemented with 0 and 100 µM of AlCl3. Root samples were taken at 0, 2, 6, 12 and 48 hours of treatment and frozen to until RNA extraction. Selective amplifications with 16 primer combinations allowed the visualization of about 2200 transcript-derived fragments (TDFs), 87 of which (4%) were differentially expressed between cultivars or Al treatments. These TDFs, named VCAL (*Vaccinium corymbosum* Aluminum), were selected, sequenced and their homologies sought in the databases. Among TDFs identified in tolerant cultivar, three of them codified for genes associated with oxidative stress, a glutation-S-transferase, calmodulin and Vp2 vacuolar H+-pyrophosphatase. The molecular characterization of these genes could be important as potential candidates for Al resistance and oxidative stress resistance in blueberry.

Supported: Millennium Nucleus for Plant Functional Genomics, Fondecyt-1080372.

65.

Establishing a platform for molecular characterization of genes involved in plant heavy metal tolerance

Kanda Alfaro[*], Darwin J.Mutarello-Wuillans[£],Rodrigo Alvarez[£], Gina Arancio[†], Andrés Zurita-Silva[£ς], Viviana R. Ordenes[£ς].

(£)Laboratorio de Biología Molecular Vegetal, Centro de EstudiosAvanzados en ZonasÁridas (CEAZA); (ς)Centro de Biotecnologíapara el Desarrollo en ZonasÁridas (BioTecZA) La Serena, Chile;(†)Universidad de La Serena, La Serena, Chile; (*)IngenieríaAgronómica, Universidad de Aconcagua, La Serena, Chile.

The main economic activity of the Coquimbo Region is mining industry. During the past decades there has been developed several mining projects that have generated tailings waste piles accumulated in multiple locations throughout the region.
Despite the high levels of heavy metals contained in such mining dumps, it appears that some species of native and introduced flora can grow and develop in this substrate considered highly toxic. These plants are adapted to metal stress and possess interesting homeostatic mechanisms that we are starting to study based on a gene-hunting strategy.
As first step, we sampled mining dump located in Andacollo (southeast of La Serena, Coquimbo) and identified the following species growing on mining dumps: *Baccharislinearis*, *Tessariaabsinthioides*, *Atriplexnummularia* and *Acacia caven*. We performed an analysis of zinc, iron and copper content in plant samples of these species and found that some of them accumulated high levels of metals compared with control plants. Our main aim is to identify plant genes involved in resistance and tolerance to heavy metal, in particular those that promote bioaccumulation.

Acknowledgements: INNOVA-CHILE Bioacumulación - 08CM01-12 and Centro de Biotecnologíaen ZonasÁridas – BIOTECZA, 06FC01IBC-71

66.

First insights into genetic diversity and phylogeography of *Nothofagus dombeyi* (Mirb.) Oerst. in Chile

Alexandra Stoll[1] & Frank Blattner[2]

[1] *Centro de Estudios Avanzados en Zonas Aridas (CEAZA),*
[2] *Taxonomy and Evolutionary Biology Institute of Plant Genetics and Crop Research (IPK Gatersleben), Alemania*
E-mail: alexandra.stoll@ceaza.cl

The pleistocenic South American ice ages marked a deep climatic impact on the austral South American region. Regarding Central-South Chile, they caused north-south directed plant migration resulting in the current distribution patterns, including an exceptional rate of endemism besides neotropical and antarctical elements. Identifying flora refuge areas and reconstructing migration pathways after the glacial retreat are two of the most challenging tasks within biogeography. Population genetics combined with plant sociology are the tools selected in this preliminary study regarding the genetic diversity of *Nothofagus dombeyi* (Mirb.) Oerst. (Coigüe) along its distribution area in Chile.
AFLP markers were applied to a total of 88 individuals from eleven populations to determine population structure. Until now Neighbour Joining and Mantel tests were used to analyse the genetic data. There was a relative low variation over the distribution area, but a very high variation within the populations. Mantel correlations between geographic and genetic distances were not significant. Nevertheless, in the Neighbour Joining tree shows very isolated groups for the coastal populations, e.g. Alerce Costero and Nahuelbuta Sector (2 populations). Also Andean populations in Conguillio and Villarica are group together, but indicating genetic exchange between them. An interesting situation is found for the coastal Qb. Pellin, which represents one of the northernmost Coigüe-populations in the coastal range. Qb. Pellin and strongly linked with Vilches, an Andean population.
Further details to those distribution patterns are expected by including Bayesian clustering and diversity and differentiation measures.
Thanks to the IPK Gatersleben for the possibility to realize the AFLP-analyses in their laboratories during July/ August 2009.

