III reunión de biología vegetal

23-24 de Octubre 2008

Centro de Conferencias "Paso Pehuenche", San Clemente, Talca

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Thursday October 23th

07:30 - 09:00 **Breakfast**

09:00 - 09:45 **Registration and poster set up.**

09:45 - 10:25 **WELCOME** (Meeting Organizer - Dr. Alejandra Moya)

10:30 - 11:30 **OPENING SEMINAR**

Dr. Tom Beeckman, Dr. Tom Beeckman
Plant Systems Biology Laboratory. VIB Institute. University of Ghent. Belgium.
"Systems biology approaches to study root development"
Host: Dr. Virginia Garretón

11:30 - 11:55 **Coffee break**

12:00 - 13:30 **ORAL SESSION 1**
Chairs: Virginia Garretón / Michael Handford

12:00 - 12:30 **Dr. Lee Meisel,** Millennium Nucleus in Plant Cell Bitechnology, Plant Molecular Genetics Laboratory, Plant Biotechnology Center, Andrés Bello University, Santiago, Chile.
"Analysis of factors associated with blue-light stimulated movement of different types of organelles"

12:30 **Daniela Fuentes**. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.
Impairment in mitochondrial complex II produces an increase of the photosynthetic activity in *Arabidopsis thaliana.* Fuentes, D[1], Nunes-Nesi[2], A, Araujo, W[2], Fernie[2,] A and Jordana, X[1]. [1]Pontificia Universidad Católica de Chile; [2]Max Planck Institut fur Molekulare Pflanzenphysiologie, Germany.

12:50 **Rodrigo Tapia**. Millenium Nucleus in Plant Cell Biotechnology, Center of Plant Biotechnology, Andres Bello University
An *Arabidopsis thaliana* mutant in the UDP-glucose glycoprotein: glucosyltransferase, a key enzyme in quality control at the endoplasmic reticulum, shows a root phenotype. Tapia, R., Reyes, F. and Orellana, A.

13:10 **Elena Vidal.** Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile.
Function of the nitrate responsive MIR393:*AFB3* module in the development of A*rabidopsis thaliana* roots. Vidal, E.A., Araus, V. and Gutiérrez, R.A.

13:30 - 15:00 **Lunch**

15:15 - 17:15 **ORAL SESSION 2**
Chairs: Raúl Herrera / Andrés Zurita

15:15 – 15:45 **Dr. Ross Whetten,** Department of Forestry and Environmental Resources, North Carolina State University, Raleigh NC USA.
"High-throughput DNA sequencing – a tool to integrate the study of ecology, evolutionary biology and economically important traits in non-model plant species"
Host: Dr. Raúl Herrera

15:45 **Sergio Diez de Medina.** Millennium Nucleus in Plant Cell Biotechnology; Plant Functional Genomics and Bioinformatics Lab, Universidad Andres Bello.
Positioning of wooliness gene candidates through a selective mapping approach in *Prunus persica.* Diez de Medina, S. and Silva, H.

16:05 **José Tomás Matus.** Laboratorio de Enología, Departamento de Fruticultura y Enología, Facultad de Agronomía, Pontificia Universidad Católica de Chile.
Genome wide-analysis of the grape MYB R2R3 subfamily and functional characterization of members with potential effects on wine quality. Matus, J.T., Aquea, F., Walker, M., Robinson, S., Ferrier, T., Barrieu, F. and Arce-Johnson, P.

16:25 **Daniela Urbina.** Millennium Nucleus in Plant Cell Biology and Biotechnology, Plant Molecular Genetics Laboratory, Center of Plant Biotechnology, Andres Bello University.
Arabidopsis ε adaptin accumulates in the early stages of mitosis. Urbina, D.C. and Meisel, L.A.

16:45 - 17:15 **Dr. José Casaretto**, Institute of Plant Biology and Biotechnology, University of Talca, Talca, Chile.
"Regulation of gene expression by a family of plant transcription factors involved in water deficit and salt stress"

17:15 – 17:40 **Coffee break**

17:45 - 19:45 **ORAL SESSION 3**
Chairs: Rodrigo Gutiérrez / Gastón Muñoz

17:45 - 18:15 **Justin Borevitz**, The University of Chicago, Chicago, IL, USA.
"Genetics of Adaptation: From Model Organisms to Model Ecosystems"
Host: Dr. Rodrigo Gutiérrez

18:15 **Viviana Ordenes.** Laboratorio de Biología Molecular Vegetal, Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile
Analysis of a boron-resistant *Arabidopsis thaliana* mutant. Ordenes, V.R.[1], Darwin Mutarello, D[1], Oetiker, N.[2], Alvarez, R.[1] and Zurita-Silva, A.[1]. [1]Laboratorio de Biología Molecular Vegetal, Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile; [2]Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile.

18:35 **Victor Polanco.** Instituto de Investigación Agropecuaria, INIA-Quilamapu.
Silencing the jasmonate cascade in transgenic grapevine plants, and its effect on defensive responses to wound and *Botrytis cinerea.* Polanco, V.[1], Olmedo, B.[2], Prieto, H.[2] and Peña-Cortés, H.[1]. [1]Instituto de Investigación Agropecuaria, INIA-Quilamapu, Chillán; [2]INIA-La Platina, Santiago; [3]Centro de Biotecnología-UTFSM, Valparaíso.

18:55 **Wendy González.** Centro de Bioinformática y Simulación Molecular, Universidad de Talca, Chile.
Structural properties of sensor histidine of extracellular pH in different stomatal inward potassium channels. González, W.[1], Morales, S. [1], Alzate, J. [1], González-Nilo, F. D. [1] and Dreyer, I [2]. [1]Universidad de Talca, Chile. [2]Universität Potsdam, Institut für Biochemie und Biologie, Heisenberg-Gruppe Biophysik und Molekulare Pflanzenbiologie, Germany.

19:15 - 19:45 **Dr. Haroldo Salvo,** INIA, Carillanca, National Institute of Agriculture Research. Biotechnology Unit. CGNA, Agri Aquaculture Nutritional Genomic Center.
"Genetic and genomic strategies in crop improvement for the food industry and a more sustainable agriculture"

20:00 - 21:30 **Dinner.**

21:30 - 23:00 **POSTER SESSION 1 (Beer and Refreshments, Odd number panels)**

Friday October 24th

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

09:00 - 11:00 **ORAL SESSION 4**
Chairs: Herman Silva / Alejandra Moya

09:00 - 9:30 **Dr. Willy Roca,** Ex-Director Recursos Genéticos y Biotecnología del CIP, y actual coordinador nacional REDBIO-Perú.
"Contribution of biotechnology to the potato crop: Evidences on its origin and diversity, and future perspectives"
W. Roca, R. Centeno, M. Ghislain. CIP, Redbio - Lima.
Host: Dr. Boris Sagredo

09:30 **Boris Sagredo.** Instituto de Investigaciones Agropecuarias, INIA Remehue.
Genomic tools for the potato breeding. Sagredo, B., Kalazich, J., Acuña, I., Rojas, J.S. and López, H.

09:50 **Lorena Pizarro.** Laboratorio de Biología Molecular Vegetal. Departamento de Biología. Facultad de Ciencias. Universidad de Chile.
Determination of the function of lycopene β-cyclase (lcyb) gene in carotenoid biosynthesis in *Daucus carota*. Pizarro, L. and Stange, C.

10:10 **Patricio Mandujano.** Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.
AtSDL, a putative sugar-alcohol dehydrogenase in *Arabidopsis thaliana*. Mandujano, P., Severin, D. and Handford, M.

10:30 - 11:00 **Dr. Claudio Pastenes**, University of Chile, Santiago, Chile.
"Plant stress physiology and wine quality"

11:00 - 11:25 **Coffee Break**

11:30 - 13:00 **ROUND TABLE**
Conversando con el sector productivo
Leader: Dr. Raúl Herrera

Sr. Gonzalo Herrera, Director Ejecutivo Fondef
Sr. Alvaro Arriagada, Enólogo, Gerente Viña Casa Donoso.

13:15 - 14:30 Lunch

14:45 - 16:15 POSTER SESSION 2 (Refreshments, Even number Panels)

16:15 - 16:45 CLOSING CEREMONY.

ORGANIZATIONAL SESSION – IV Plant Biology Meeting

16:45 - 18:00 MEETING RECEPTION

OPENING SEMINAR

INVITED SPEAKER

Lateral root initiation: auxin drives formative divisions.

Tom Beeckman

Department of Plant Systems Biology (VIB- University Ghent), Technologypark 927, B-9052 Ghent, Belgium.
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Plants, in contrast to animals, can form new organs during their entire life span. This permanent organogenetic capacity requires the preservation of stem cells in the adult plant body. In the root, *de novo* organogenesis is known to occur outside the meristem in a distinct monolayer of cells, the pericycle. Up to now, there is a striking lack of information how or whether the concept of stem cells applies to lateral root formation.