67.

¿Cuantas orejas tiene el zorro?
- Advances in taxonomical revision of the genus *Aristolochia* in Chile –

Alexandra Stoll[1], Steffen Hahn[2] & Lars Symmank[3]

[1] Centro de Estudios Avanzados en Zonas Aridas (CEAZA), [2] Jardín Botánico Universidad de Talca, [3] Institut für Botanik, Technische Universität Dresden, Alemania
E-mail: alexandra.stoll@ceaza.cl

Aristolochiaceae are distributed worldwide with the highest diversity in tropical and subtropical regions. This project is related to two of the southernmost *Aristolochia* species. *Aristolochia bridgesii* (Klotzsch) Duchr. and *Aristolochia chilensis* Bridges ex Lindl. (Oreja de zorro) are endemic in Chile, growing in the semi-arid and Mediterranean climate zone (Atacama to Metropolitan Region).
Findings in 1999/ 2006 extended the known distribution area of the genus *Aristolochia* in Chile to the Maule Region (250 km S). But the collected individuals and others from North Chile do not match with the description of Chilean *Aristolochia* species. Leading to the question, how many *Aristolochia* species exist in Chile? A combination of traditional taxonomy and phylogenetic methods was chosen to answer this question.
Morphological analyses indicate hybridization between the two known species. But this explanation is only applicable in the northern distribution area, where *A. bridgesii*, *A. chilensis* grow together, not for the populations in the Maule Region, where both parent species do not occur. To clarify the relationship within the *Aristolochia* genus in Chile, highly variable non-coding regions of the chloroplast genome (trnK-matK-Region) were sequenced. Eight accessions widespread over the distribution area were sampled. Phylogenies were constructed using maximum parsimony. Results from the first dataset (trnK-matK spacer) reveal a very close relationship between the Chilean accessions. *A. chilensis* accessions group with *A. bridgesii* as well as with accessions representing the populations from the Maule Region. This is interpreted as indicator for the existence of only one, highly variable *Aristolochia* species in Chile. The analyses of the whole trnK-matK-Region are expected to substantiate these preliminary results.

68.

Asymbiotic seed germination and *in vitro* seedling development of *Chloraea cristata* Lindl., an endemic Chilean orchid.

Marta Vargas S.[1], Ana Sandoval[2], Viviana Ordenes[1], Rodrigo I. Álvarez[1], Pedro León-Lobos[2], Phil Seaton[3] & Hugh Pritchard[3] and Andrés Zurita-Silva[1]

[1]*Laboratorio de Biología Molecular Vegetal, Centro de Estudios Avanzados en Zonas Áridas - CEAZA, Benavente 980, La Serena, Chile.*
[2] *Banco Base de Semillas. Instituto de Investigaciones Agropecuarias, INIA.*
[3] *Seed Conservation Department, Royal Botanic Gardens Kew*

Chloraea cristata Lindl. is an endemic Chilean orchid with both ornamental and ecological value. Procedures for asymbiotic seed germination and seedling acclimatization were developed for this species. Seeds were collected at Los Vilos, Coquimbo region. Three germination media (Murashige & Skoog, Nitsch & Nitsch and Gamborg 5) were tested for their effectiveness in promoting seed germination and protocorm development. Effects of active carbon on *in vitro* seedling development were also assessed. Germination and advanced seedling development only occurred on the Gamborg 5 (half-strength) medium with active carbon. We induced the early stages of development i.e. testa imbibition, embryo elongation, and protocorm formation after 3 weeks of culture. Protocorm rhizoids were observed after 5 weeks of *in vitro* culture. The emergence of the first leaves was observed after 18 weeks of culture. Asymbiotic orchid seed germination techniques have been used for the production of commercially important orchids, and also have been shown to be an efficient tool for the orchid production for conservation and sustainable use purposes.

Acknowledgements: Orchid Seed Storage for Sustainable Use Project (OSSSU), Darwin Initiative. Millennium Seed Bank Project, Royal Botanic Gardens Kew. Centro de Biotecnología en Zonas Áridas – BIOTECZA, 06FC01IBC-71

69.

Germination potential and vegetative propagation of *Guindilia trinervis* Gillies ex Hook. et Arn.

D. Prehn[1], M. Gebauer[1], M. Jordán[3], G. Parada[2] and R. San Martín[2]

1 Fac. Agronomía, P. Universidad Católica de Chile.
2 Dpto. Ing. Química y Bioprocesos, P. Universidad Católica de Chile.
3 Escuela de Biotecnología, Universidad Mayor.

Guindilia trinervis (*Sapindaceae*) is a frequent native evergreen shrub that grows in the Andes mountains above 1500 m.a.s.l. in the Central Region of Chile. It is also found at high elevations in the costal range. Research accomplished at the Universidad Católica de Chile showed that the fruits contain about 65-70% oil, suitable for biodiesel production. Preliminary estimates show that wild plants could produce about 500-600 L oil per hectare. Our hypothesis is that the yield of seeds can be improved by selecting elite plants and propagate them vegetatively in order to make the oil production more competitive. Vegetative propagation studies to achieve multiplication have not been reported so far and botanical description is scarce. In this investigation, different aspects of propagation were studied. Germination of seeds ranged from 78 to 99% after 20 days of incubation at 20°C and gave rise to seedlings when transferred to peat moss. Vegetative propagation by single node cuttings excised from 3 month old seedlings revealed a high rooting ability. Dipping the base of the explants for 15 min in a 50 mg l^{-1} 3-indol-butyric-acid (IBA) solution generated 85% of rooted explants with an average of 4,6 roots/explant. Sprouting was also favored by the IBA treatment. Rooted plantlets were transplanted to peat moss and kept in the greenhouse at room temperature. *In vitro* culture of shoot tips initiated roots in 25 to 82% of the cases. Roots formed at the basal end of the shoots producing plantlets when cultivated in ½ strength MS medium in the presence of 1.0 mg l^{-1} IBA and reduced sugar. Sub-apical nodal sections did not grow roots unless excised from *vitro* grown plantlets and recultured on fresh medium. Callus formed profusely at the base of most explants, especially when recultured. *In vitro* rooted plantlets could be acclimated succesfully. In this investigation we show that *G. trinervis* can be propagated asexually by cuttings *in vivo* as well as *in vitro*. Studies on vegetative propagation of field plants are underway.

70.

Jatropha: Development of a new oil crop for biofuel

G. Martínez, A. Fuente, C. Torres, C. Kremer, ,
K. Vázquez, F. Labra, F Medina, N. Magner, and M. Paneque.

Facultad de Ciencias Agronómicas. Universidad de Chile. Santiago. Chile

The interest in using Jatropha curcas L. as a feedstock for the production of bio-diesel is rapidly growing. The properties of the crop and its oil have persuaded investors, policy makers and clean development mechanism project developers to consider jatropha as a substitute for fossil fuels to reduce greenhouse gas emissions. However, jatropha is still a wild plant of which basic agronomic properties are not thoroughly understood and the environmental effects have not been investigated yet. Despite the interest that is being shown in the large-scale cultivation of jatropha, genetic resources remain poorly characterized and conserved as there has been very little plant breeding for improved traits. The aim of this study is to determine the potential of the plant for the production of biodiesel as a renewable energy source and to use the meal obtained after the extraction of oil as a source of animal food. We present the studies carried out to introduce the crop in Chile which include the identification of potential sites of adaptation, development of in vitro propagation methods and establishment of 10 pilot areas for the selection of elite clones. Financed by FIA-PI-C-2007-1-A-009

Contact: M. Paneque, Laboratory of Environmental Biotechnology and Bioenergy. Department of Environmental Sciences and Natural Resources. Facultad de Ciencias Agronómicas. Universidad de Chile, Santa Rosa 11.315, La Pintana, Santiago, Chile. Tel. (+56 2) 978 5863. Fax (+56 2) 978 5929. E-mail: mpaneque@uchile.cl.

71.

Design and use of a temporary immersion bioreactor to induce the phenylpropanoid pathways on *Deschampsia antarctica.*

Sequeida[1], A., Tapia[2], E., Prieto[4], H., Zamora[3], P., Castro[4], A., and Zuñiga[3], G.E

[1] *Biotechnological Engineering Department, Universidad Tecnológica de Chile INACAP, Santiago de Chile, Chile.*
[2] *Biotechnological Engineering Department, Universidad Técnica Federico Santa Maria, Valparaiso, Chile.*
[3] *Biology Department, Chemistry and Biology Faculty, Universidad de Santiago de Chile, Santiago de Chile, Chile (USACH).*
[4] *Biotechnology Laboratory, INIA – La Platina Station.*