The Root Development group of the Department of Plant Systems Biology is interested in unraveling the very early events by which root pericycle cells are activated to undergo the first formative divisions. Being in search of the factors controlling these divisions, we performed transcript profiling experiments covering the changes in gene expression prior to and during the first formative divisions. We came across several candidate genes of which some were also found to be involved in the stem cell maintenance process in the primary root tip. Furthermore, we set-up a chemical genetics screen as an alternative and conditional approach to identify components involved in lateral root initiation that might have been missed due to embryo lethality by former classic mutagenesis approaches.

ORAL SESSION 1

INVITED NATIONAL SPEAKER

Lee Meisel

Analysis of factors associated with blue-light stimulated movement of different types of organelles.

Millennium Nucleus in Biotechnology and Plant Cell Biology, Plant Molecular Genetics Laboratory, Plant Biotechnology Center, Ecology and Natural Resources Faculty, Andrés Bello University, Santiago, Chile.
e-mail: lmeisel@unab.cl

Chloroplast movement in response to light was the first example of how organelles can move in cells. However, the mechanisms by which chloroplast move in response to exogenous stimulus are still poorly understood. Even less is know about how exogenous stimuli may affect the movement of other organelles in the plant cell.

It has been demonstrated previously that chloroplast positioning is an actin dependent process. Furthermore, chloroplasts have been shown to interact with the actin cytoskeleton. To decipher the factor(s) associated with chloroplast-actin interaction we have established a quantitative in vitro adhesion assay which has enabled us to quantify the number of chloroplasts that are capable of adhering to actin filaments. Combining this quantitative *in vitro* adhesion assay with a genetic mutant screen in Arabidopsis, we demonstrate that loss of function mutants, *arp2* and *arp3,* have a reduced number of chloroplasts that adhere to actin *in vitro*. These results suggest that the Arp2/3 complex participates in the adhesion of chloroplasts with actin.

We have also established an *in vivo* assay which enables us to detect chloroplast movement after 15 seconds of blue light stimulation. Using this *in vivo* assay we have confirmed that blue-light stimulated chloroplast movement is impaired in *phot1, chup1*, and *arp3* mutants. However, *arp2* and *cry1*mutants are not impaired in this movement. The impaired chloroplast movement in *arp3* mutants but not *arp2* mutants suggests that reduced interaction between chloroplasts and actin does not necessarily affect blue-light stimulated movement.

Using a similar *in vivo* assay, we have also demonstrated that mitochondria and nuclei move in response to blue-light stimuli. Drugs that alter the stability of the actin cytoskeleton affect the movement of mitochondria and nuclei in response to blue light, suggesting that the movements of these organelles are also dependent upon the actin cytoskeleton. These results suggest that a more generalized blue-light stimulated repositioning of multiple types of organelles may exist.

Financed by ICM P06-065-F and UNAB DI-14-08/R.

Impairment in mitochondrial complex II produces an increase of the photosynthetic activity in *Arabidopsis thaliana*.

Fuentes[1], D., Nunes- Nesi[2], A., Araujo[2], W., Fernie[2], A and Jordana[1], X.

[1]*Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.*
[2]*Max Planck Institut fur Molekulare Pflanzenphysiologie, Germany.*

Succinate dehydrogenase (complex II), is an enzymatic complex that participates in both the tricarboxylic acid cycle and the electron transport chain. In plants it contains two perypherical subunits (SDH1 y SDH2) and two integral membrane subunits (SDH3 y SDH4). Our group has obtained one heterozygous SDH1-1/sdh1-1 mutant line and two *SDH1-1* silenced lines. In this work we used this plants to study the role of complex II in photosynthetic. We observed that the mutant and the silenced lines are larger than control plants. Non alterations in photosynthetic electron transport rate and photochemical quenching were not observed in these plants, but the stomatal conductance and CO_2assimilation increased. We performed a metabolic profiling of the mutant and silenced lines to have a clearer view of the processes altered in our plants, and the results will be discussed.

These results show that the impairment in mitochondrial complex II produces an increase in photosynthesis, probably mediated by the alteration of the stomatal function.

Supported by Fondecyt 1060485, Beca de pasantías al extranjero CONICYT and VRAID and Beca de apoyo a la tesis doctoral CONICYT. Millenium Nucleus in Plant Functional Genomics P06-009-F

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

Tapia, **R**., Reyes, F. and Orellana, A.

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

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. The role of this enzyme *in vivo* has been mostly analyzed in unicellular but not in multicellular eukaryotes. 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 gene encoding for this enzyme. Homozygous insertional mutant lines were isolated and these plants were characterized. Since the highest level of the UGGT mRNA was found in root tissue, we analyzed the structure of the root in the mutant plants. Shorter roots were observed in the mutant population in comparison to the wild type, suggesting an important role for the UGGT in root cell elongation and protein secretion in *Arabidopsis thaliana*.

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

Function of the nitrate responsive MIR393:*AFB3* module in the development of *Arabidopsis thaliana* roots.

Vidal, E.A., Araus, V. and Gutiérrez, R.A.

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

Plant roots are highly plastic and adapt their growth and development in response to nutritional cues. Regulation of primary and/or lateral root growth represents a mechanism to optimize nutrient and water acquisition from soils.

Nitrate, the main nitrogen source available in agricultural soils, stimulates primary and lateral root development. The molecular mechanism involved in controlling this phenotypic response is not completely understood, but it is known it involves modulation of plant hormone signaling pathways. Previous studies indicate that auxin signaling components are regulated by nitrogen treatments. Detailed time course experiments indicate that the expression of the auxin receptor *AFB3* is rapidly and strongly induced by nitrate treatments. *AFB3* and other auxin receptors are known targets of miR393. We found that this microRNA is nitrate responsive and might be regulating AFB3 levels, working as a feedback loop probably regulated by an organic N signal. This prompted the hypothesis that auxin signaling modulation -by a pathway involving miR393-, might be one of the mechanisms controlling root architecture in response to nitrate. Analysis of an *AFB3* T-DNA mutant (*afb3-1*) showed that *AFB3* is key to lateral root development in response to nitrate supply. Given that lateral root development has been shown to be a highly cell specific process, we are currently analyzing miR393:*AFB3* module regulation by nitrate in specific root cell types to find new components of this signaling pathway.

Acknowledgments : This work is funded by Núcleo Milenio en Genómica Funcional de Plantas P06-009-F, FONDECYT 1060457 and ICGEB CRPCHI0501 grants to Dr. R.A.G. and CONICYT Doctoral Fellowship to E.A.V.

ORAL SESSION 2

INVITED SPEAKER

Ross Whetten

High-throughput DNA sequencing – a tool to integrate the study of ecology, evolutionary biology and economically important traits in non-model plant species.

Department of Forestry and Environmental Resources
North Carolina State University, Raleigh NC USA 27695-8008

Recent advances in technology for determining DNA sequences of many fragments in parallel reactions allow new kinds of research to be conducted on non-model plant species. Transcriptome analysis can be carried out on small samples of total RNA using PCR-based cDNA synthesis and efficient normalization methods, allowing discovery of many if not all the expressed genes in any species of interest. Quantitative analysis of gene expression can be carried out as well, by counting the frequency of specific DNA sequences in non-normalized samples obtained from different tissues or developmental stages. Genome organization can be explored by sequencing DNA fragments selected based on methylation status or nucleotide sequence, and complete sequencing of the entire genome or a specific subset of the genome is possible for species of particular interest. Comparative analysis between species can now be extended to many more genes and many more species than has previously been possible. These technological advances make possible genomic research in species that are not widely used as laboratory models, but have significant research interest from the perspective of ecology, evolutionary biology, or economic importance.

Positioning of wooliness gene candidates through a selective mapping approach in *Prunus persica.*

Diez-de-Medina, S. and Silva, H.

Millennium Nucleus in Plant Cell Biotechnology; Plant Functional Genomics and Bioinformatics Lab, Universidad Andres Bello, República 217, 837-0146 Santiago, Chile.

One of the Chile's major problems for exporting fruits is the distance to the foreign markets and reaching those markets with good quality fruits. The prolonged storage of fruits such as peaches and nectarines at low temperature can trigger a disorder know as chilling injury. One of the most important problems associated with chilling injury is woolliness. In order to address this problem, four cDNA libraries including different post harvest conditions were constructed as well as 50,000 ESTs were sequenced. Differential expression analysis was performed among all the libraries. Woolliness has been associated with cell wall loosening therefore we searched for genes related to cell wall metabolism, carbohydrate metabolism and genes related to stress response. Under this approach, two groups of unigenes were selected from the differentially expressed ESTs libraries. The goal was to develop molecular markers to be positioned to a Texas (almond) x Earlygold (peach) F2, *Prunus* reference map through selective mapping (Bin mapping). These markers will allow us to search for common regions present in woolly fruits as well to employ these new markers in further evaluations of peach populations that segregate for the woolliness phenotype. Intron flanking PCR molecular markers were developed in base of the selected unigenes to accomplish the positioning on the genetic map.