Deschampsia antarctica, which is characterized by high levels of free endogenous phenylpropanoids derived phenolics compounds, is the only Gramminea endemic to the Antarctic territory. This work is the first report describing the exclusive design, use and evaluation of a temporary immersion bioreactor as a new high efficiency platform to improve biomass propagation, enhancing secondary metabolite production through UV-B light elicitacion. The design and implementation of the bioreactor significantly increased biomass growth of *D. antarctica* using six immersions per day of three minute duration as first operation conditions. At day 14 of culture, biomass was duplicated in relation to initial inoculum. Among the different UV-B elicitation treatments, best results were obtained with 0.5 h irradiance in 6 h intervals, in which total phenolics and antioxidant activity were increased more than 3 and 1.5 fold respectively. Once these results were obtained, the bioreactor was used in a Two Stage operational system, a first stage to obtain the optimal biomass concentration during 14 days of culture in absence of UV-B light, followed by 7 days of best induction treatment with UV-B light. Under these conditions, metabolite concentration and biological activity showed no significant difference, but the biomass was considerably maximized.

Funded by DICYT Universidad de Santiago de Chile.

72.

An *Arabidopsis thaliana* mutant in the UDP-glucose glycoprotein: glucosyltransferase, a key enzyme in quality control at the endoplasmic reticulum, shows a root phenotype.

Rodrigo Tapia, **Francisca Blanco**, Francisca Reyes and Ariel Orellana.

Millenium Nucleus in Plant cell Biotechnology, Center of Plant Biotechnology, Andres Bello University, Santiago, Chile

The presence of a single terminal glucose residue on N-linked oligosaccharides of newly synthesize polypeptides is used as a signal for folding assistance in the endoplasmic reticulum (ER) lumen. The enzyme responsible for the recognition of misfolded proteins and the addition of the glucose residue is the UDP-glucose glycoprotein: glucosyltransferase (UGGT). Thus, this enzyme is a key step in the quality control process that occurs in the ER to ensure the proper folding of proteins. Little is known about the role of this enzyme in multicellular eukaryotes and even less is known in plants. Therefore, we decided to analyze the role of this enzyme in the model plant *Arabidopsis thaliana.* First, we detected biochemically the UGGT activity and found that was present in ER enriched fractions from *Arabidopsis*. The analysis of N-linked oligosaccharides produced by UGGT, confirmed the specificity of the enzyme. The *Arabidopsis* genome contains only one putative gene encoding for this enzyme. Mutants in UGGT are smaller than wild type. Roots seem to be the more affected organ, coinciding with the higher content of the mRNA for UGGT. Microscopic analysis reveals that root architecture was altered. Moreover, mutant plants are less tolerant to stress. These results suggest an important role for UGGT in root cell elongation probably due to an alteration in protein secretion.

Supported by Fondecyt 1070379, PCB-MN ICM P06-065-F, PFB-16.

73.

Analisys of the spatio-temporal expression pattern of the AtUTr1 and AtUTr3 during the root development.

Adrian Moreno*, Gonzalo Cisternas and Ariel Orellana.

Núcleo Milenio en Biotecnología Celular Vegetal, Centro de Biotecnología Vegetal, Facultad de Ciencias Biologicas, Universidad Andrés Bello.
Contact: a.moreno@uandresbello.edu; aorellana@unab.cl

We generate transgenic plants harboring the AtUtr1 and AtUTr3 promoter fused to uidA gene (GUS). These plants AtUTr1pro:GUS and AtUTr3pro:GUS respectively shown an strong GUS activity in the pericycle cells, xylem associated cells and in the root cap. Interestingly we observed that once the lateral root development start the GUS activity observed in the pericycle cells was diminished after the lateral root emerged and start to grow, the GUS activity reappears in the pericycle and xylem associated cells of the mature lateral root. Several reports associated this kind of phenomena with cell cycle progression in the cells of the pericycle. Actually we are working on determined the effect of ER stress over the pattern of expression during the root development due previous report indicated that AtUTr1 and AtUTr3 are highly up-regulated during the unfolded protein response.

Financing from *Beca de Postgrado CONICYT, Núcleo Milenio PCB-P06-065-F and Proyecto Basal PFB-16.

74.