This research was supported by ICM P06-065-F and Consorcio Biofrutales SA.

Genome wide-analysis of the grape MYB R2R3 Subfamily and functional characterization of members with potential effects on wine quality.

Matus[1], J.T., Aquea[2], F., Walker[3], M., Robinson[3], S., Ferrier[4], T., Barrieu[4], F. and Arce-Johnson[2], P.

[1]*Facultad de Agronomía e Ingeniería Forestal, P. Universidad Católica de Chile.*
[2]*Facultad de Ciencias Biológicas, P. Universidad Católica de Chile.*
[3]*CSIRO Plant Industry and CRC for Viticulture, Australia.* [4]*Institut des Sciences de la Vigne et du Vin Centre INRA-Bordeaux, Francia.*

Flavonoids (anthocyanins, proanthocyanidins and flavonols) are the most abundant pigmented compounds found in grape berry tissues. These molecules are considered as quality parameters for the analysis of grape musts and wines as they define colour density, astringency, bitterness and because of their beneficial properties on human health. Their synthesis arises from the phenylpropanoid pathway, which is tightly regulated by the combinatorial interaction of at least three families of regulatory factors; MYB, bHLH and WD repeat proteins. Although the MYB Superfamily constitutes the most abundant group of transcription factors described in plants, few *MYB* genes have been identified and characterized in grapes. While searching in the Grape Genome Browser (www.genoscope.cns.fr), we found 108 members of the grape R2R3 *MYB* gene Subfamily. MYB factors have been found to control a wide diversity of physiological functions in model species. Grape MYB clades concerning flavonoid synthesis were found to be expanded when compared to the Arabidopsis and rice genomes. Two anthocyanin MYBA-related and three TT2 proanthocyanidin-related loci were identified in different chromosomes. *MYB* gene abundance, homology and orientation within these loci suggest that these MYB clades suffered multiple tandem duplication events. Different gene models were isolated and their expression was studied in different grapevine organs. VvMYB4 is being characterized in order to establish its contribution towards the regulation of flavonoid synthesis. This factor is a repressor of *UFGT* gene expression, as revealed by means of transient dual luciferase assays. MYB24 is possibly involved in anther and fruit development and is also being functionally characterized.

Arabidopsis ε Adaptin accumulates in the early stages of mitosis.

Urbina, D. and Meisel, L.

Millennium Nucleus in Plant Cell Biology and Biotechnology; Plant Molecular Genetics Laboratory, Center of Plant Biotechnology, Andres Bello University, Av. República 217, 837-0146 Santiago, Chile.
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The secretory pathway is a principal player during plant cell division. In mammalian polarized cells, AP-4 is a heterotetrameric complex which is necessary for apical protein sorting. In this work, we are investigating the role of ε adaptin, a putative AP-4 protein complex component, during plant cell division in *Arabidopsis thaliana.* Using polyclonal antibodies against ε adaptin of *Arabidopsis thaliana*, we have determined that this protein co-fractions with crude microsomal extracts. This protein and its transcript are accumulated in high levels in cells that are undergoing division such as the floral apex. We have bioinformatically identified MSA elements in the ε adaptin promoter sequence, a cis elements which has been associated with inducing gene expression during cell division. Additionally, whole mount immunolocalization of Arabidopsis plants reveals that this accumulates in cells that are in the early stages of mitosis. These results suggest that ε adaptin participates in plant cell division.

Financed by "Beca de Postgrado CONICYT", PCB-P02-009-F, ICM P06-065-F

INVITED NATIONAL SPEAKER

Casaretto, José A.

Regulation of gene expression by a family of plant transcription factors involved in water deficit and salt stress.

Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Chile.
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Dehydration is one of the most critical conditions for plants since it reduces growth and ultimately survival. Perception of water deficit occurs from seed maturation and germination to vegetative and reproductive growth and triggers signal cascades that include the phytohormone abscisic acid (ABA) and the activation of genes involved in adaptation processes. Among these genes, a family of bZIP transcription factors, named ABI5/AREB/ABF, and upregulated by ABA, drought and salt stress, are key regulatory points of such signal transduction cascades. Roles for these transcriptional regulators will be exemplified in: (1) the acquisition of seed desiccation tolerance, which is attained in part by the presence of highly hydrophilic proteins during late embryogenesis that are regulated by ABA and this small group of bZIP factors. These transcription factors recognize regulatory elements in target promoters forming special transcriptional complexes with other regulatory proteins. (2) The response of vegetative tissues to abiotic stresses such as drought and salinity. Two tomato (*Solanum lycopersicum*) cDNA sequences, named *SlAREB1* and *SlAREB2,* which share high homology with these group of bZIP transcription factors were identified and characterized. The expression of both genes is affected by dehydration and salinity, though that of *SlAREB1* showed the most significant induction. Transgenic tomato plants over-expressing and down-regulating *SlAREB1* were generated and revealed that these bZIP factors do play a role in conferring stress tolerance.

ORAL SESSION 3

INVITED SPEAKER

Justin Borevitz

Genetics of adaptation: from model organisms to model ecosystems.

The University of Chicago

We present fine scale population structure in the annual selfing plant Arabidopsis thaliana as local and patchy with phenotypic variation due to new mutations and new allelic combinations. Whole genome association mapping is being used to identify causative loci. Strong selection in a heavily disturbed and fragmented landscape, leads to population explosion and collapse, however genetic variation often remains. Restricted gene flow in isolated populations, and a changing environment, may have similar destabilizing consequences on the distribution of genetic variation for many species occupying the same habitat. New sequencing technologies and the low cost of genotyping allows emerging model organisms to be developed and soon model plant communities will be characterized at the genetic level. Thus studies of the genetics of adaptation to environmental change can be replicated across species that together shape their biotic environment and feed back on their light, temperature, soil, and moisture conditions. The genetic and species successional process of ecosystem evolution is being studied with high density remote sensing in remnant and reconstructed prairies near Chicago. This work should enable breeding for natural selection to restore biodiversity and ecosystem integrity as we adapt to global climate change.

Analysis of a boron-resistant *Arabidopsis thaliana* mutant.

Ordenes[1], V.R., Mutarello[1], D., Oetiker[2], N., Alvarez[1], R. and Zurita-Silva[1], A.

[1]*Laboratorio de Biología Molecular Vegetal, Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile.*
[2]*Licenciatura en Bioquímica, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile.*

Boron (B) plays a uniquely important role as essential plant nutrient and has high agronomic impact. The range of B deficiency and toxicity is rather small and is affecting soil and crop productivity in many areas of the world. Application of B-enriched fertilizers may however reduce the problem of deficiency, but toxic levels of B will remain a serious problem especially in arid zones, reducing crop yields and quality. Despite decades of research, only few is known about B plant biology and there is the continuing need to understand more about B function and uptake mechanisms. Toward this goal, we identified and investigated a mutant of *Arabidopsis thaliana*, which is more resistant to high boron levels than wild type plants. The expression of the mutated gene was analyzed by real-time quantitative PCR and displayed higher expression in mutant plants. The gene is predicted to be involved in cell wall modification. Given the fact that B plays an important role in plant cell wall integrity, our research reveals new insights of cell wall modifying enzymes and their possible role in a resistance mechanism to toxic B levels.

Project funded by INNOVA-CORFO, 05CR11PAT-19

Silencing the Jasmonate Cascade in Transgenic Grapevine Plants, and its Effect on Defensive Responses to Wound and *Botrytis cinerea.*

Polanco[1], **V**., Olmedo[2], B., Prieto[2], H. and Peña-Cortés[1], H.

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Plant responses to stress conditions involve an increase of endogenous levels of JA, which is essential to activate and regulate the expression of genes associated to the plant defence. In plants, JA is synthesized by the octadecanoid pathway, which employs at least five enzymes (lipase, lipoxygenase (LOX), allene oxide synthase (AOS), allene oxide cyclase (AOC), and 12-oxophytodienoate reductase 3 (OPR3). Molecular and biochemical analyses have led to the identification of almost all the genes of the octadecanoid pathway in three model plants, *Arabidopsis*, rice and tomato, however in grapevine, an important fruit crop, this pathway remains poorly characterized. Here we report the cloning and characterization of *Arabidopsis* orthologous genes (*AOS*, *AOC* and *OPR3*) from grapevine plant, named *VvAOS, VvAOC* and *VvOPR3*.

Reports indicate that the reaction catalyzed by OPR3 being one of the major regulation steps in the JA biosynthetic pathway. We silenced the expression of *VvOPR3* gene by RNA antisense technology in grapevine plants (*Vitis vinifera* cv Thompson seedless), in order to gain a broader insight about the involvement of JA in response of grapevine plants to stress conditions. Transgenic grapevine plants (anti-*VvOPR3*) exhibited strong susceptibility to mechanical wound and the fungus *Botrytis cinerea*. Analysis of transcript profiles in anti-*VvOPR3* plants showed that the induction of genes previously known to be JA-dependent is inhibited after stress conditions.

We conclude that an increased susceptibility to wound and fugal attack can be observed in the absence of *VvOPR3* transcripts. Our work contributes to knowledge of JA pathway in grapevine, the main commercial crop of Chile, and its future applications in control of pests and diseases.

Financed by INIA-Quilamapu-CONICYT-(PSD56); Center of Biotechnology-UTFSM; MECEUCV0206.

Structural properties of sensor histidine of extracellular pH in different stomatal inward potassium channels.

González[1], W., Morales[1], S., Alzate[1], J., González-Nilo[1], F.D., and Dreyer[2], I.

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The uptake of potassium mediated by an apoplasm acidification is a requirement for stomatal opening in plants. This acidification is perceived by voltage gated inward potassium channels (K_{in}) that open their pores with lower energy cost. The sensor units of extracellular pH in stomatal K_{in} channels are histidines exposed to the apoplasm. But, in *Arabidosis thaliana* K_{in} channel KAT1, mutations in the unique histidine (H) exposed to the solvent, do not affect pH dependence. H267 is placed in the pore of KAT1 channel, as part of the consensus sequence TVGYGDXH. KAT1 channel presents a phenylalanine (F) in X place of this sequence, meanwhile the rest of K_{in} channels present a leucine in X position. A homology model of KAT1 channel shows that F266 and H267 side chains are less than 5Å of distance. It was proposed the hypothesis that H267 of KAT1 channel cannot sense pH changes because F266 displace its pK_a to undetectable values. This hypothesis was corroborated experimentally using site directed mutagenesis and two electrode voltage clamp technique. It was established using Quantum Mechanics-Molecular Mechanics calculations (QM/MM) that if X position is a leucine, the sensor histidine could be protonated and unprotonated. Meanwhile, if X position is a phenylalanine these events could not happen, because phenylalanine is establishing a cation-pi interaction with H267, displacing the pK_a of this residue.

Thanks to: WG CONICYT scholarship and AT24071103 grant, FDGN ACT/24 grant.

INVITED NATIONAL SPEAKER

Haroldo Salvo-Garrido

Genetic and genomic strategies in crop improvement for the food industry and a more sustainable agriculture.

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Crops are important for the food chain and manufacturing. However, quality, quantity, efficient agronomic managements, environmentally friendly and sustainable agriculture systems are a real concern that has to be taken into account when modern varieties are developed to suite these challenges. Genetic and genomic strategies are crucial, since different chromosomal segments have to be carefully manipulated , in order to meet the specific demands imposed by the agro food chain. Furthermore if we consider the little time available to achieve these goals.

At the INIA and CGNA, we have began to conduct specific research on ,yellow lupin, canola, flax, wheat (durum and bread wheat) and rice, with the aim of understanding the genetic control underlining important traits related to food quality and agronomic performance. Together with the private sector we have established molecular strategies to introgress important agronomic traits and genes that will lead to a more sustainable agriculture. Different ongoing strategies applied to modern plant breeding including genome analysis, proteomic, genetics tools, genome selection, practical application of Marker Assisted Selection (MAS), and Graphical genotyping will be presented . These strategies, for example, help to identify genomic regions of agronomic/nutritional interest; facilitate the management of these identified regions and conclude with the corresponding field evaluation to evaluate plant performance. Results will include the release of eleven crop varieties where the application of MAS and graphical genotyping has allowed the introgression and recovery of elite genetic backgrounds up to 95-98% within a minimum span of time of 20 months. Genome coverage compared to the corresponding SSR consensus maps was 93% in wheat (2390cM) and 95% in rice (1448cM). Field comparisons have shown no differences in fitness and productivity in introgressed-elite versus elite varieties.

This work has been conducted in collaboration with INIA, CGNA, BASF, AquaChile, FONDEF, FONDECYT and CORFO.

ORAL SESSION 4

INVITED SPEAKER

Roca, W., Centeno, R. and Ghislain, M.

Contribution of biotechnology to the potato crop evidences on its origin and diversity and future perspectives.

CIP, Redbio - Lima

In terms of world total cropping area and consumption as a food by humans, potato is fourth and third, respectively; the latter only after rice and wheat. Current yearly world potato production is over 300 million tons, with more than 50% grown in developing countries, and is consumed by nearly 1000 million people in 150 countries. Estimated projected potato production growth is among the highest among food crops , 2.5 % yearly for the next twenty years.

Recent advances in biotechnology have allowed to add precision and efficiency to understand potato genetic structure and diversity , and advance knowledge on long-term issues concerning the evolutionary history and phylogeny relationships of potatoes, as well as to add clarity and shed light into otherwise intractable issues. Noteworthy has been the use of molecular genetic markers to advance new hypotheses on the origin and domestication, and on the early movement of the potato away for its South American home.

Modern biotechnology has also contributed to the characterization, evaluation and exploitation of wild and cultivated potato genetic resources for exploring and identifying potential new sources to approach otherwise intractable biotic and abiotic production constraints. Recently, similar approaches have allowed to link genetic variation to metabolic profiles in nutritional and health related applications. Special attention has been given to productivity and utilization constraints prevalent in resource-limited potato farming circumstances.

Genomic tools for the potato breeding.

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The potato crop specie (*S. tuberosum* 2n=4x=48), from the south America Andes, is one of the most important crop worldwide. In Chile it has high economic and social importance, with more than 91.000 farmers; it is cultivated around 80.000 ha with a total production of 1.3 millions ton. Currently there is a big demand for potato varieties with both higher tolerances to biotic and abiotic stresses and less dependence on agrochemical input. Also the consumers are increasing their demand for potatoes with better nutritive and nutraceutical properties. By the other hand, the increasing necessity for bio-fuel, biopolymer, food for the livestock animals, and special pharmaceutical compounds, require new special potato varieties. The development of a new potato variety is a complex and tedious process. Usually after a cross between adequate progenitors it is required around 10-12 years of selection and agronomic evaluation before to release a new potato variety. Fortunately, the modern biotechnology is generating new tools that are facilitating the breeding process, such as the transgenic and/or molecular assisted selection (MAS) methods. The potato breeding program of INIA-Chile had been implementing MAS methods to increase the frequency of resistance genes to PVY, PVX, PLRV and golden nematode within the breeding populations. In this moment the INIA potato breeding program is participating in two international genomic initiatives: the "Potato Genome Sequencing Consortium" and the "Development of the potato DArT", both projects will generate a powerful genomic platform that will facilitate the exploration and exploitation of the whole potato specie and other plant relatives.

Determination of the function of lycopene β-cyclase (lcyb) gene in carotenoid biosynthesis in *Daucus carota.*

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In plants, β-carotene, a carotenoid synthesized in plastids, is the precursor of abscisic acid and is involved in light harvesting. In animals, β-carotene and α-carotene, are precursors for vitamin A and retinol. The enzyme lycopene β-cyclase (LCYB) is directly involved in the synthesis of β-carotene and α-carotene. In some plants, such as tomato, two genes code for LCYB. These are lcyb and cycb, which are expressed in leaves and fruits, respectively. Cycb has a high sequence identity with the capsanthin capsorubin synthase gene (ccs) of pepper, which codes for a CCS with proven LCYB activity.

Daucus carota (carrot) accumulates high levels of β-carotene in the photosynthetic organs and in its modified root. Recently, a complete cDNA sequence for lcyb and ccs has been described in *D. carota*, which are predicted to code for a protein with LCYB activity. To determine if lcyb has an organ-specific LCYB function in *D. carota*, we silenced lcyb gene by means of post-transcriptional gene silencing. To achieve this objective, we over-express a conserved antisense cDNA fragment of *D. carota* lcyb. Transgenic lines with different silencing levels, determined by quantitative RT-PCR, have a significant β-carotene and carotenoid reduction in their leaves and modified roots, suggesting that lcyb gene has a non organ specific LCYB function in *D. carota.* The decrease in lcyb in transgenic lines, induces also a significant decrease in the expression of other carotenogenic genes such as ccs. Therefore, we suggest that β-carotene or other products synthesized downstream of the pathway, such as abscisic acid, may positively regulate the expression of upstream carotenogenic genes.

AtSDL, a putative sugar-alcohol dehydrogenase in *Arabidopsis thaliana*.