Phenotypic characterization of a Ruby Seedless × Sultanina progeny using a multivariate approach

Correa J.[1,2], Hinrichsen P[1]., Riquelme A[2]., and Pinto M [1]

1.- Instituto de Investigaciones Agropecuarias
2.- Universidad de Chile

With the aim to characterize phenotypically a progeny (n = 144), from a Ruby Seedless × Sultanina crossing, 31 variables were measured at flowering time during the 2008-2009 season. Variables included: biometric and phenological parameters; sprouting, vigor and fertility. A Principal Component Analysis (PCA) gave in total 31 components where the first 13 components accounted for 92% of total variance. From these last the first one accounted for 24.3% of the variation and was mainly associated to features dealing with phenology, flowering percentage of primary and secondary shoot and the percentage of proximal sprouting. The second component accounted for 15.7% of the variance and was associated to the location of the tendrils in the primary and secondary shoot and the flowering percentage of primary shoot. In order to perform a Cluster Analysis (CA), we scored each observation from each component. As result we obtained 10 clusters (> 90% similarity). In average the cluster with more vigor reached approximately 0.9 cm in cane diameter; the most fertile reached more than 10 inflorescences per cane. Most of the inflorescences (≈60%) were distributed in the distal half of the cane. Clusters with a high proximal fertility (> 80%) had in turn a low total fertility (< 5 bunches/cane). Flowering time for the earliest clusters occurred between the 2-3 weeks of November. After this analysis we concluded that phenology and fertility were the characters that contributed the more to the total variance measured. These results confirm that this progeny can be used to identify the genetic determinants for these traits.
Genoma FONDEF G07I-1002

75.

Relationship between the rate of chlorophyll accumulation and the ELIPs expression during the transition to the autotrophy stage of grapevine leaves.

Olivares D [1, 2,], Sáenz-Diez D[1,], Sierralta W.D[1,], y Pinto M.[1, 2]

1/ Universidad de Chile 2/ Instituto de Investigaciones Agropecuarias – INIA.

In leaves transition to the autotrophy stage is characterized by the accumulation of chlorophyll and by the assembling of photosystems. Chlorophyll is synthesized in the chloroplast membranes and then transported to the thylakoid membranes presumably bound to early light inducible proteins (ELIPs). The relationship between the rate of chlorophyll accumulation and the ELIPs expression during the transition to the autotrophy stage in grapevine leaves has not been studied yet. In this study two kinds of leaves were characterized: heterotrophic (young leaves) and autotrophic leaves (adult leaves). The heterotrophic leaves presented a growth rate higher than the autotrophic leaves; however under saturating light its CO2 assimilation rate was negative. The chlorophyll accumulation rate was high in heterotrophic leaves but almost nil in autotrophic leaves. This has a positive correlation with the level of ELIPs expression which in turn was also high in heterotrophic leaves and nil in autotrophic leaves. The proposed function of the ELIPs as scavengers of free chlorophyll molecules is discussed.

Fondecyt Nº 1070788.

76.

Reduction in photochemical efficiency and nutritional state in upland and lowland rice subjected to toxic levels of different sources of iron

Caroline Müller, Laíse R. Souza, Eduardo G. Pereira, Daniel T. Pinheiro, Advânio I. S. Silva, Marco Antonio Oliva, **Andréa Miyasaka de Almeida**
Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Brazil,
amalmeida@ufv.br

Plants of lowland and upland rice (*Oryza sativa*) were subjected to toxic levels of different iron sources. Seeds were mechanically scarified, surface sterilized, sown in paper for 14 days. Seedlings were transferred to Hoagland nutrient solution, pH 4.0. After 30 days, plants were treated with 7mM divalent iron source: ferrous sulfate ($FeSO_4$ with EDTA w/w) and trivalent iron sources: ferric chloride ($FeCl_3$ with EDTA w/w) and ferric citrate. After 7 days of treatment, chlorophyll a fluorescence parameters were evaluated using a modulated fluorometer Imaging-PAM (Heinz Walz, Effeltrich, Germany), net photosynthetic assimilation rate was determined by infrared gas analyzer, nutrient content was quantified by atomic absorption spectrometry and Pearls Prussian blue method was employed for the histolocalization of iron. The iron promoted significant decreases of on electron transport rate and photosystem II (PSII) effective quantum yield (Y(II)). These reductions probably occurred due to damage in PSII reaction center as, indicated by the increase on basic fluorescence (F_0) and reduction on open reaction centers estimated by the photochemical quenching coefficient (qL). Ferric chloride caused a minor decrease in the ETR and Y(II) without significant changes in F_0. The ferric chloride did not affect the chlorophyll a fluorescence in the analyzed conditions. Iron in excess conducted to reduction of net CO_2 assimilation rate in all conditions. The result shows that the ferrous sulfate affected more PSII because iron is in a more available form for the plants when compared with other iron sources used.

Support: CNPq, CAPES/PROCAD, FAPEMIG