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

Sugar alcohols, including sorbitol, mannitol and xylitol, perform a variety of roles in plants, including as a means of transporting carbon long-distance in the phloem and as compatible solutes. The synthesis and breakdown of these compounds has been studied extensively, and several NAD-dependent sorbitol dehydrogenase (*SDH*) genes have been cloned and characterised from plants. Although sorbitol is the main substrate of SDH, other sugar alcohols are also oxidised. In *Arabidopsis thaliana*, a species which possesses low basal levels of several sugar alcohols, we have identified a putative SDH (AtSDL). AtSDL shares >75% amino acid identity with known plant SDHs and structurally and catalytically important residues are conserved. Our aim is to biochemically characterise this enzyme. RT-PCR assays demonstrate that *AtSDL* is expressed in multiple plant organs, including leaves, roots, stems and seeds, findings which are supported by *in silico* analyses. A more-detailed expression profile will be determined in *Arabidopsis* plants which have been transformed with an AtSDL promoter-GUS construct.Transient expression of AtSDL-YFP in tobacco leaves indicates that the protein is localised in the cytosol, consistent with the subcellular localisation of previously-described SDHs and with *in silico* analysis of the protein sequence. In order to determine the substrate specificity of the enzyme, AtSDL has been over-expressed in different strains of *Escherichia coli* and in tobacco, both as a native protein and as an epitope-tagged version. The oxidation of potential sugar alcohol substrates found *in vivo*, including sorbitol and xylitol will be analysed spectrophotometrically. To this end, three-dimensional modelling and molecular docking studies of the protein indicate that NAD is an acceptable cofactor, a finding which will be confirmed experimentally.

INVITED NATIONAL SPEAKER

Claudio Pastenes

Plant stress physiology and wine quality.

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Agricultural practices consist, widely, in reducing the extent of biotic and abiotic constraints in order to increase yield and quality of plant products. An exception of this golden rule is the production of grape berries for enology. It is well known that water stress would reduce berry size increasing the skin to pulp ratio and, also, restrict plant growth improving the microclimate at the fruiting zone. Besides the change in berry size, secondary metabolism is triggered in berry skins by water stress, further improving quality. Of course, a trade-off between quality and yield occurs. We have investigated the effect of water stress on the expression of genes coding for enzymes involved in the phenylpropanoid pathway in grape berries of Carmenérè, such as chalcone synthase 1 and 2 (CHS1, CHS2), phenylalanine ammonia-lyase (PAL), UDPG flavonoid-3-*O*-glucosyltransferase (UFGT) and dihydroflavonol 4 –reductase (DFR). Also, the expression of leucoantocyanidin reductase (LAR) a key enzyme for tannin formation in berry skins, of great value as anthocyanin cofactors stabilizing colour for wine aging. Gene expression for transcription factors such as MyB and MyC have been also under assessment, together with abscisic acid (ABA) content in berries, known to modulate the expression of such transcription factors linked to the expression of genes for flavonoid biosynthesis. Such results will be discussed regarding the final concentration of the anthocyanins on their various forms: glucosilated, acetilated and coumaroilated and also of tannins. The impact of water stress at the plant level has also been assessed by means of photosynthesis, chlorophyll content and its correlation with gene expression of pheophorbide (pheide) *a* oxygenase (PaO) and SAG12, the former being sequenced in *Vitis vinifera L*.

Poster Session

Poster presentation 1: Biochemistry and Molecular Biology

Expression analysis of the *LEAFY* and *SUPPRESSOR OVEREXPRESSION of CONSTANS1* homologues, *VvLFY* and *VvSOC1* from *Vitis vinifera* cv. Carmenèré.

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Grapevine (*Vitis vinifera* L.) is a woody perennial crop with a peculiar plant architecture and mode of reproductive development. At regular times during the adult plant shoot development and within latent buds the shoot apical meristem generates an extra-axillary structure called "uncommitted primordia" (UP). The first 1–3 UP formed around summer will often undergo repeated branching and develop into an immature inflorescence (flowering induction) establishing the number of inflorescence primordia per bud (fruitfulness) before the plant dormancy. Bud fruitfulness can determine the quality and quantity of crop production and also affects the management of the vineyard. Therefore, to know which genes are expressed during the grapevine flowering induction can help us to develop new genetic and management strategies to improve their culture and breeding. In this work, we analyzed the expression profiles of *VvLFY* and *VvSOC1*, the grapevine homologs of the Arabidopsis *LEAFY* and *SOC1* genes, during two growing seasons covering important developmental events as flowering induction, dormancy and flower and fruit develop. An increase in the *VvLFY* and *VvSOC1* transcripts was observed at the flowering induction period suggesting a role of them during this process. Furthermore, during the dormancy period a gradual reduction in the *VvLFY* and *VvSOC1* transcripts amounts was evidenced, but then, at the bud burst and flower development the *VvLFY* expression increased suggesting a role of it in these development transitions. Our results are discussed with the previously reported in other grapevine varieties and angiosperm species.

Acknowledgments: Universidad de Talca (R.A. and N.C. PhD fellowships) and Proyecto Biofrutales SA

Poster presentation 2: Biochemistry and Molecular Biology

Functional analysis of a SA-inducible Lectin Like Protein (LLP) in the stress defense reponse in *Arabidopsis*.

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Salicylic acid (SA) is a crucial hormone for the establishment of defense responses to biotic and abiotic stress. Genes activation mediated by this hormone is essential for these responses. We previously identified a group of early SA-inducible genes by transcriptomic analysis in *Arabidopsis*. Within this group, *LLP*, coding for a lectin like protein, has the highest level of activation. Our previous work indicates that *LLP* is transcriptionally activated by SA and by inoculation with *Pseudomonas syringae* pv tomato (Avr Rpm1). The product of *LLP* has not been associated to any biological function.

To evaluate the function of LLP in microbe-induced defense responses, we developed transgenic lines over-expressing LLP fused to c-myc epitope or to GFP, by using Agrobacterium-mediated transformation. Homozygote lines were isolated and the level of LLP expression was determined by Northern blot. We also isolated and characterized a homozygote and null T-DNA insertion line for *LLP* gene. By using these genetic tools, we are currently evaluating the role of LLP in the defense response to *Pseudomonas syringae* pv tomato (virulent and avirulent races). A first round of experiments suggests that *LLP* is involved in the defense response against *Pseudomonas.* Furthermore, our preliminary results of subcellular localization, by using confocal microscopy, indicate that LLP-GFP is located in the periphery of the plant cell, probably associated to the plasma membrane.

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

Poster presentation 3: Biochemistry and Molecular Biology

Quantitative analysis of viral load in GFLV infected plants.

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[2]*Universidad Andrés Bello, Facultad de Ciencias de la Salud.*

To date, more than 1000 plant viruses have been described and represent one of the mayor pathologic problems in plants. Grapevines are not exempted from this problem and 12 of the 60 grapevine viruses have been found in Chilean vineyards causing important losses in quality and productivity. Due to the lack of natural resistance in grapevines against most of the viruses, significant effort has been done in order to produce new resistant varieties. To evaluate these new varieties it has became necessary to manipulate the viruses in the laboratory in order to use them to challenge the plants and to be able to quantify the protection in the new varieties in comparison to the wild type.

In the laboratory we have been working for many years with GFLV, one of the more important grapevine viruses. We optimized several methods to detect and quantify the amount of virus in infected plants. In this work we present a comparison of several methods designed to quantify virus load, such as: quantitative ELISA, qRT-PCR and immuno capture coupled to qRT-PCR. The comparison was done in order to found the best way to quantify not only the amount of virus present in a plant but also to correlate it with the real infectivity of an inoculum.

Financed by: Conicyt PFB16 and UNAB DI-03-08/I

Poster presentation 4: Biochemistry and Molecular Biology

An optimized transient expression assay to study stress- and ABA-regulated gene expression in the solanaceae.

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A simple transient expression assay was optimized for rapid analysis of genes regulated by transcription factors in tomato and tobacco by means of Agrobacterium-mediated transformation. Transgene expression was achieved by infiltrating Agrobacterium cells via hand inoculation or vacuum infiltration. Expression of GUS, GFP and a bZIP transcription factor was assessed in both tomato and tobacco leaves, reaching high levels at 48 to 72 hours after infiltration. This approach was intended to investigate possible roles of transcription factors in controlling the expression of genes that respond to abiotic stress. A member of a group of bZIP transcription factors that participate in abiotic stress and abscisic acid signaling was previously identified in tomato (*Solanum lycopersicum*), *SlAREB1*, and was used in this transient assay. Total RNA samples isolated from control leaves and leaves infiltrated with Agrobacterium harboring a *35S-SlAREB1* construct were used to evaluate the expression of ten stress tolerance-related genes through RT-PCR analysis. Tobacco leaves expressing *SlAREB1* showed up-regulation of the transcription factor *PHI-2,* the stress responsive gene *RD29B,* the Lea genes *ERD10B* and *TAS14* and a trehalose-6-phospate phosphatase (*TPP*) gene. Further comparison between transcript profiles from these samples by means of cDNA-AFLP or microarray technology will allow the identification of more target genes for this transcription factor involved in abiotic stress response.

Supported by Fondecyt 1060843 and IFS C-4075

Poster presentation 5: Biochemistry and Molecular Biology

Identification of a novel boron transporter in citrus associated to boron tolerance.

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

Boron is an essential micronutrient for normal growth and development of plants. Excess or deficiency of this element is a major problem affecting productivity in crops worldwide. Understanding the mechanisms involved in boron transport should allow developing new technologies for improving mineral nutrition. Citrus species are one of the most important crops in Chile. They are cultivated from the II Region in the north to the VI Region in the south, therefore they tolerate differential boron concentrations in soil and posses diverse mechanisms which alleviate boron deficiency or toxicity. Using *Citrus macrophylla* rootstock and based on the sequences described in literature, we identify and characterized a putative boron transporter gene *CmBOR1* (EF581174). This gene has an open reading frame of 2145 bp encoding a deduced protein of 714 aminoacids. CmBOR1 is homologous to AtBOR1, both with ten trans-membrane domains and it groups with several deficiency boron transporters. *CmBOR1* is mostly expressed in roots and stems but also is detected in fruit and floral tissues. Gene fusions of CmBOR1 to GFP indicated protein localization in the plasma membrane. Complementation studies in yeasts are now in progress to functionally characterize this boron transporter.

Aknowledgements: INNOVA CHILE 204-4037 proyect and Nucleo Mileno en Genomica Funcional de Plantas.

Poster presentation 6: Biochemistry and Molecular Biology

A fruit specific complex II iron-sulfur subunit is conserved among angiosperms.

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In *Arabidopsis thaliana*, mitochondrial respiratory complex II subunits are all encoded in the nuclear genome. The iron-sulfur subunit is encoded by three nuclear genes, namely *SDH2-1, SDH2-2* and *SDH2-3*. Interestingly, *SDH2-3* has specific properties which set it apart from the other two genes: it is structurally different, is specifically expressed in the embryo during seed maturation, is abundant in dry seeds and its expression declines during germination. Previous work from our laboratory has identified *SDH2-3* orthologs in monocot plants, termed *SDH2-3*-like genes. In addition, the *SDH2-3*-like mRNA steady-state levels during post-germinative growth were examined by Northern blot and shown to have the same expression pattern as *SDH2-3*. We therefore set out to study whether there is a conservation of SDH2-3-like proteins in plants, either at the amino acid sequence level, expression pattern or both. For this purpose, we have identified, analyzed and characterized deduced amino acid sequences of non SDH2-3-like and SDH2-3-like proteins in various plants species and integrated them into an unrooted phylogenetic tree. In addition, we have characterized *in silico*, promoter sequences and the exon-intron structure of genes encoding SDH2-3-like proteins. Finally, we have evaluated the expression pattern of the *SDH2-3*-like gene of *Vitis vinifera* by RT-PCR. We have found a conservation of SDH2-3-like proteins among various plant species, suggesting an important role for this subunit in higher plants.

Fondecyt regular Project 1060485 and Millennium Nucleus for Plant Functional Genomics P06-009-F.

Poster presentation 7: Biochemistry and Molecular Biology

Effects of the SDH2-3 iron-sulfur protein inactivation on seed germination in *Arabidopsis thaliana.*

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Three nuclear genes encode the iron-sulfur protein, a component of mitochondrial complex II in *Arabidopsis thaliana*. Transcript level analysis has revealed that while *SDH2-1* and *SDH2-2* are expressed only in vegetative tissues, *SDH2-3* expression is seed specific. Transcripts appear during embryo maturation, are abundant in dry seeds and decline during germination. These data and subsequent analysis of *cis* elements present on the *SDH2-3* promoter, suggest that this complex II specific isoform could have a role during seed development and germination. During post-germinative growth, SDH2-3 appears to be replaced by SDH2-1 and/or SDH2-2 as complex II iron-sulfur subunit.

Due to the seed specific expression pattern of *SDH2-3*, we evaluated the effects of it's inactivation on seed development and germination using two *sdh2-3* insertional mutants and a *sdh2-1/sdh2-3* double mutant. Germination kinetics was determined on basal medium, medium containing different salt concentrations and after treatments with peroxide. In all cases we observed that mutant seeds germinated slowly when compared with wild type seeds moreover sdh2-1/sdh2-3 double mutants show a greater delay in germination. After germination, mutant seedlings developed normally although they are smaller than wild types at 8 days post imbibition. Normal development is probably due to compensation by the other genes, and preliminary results indicate that *SDH2-1* transcripts increase in *sdh2-3*mutant seeds. Finally, a decrease of complex II activity was observed in mutant embryos at different development stages.

Proyecto Milenio P06-009-F

Poster presentation 8: Biochemistry and Molecular Biology

Identification of new mutations within the *erg27* gene in fenhexamid-resistant *B. cinerea* isolates from Chile.

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Fenhexamid is a fungicide highly active against *Botrytis cinerea* that belongs to the hydroxyanilide family. It inhibits the 3-ketoreductase enzyme enabling the C4 demethylation in the ergosterol biosynthesis pathway. This enzyme is encoded by the *erg27* gene. Chilean isolates with different fenhexamid resistance levels (n=20) were sequenced and compared to French isolates that were also resistant to this fungicide. The highly resistant isolates ($HydR3^+$) showed EC_{50} values for conidial germination and mycelium growth of > 5 $\mu g \cdot mL^{-1}$ and > 2 $\mu g \cdot mL^{-1}$, respectively. As in French isolates, all of them exhibited alterations the phenylalanine at position 412 in the C-terminal end of the protein (trans-membrane domain), a mutation that is responsible for the fenhexamid resistance (Fillinger *et al.*, 2008). In 93.3% of the Chilean HydR3+ (14) isolates the phenylalanine was replaced by a serine, but only in one isolate by a valine. A substitution by isoleucine as in the French isolates was not detected in the Chilean strains. In addition to mutations at position 412, some $HydR3^+$ isolates also presented mutations at three other positions: P238S, a neutral modification that can also be found in sensitive strains (Albertini and Leroux, 2004, Fillinger *et al.,* 2008); the previously identified mutation in french isolates N369D, or the deletion of proline 298 that has not been reported before.

The weakly-to-moderately resistant isolates ($HydR3^-$) (n=5) presented EC_{50} values for conidial germination and mycelium growth that fluctuated between 0,7 – 2,6 $\mu g \cdot mL^{-1}$ and 0,4 – 3 $\mu g \cdot mL^{-1}$, respectively. These isolates presented six altered loci in the sequenced region of the *erg27* gene, corresponding to aminoacid changes between position 199 and 408 of the protein, some of which were also present in the highly resistant $HydR3^+$ isolates, but the phenylalanine at position 412 remained unchanged. The newly identified mutation I199L was only present in one isolate (HydR3-); the mutation in position 408 (tyrosine for serine) was also detected in a single isolate. In the Chilean isolates, the mutation of S336, close to the catalytic site, produced a change to alanine, different from the substitution detected previously in France (change to cysteine). The other new mutation detected in position 298 (proline deletion) was identified both in HydR3+ isolates and in HydR3- isolates. The potential effect of these new mutations of *B. cinerea* Chilean isolates in the fenhexamid resistance phenomenon and its consequences in the management of the disease in Chile is unknown.

Research supported by grant InnovaChile-CORFO: 07CN13IBM-14

Poster presentation 9: Biochemistry and Molecular Biology

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

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For the synthesis and/or modification of glycoproteins and polysaccharides in the lumen of the Golgi apparatus, specific transporter proteins are required for the import of nucleotide-sugars synthesised in the cytosol. We have identified GONST3 and 4 from *Arabidopsis thaliana*, which possess the molecular characteristics of nucleotide-sugar transporters (NSTs) and phylogenetic analysis suggests that GONST3 and GONST4 arose early in the evolution of NSTs. Our work is focussed on determining the substrate specificity of these NSTs and on analysing their role *in planta*. To achieve these aims, GONST3-His and GONST4-His vectors were made. Using *Agrobacterium*, tobacco leaves were transiently transformed and expression of GONST3-His and 4-His was confirmed by immunoblotting. After subcellular fractionation, the substrate specificity of GONST3 and 4 is being determined. A possible substrate of GONST3 and 4 is GDP-galactose which is unavailable commercially. Therefore, we have synthesised and HPLC-purified GDP-galactose enzymatically from GDP-mannose for use in transport assays. In addition, we propose to lower and raise the expression levels of *GONST3* and *4* and to analyse the phenotype(s) of the resulting lines. Constructs were made and *Arabidopsis* transformed to lower expression of *GONST3* and/or *4* by RNAi. Moreover, to study the effect of over-expressing an NST in plants, *Arabidopsis* has been transformed with GONST3-His or GONST4-His and the presence of the insert has been confirmed at the molecular level. To determine the expression profiles of these NSTs, *Arabidopsis* was transformed with promoter-GUS fusion constructs. Additionally, GONST3-GFP and GFP-GONST4 vectors were made to analyse their subcellular localisation and membrane topology. Current progress will be presented.

Funding: Fondecyt Iniciación 11060470.

Poster presentation 10: Biochemistry and Molecular Biology

Determination of the funtion of z-carotene desaturase (ZDS) genes in the biosynthesis of carotenoids in *Daucus carota*.

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

In plants, carotenoids are synthesized in plastids, are precursors of abscisic acid and are involved in photosynthesis and photoprotection. In animals, the carotenoids β-carotene and α-carotene are precursors of vitamin A. The enzyme z-carotene desaturase (ZDS) is directly involved in the synthesis of lycopene, a limiting step during β-carotene biosynthesis.

Daucus carota (carrot) accumulates high levels of β-carotene in leaves and in the modified root. Recently, two complete cDNA sequences for zds (zds1 and zds2) have been described in this species, which share 89% nucleotide identity in the coding region and 20% in the 5'UTR and 3'UTR. Most plant species have only one zds gene. However, other enzymes such as lycopene β-cyclase and phytoene synthase are coded by two genes in tomato and their function is associated specifically to leaves or fruits. To determine whether zds1 and zds2 have an organ specific function or redundant activity in *D. carota*, we are using post-transcriptional gene silencing to specifically reduce zds1 or zds2 transcript levels. To achieve this objective, we have used Gateway technology to over-express a double-stranded RNA hairpin with the 3'UTR region of *D. carota* zds1 and zds2 to obtain a specific silencing effect upon each gene.

Transgenic lines were regenerated by means of somatic embryogenesis and 15 transformants harbouring the zds2 construct were obtained and analysed at the molecular level. Transgenic lines with transcript reduced levels of zds2 gene showed an altered carotenoid content in levels and modified roots. Specific carotenoid composition done by means of HPLC analysis will be also presented.

Poster presentation 11: Biochemistry and Molecular Biology

Structural and functional characterization of *VvZIP* gene coding for a zinc transporter in *Vitis vinifera* cv. Carménère.

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Instituto de Biologia Vegetal y Biotecnologia, Universidad de Talca.

Zinc is an essential micronutrient in grapevine. Deficiency in this microelement affects fructification and berry development in *Vitis vinifera* cv Carménère and other grapevine cultivars, leading to a poor fruit setting and the development of parthenocapically-produced small sized seedless berries. Zinc deficiency could caused either by low availability or by low absorption and mobilization of this metal. In plants, the uptake mechanisms and distribution of zinc involve the participation of a wide variety of transporters controlling the Zn homeostasis. A group of them, the ZIP family is related with the uptake from this metal at the root level and the genes coding for these proteins have been isolated from several plant species. We have isolated and characterized the homologous genes from *Vitis vinifera* cv Carménère (VvZIP), which codes for a protein highly similar to ZIP transporters. Bioinformatics as well as mutant yeast complementation studies indicate that the encoded protein corresponds to a zinc uptake transporter. The VvZIP gene is expressed in aerial tissues including flowers and normal seeded fruits and was found to be repressed in seedless grapes. The results suggest an association of the parthenocarpic fruit development with alterations in the transport and homeostasis of zinc.

Poster presentation 12: Biochemistry and Molecular Biology

Isolation and characterization of a proton-pump, EVP1, involved in *Eucalyptus globulus* abiotic stress tolerance.

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Plant vacuoles constitute 40-90% of the total volume on a mature plant cell. In the vacuolar membrane, a Na^+/H^+ antiporter is present that sequesters Na^+ causing a reduction in the cytoplasmic concentration of this ion. The proton exchanger uses the electrochemical gradient generated by two proton transporters (ATPase and vacuolar pyrophosphatase) to maintain the internal water balance.

The role of the vacuolar pyrophosphatase pump in the process of plant adaptation to salt stress was shown by the complementation of the *ena1* yeast mutant and Arabidopsis AVP1 mutants with the AVP1 (*Arabidopsis* vacuolar pyrophosphatase 1) gene, restoring salt tolerance to both salt-sensitive organisms.

Our hypothesis proposes the existence of an AVP1 ortholog within the *Eucalyptus globulus* genome, which would have a role in the plant's response to salt and water stress. Thus, we isolated from an *E. globulus* cDNA library the vacuolar pyrophosphatase gene, named EVP1. The predicted amino acid sequence had more than 85% identity with the sequences described for *Vitis vinifera* and *Prunus persica*, exhibiting the structural characteristics of a vacuolar pyrophosphatase protein. The expression pattern of the EVP1 has been studied in *E. globulus* plants subjected to different types of abiotic stress through different time periods. The results of this work will be presented.

Poster presentation 13: Biochemistry and Molecular Biology

Vitis vinifera TRAUCO, a Trithorax group homologue gene is expressed during grapevine development.

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Trithorax group genes are members of a conserved machinery that maintains gene expression patterns by means of chromatin remodeling. In plants, these factors are involved in key processes during life span. In this work we describe the molecular characterization of *Vitis vinifera TRAUCO* homologue gene (*VvTRO*). This gene has been described initially in the model plant *Arabidopsis thaliana* as an essential gene. The main characteristic of this gene is a SPRY domain in the carboxi-extreme and it is expressed during embryo development and flower organs in *Arabidopsis*. *VvTRO* is a single copy gene localized in the chromosome 6. The genomic sequence is 4211 bp distributed in two exons and one intron. The open reading frame is 1170 bp that codifies a protein of 389 aminoacids, with a nuclear localization signal and a SPRY domain. Using Real Time PCR we detected *VvTRO* expression in different tissues in *Vitis vinifera cv. Cabernet sauvignon* (root, leaf, flower and seed). A chronological study of grape flower progression revealed that *VvTRO* expression is high in the firsts weeks of the flower organ formation, corresponding to the flower primordium. After this stage, *VvTRO* decreases and remains constant until the flower and pollen maturation, corresponding to the stages of four to twelve weeks of flower development. We also assessed *VvTRO* expression during berry development. Its expression increases from veraison until maturation. No expression was observed during GLRaV3 viral infection. The *VvTRO* expression pattern is similar to the one observed for the genes of floral identity of *Vitis vinifera*.

Poster presentation 14: Biochemistry and Molecular Biology

Gene expression of PR10 in fruits and leaves of *Fragaria chiloensis* in response to *Botrytis cinerea* infection.

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One of the main strength of the modern agricultural productive system is the reduction of agrochemical and fungicide sprays which affects the environment, incrementing health problems. In recent years, biotechnology has driven the exploration and study of several native species among the Chilean strawberry *Fragaria chiloensis*, which is a natural reservoir or primary source of genes for genetic improvement of this specie and the cultivated *Fragaria* x *annanassa*. Within all the positive characteristic of the Chilean strawberry, the tolerance to pest and diseases is one the most remarkable. One of them, it is its great tolerance to the fungus *Botrytis cinerea Pers*., which it is one of the most important post harvest problem in the commercial strawberry. Generating a Suppressive Subtractive Hybridation (SSH) several genes involved in defence were identified, among others with unknown function which would modify the plant response to the pathogen. One of the proteins determined presented a high homology to a pathogenesis related proteins, named PR10fch, which has presented a relative higher expression, compare to a healthy control, in *B. cinerea* chilean strawberry infected leaves and fruits. Our results showed the first approach among the plant-pathogen interaction at molecular level presented in the Chilean strawberry for the disease gray mold.

Poster presentation 15: Biochemistry and Molecular Biology

Changes in alcohol acyltransferase activity and expression level during ripening of Chilean strawberry fruit (*Fragaria chiloensis* L. Duch*.)*.

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Aroma of a fruit is determined by different volatile compounds, whose synthesis depends on several factors, such as growth conditions, ripening stage and post-harvest conditions. Volatiles are considered of great importance to determine fruit quality, as they influence the final acceptance of consumers. Among volatiles, esters are one of the most important due to their major sensory impact, and in strawberry fruit they deliver the characteristic sweet and floral fragrance. Esters are synthesized by the enzyme alcohol acyltransferase (AAT) by means of a transacylation of an acyl-CoA to an alcohol. AAT enzyme has been studied in both flowers and fruit. The Chilean strawberry fruit *(Fragaria chiloensis)*, the parent of the commercial strawberry (*Fragaria* × *ananassa)*, presents an exceptional organoleptic quality that is reflected by its great aroma and flavor. So far, few studies have been developed in this specie. During ripening of the Chilean strawberry a rapid increase in the production of some esters of high sensory impact is observed, which coincides with the increase in the activity of AAT. In addition, an increase in the level of expression of AAT gene was recorded by real-time PCR. The expression of AAT gene is fruit specific and increases dramatically as the fruit ripens. These results suggest an important participation of AAT in the generation of esters during the ripening of the Chilean strawberry fruit.

Acknowledgements to Anillo PBCT Ciencia y Tecnología ACT-41 Project.

Poster presentation 16: Biochemistry and Molecular Biology

Functional promoter analysis of GRXC9 gene coding for a glutaredoxin in *Arabidopsis*.

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Glutaredoxins are small disulfide oxidoreductases that use glutathione (GSH) to reduce disulfide bridges and S-glutathionylated cysteines from proteins, protecting them from oxidative damage. GRXC9 was identified in our group as one of the GRXs highly induced by salicylic acid (SA), a hormone involved in the stress defense response, and by an avirulent bacterium. Expression analyses indicate that GRXC9 is induced by SA without requirement of de novo protein synthesis and the coactivator NPR1, but requiring TGA2/5/6 subclass of transcription factors, being therefore classified as an early NPR1-independent SA-induced gene. In silico promoter analysis for this class of genes, by using MotifSampler software, allow to identify the as-1 like element (TGACGTCAnnnnTGACGTCA) over-represented in their promoters. This element was previously described as a SA-responsive element.

GRXC9 promoter contains two as-1 like elements in its proximal region. We analyzed the function of these elements, by evaluating the SA-induced expression of eGFP-GUS reporter genes controlled by the full-length promoter (-1860 to +26) as well as truncated and site-directed mutagenized versions of the promoter, using a stable Agrobacteriummediated transformation system. Our results indicate that the distal element as an important motif for transcriptional activation by SA.

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

Poster presentation 17: Biochemistry and Molecular Biology

Verification of Cherry Varieties using *Prunus* SSR Molecular Markers.

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Chilean sweet cherries (*Prunus avium*) have recently become one of the most competitive export fruit crops. The cherry fruit industry has the potential to expand and increase the production, quality and volume of exported cherries. Towards this end, a Chilean Cherry Breeding program has been established between investigators at Universidad Catolica de Valpariso and Andres Bello University. In order to begin this breeding program, Cherry varieties that are in Chile as well as new varieties that will be imported to Chile must be verified molecularly. In order to molecularly verify these varieties, we have created a database of over 234 *Prunus* SSR molecular markers based upon literature searches as well as personal communication. These molecular markers are being used to verify 13 sweet cherry varieties that will be used in this breeding program.

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

Poster presentation 18: Biochemistry and Molecular Biology

Identification of genes involved in the perception of daylenght and study on their daily expression profile in grapevine leaves.

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Photoperiodism defined as the ability to recognize and respond to changes in daylength constitutes one of the main mechanisms by which plants adapt to seasonal changes. Some of these responses are flowering, tuberization, growth cessation and dormancy. In grapevine there are at least two photoperiod-depending processes: (i) flowering, which is induced by increasing photoperiod and (ii) growth cessation and bud dormancy which are induced by decreasing photoperiod.

Phytochrome (Phy) signaling pathway is the first step in photoperiodic dependant responses, since Phys sense light and regulate the activity of different proteins and the expression of several genes. Here, we identified two phytochrome genes, PhyA and PhyB, in leaves of *V. vinifera* cv. Thompson Seedless and studied their daily expression pattern under long day (LD) and short day (SD) conditions. Under LD these genes presented a diurnal oscillation in their expression, but under SD their expression remained constant along the whole daily cycle. In addition to Phys, we identified *Arabidopsis thaliana* orthologues of CONSTANS (CO), Flowering Locus T (FT) and SOC1 (VvMADS8) genes in *Vitis*, all of which are also involved in daylength perception. VvCO showed a diurnal rhythm with maximum expression occurring at dawn and minimum expression at dusk. This pattern of expression was maintained under SD and LD conditions, indicating a possible circadian regulation of VvCO. In contrast, VvFT expression remained constant along the daily cycle under either, LD and SD conditions. However, abundance of VvFT transcript was significantly higher under LD than under SD conditions. VvMADS8 also presented differences in its diurnal expression profile and throughout the seasons.

Poster presentation 19: Biochemistry and Molecular Biology

Role of glutaredoxin S13 in the defence response of *Arabidopsis thaliana* to oxidative stress.

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Salicylic acid (SA) is one of the signals produced in response to different types of stress in Arabidopsis and plays a central role in activating defence genes. One of the genes early activated by SA corresponds to *AtGrxS13*, coding for the monothiolic glutaredoxin S13. Glutaredoxins are small disulfide oxidoreductases that use glutathione (GSH) to reduce disulfide bridges and S-glutathionylated cysteines from proteins. It has been reported that GRXs are able to modulate the redox state of the cell through the direct reduction of DHA to ASC and regulating the activity of antioxidant enzymes such as DHAR and peroxiredoxin through glutathionylation. Results from our group indicate that *AtGrxS13* is transcriptionally induced by SA and by inoculation with the bacteria *Pseudomonas syringae* pv tomato (Avr Rpm1). To determine the function that this protein plays in the defence response to stress, we have obtained homozygous transgenic lines that overexpress and silence *AtGrxS13*. Basal and stress-induced levels of ROS and glutathione in these transgenic lines are being evaluated. Preliminary results suggest that AtGrxS13 could be involved in stress-defense responses. We have also studied the subcellular localization of this protein, by expressing AtGrxS13 fused to GFP in transient and stable transformation assays.

Financed by: Fondecyt-Conicyt No. 1060494 and Millennium N.

Poster presentation 20: Biochemistry and Molecular Biology

Pectin disassembly of chilean strawberry fruit and the search for a related rhamnogalacturonase gene sequence.

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Chilean strawberry (*Fragaria chiloensis*) fruit shows a faster softening rate compared to *Fragaria* ×*ananassa* cv. Chandler during ripening. We have analyzed the changes of cell wall composition through developmental fruit stages in both strawberry species. Both species displayed different pectin degradation profiles, mainly between the transition from the large green (LG) to the ripe (R) stages. The fast softening rate observed in *F. chiloensis* fruit seems to be related to a rapid solubilization of covalently-bound pectins, whereas softening of *F.* ×*ananassa* showed a greater depolymerization of these polymers than the Chilean strawberry. In addition, *F. chiloensis* fruit showed a marked increment in neutral sugar content at the turning stage, probably due to solubilization of large pectin fragments with neutral sugar ramification, which could be related to a pectinase activity like rhamnogalacturonase (RGase). RGase activity has been detected in apple, grape and tomato fruits, but no plant RGase protein or gene sequence has been published. In GenBank database, we found a putative polygalacturonase (PG) sequence of *Arabidopsis* that appears to be most closely related to the *Aspergillus* RGase gene sequence. This sequence was aligned with *FcPG1*, a Chilean strawberry PG, and the non-homologous regions were used as target sequences for primers design. With the use of three primer pairs, we have achieved to clone amplicons flanked by the gene specific primers. We plan to perform an enzymatic assay for RGase and Northern-blot analysis to clarify the participation of RGase during softening of both *Fragaria* species.

Acknowledgements to PBCT Project "Anillo de Ciencia y Tecnología" ACT-41.

Poster presentation 21: Biochemistry and Molecular Biology

The transcription factor *AtbZIP60* regulate the response to the accumulation of unfolded proteins through an unconventional splicing in *Arabidopsis thaliana*.

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The IRE1 pathway that is turned on during the unfolded protein response (UPR), involves the unconventional splicing of a transcription factor. In the present work we evaluated whether *AtbZIP60* corresponds to the transcription factor that is generated when the signaling pathway triggered by IRE1 is activated during UPR in plants. In order to test this hypothesis, we modeled the secondary structure of the transcript for *AtbZIP60* using bioinformatic tools. This analysis predicted the formation of a double loop that could be susceptible to an IRE1 mediated splicing. The formation of splicing variants was experimentally analyzed by RT-PCR using RNA extracted from seedlings treated with tunicamycin. Under normal conditions a single transcript for *AtbZIP60* was observed; however, when UPR was induced, this product decreased and a concomitant increase in a product with a lower molecular weight occurred. Sequencing analysis of both products showed a difference in 23 nucleotides. In addition, both sequences predicted proteins with a DNA binding domain.

We propose that the transcript produced by the splicing of *AtbZIP60* is the responsible for the transcription of the genes activated by the IRE1 pathway in plants.

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Poster presentation 22: Biochemistry and Molecular Biology

Chemical and genetic characterization of flaxseed cultivated in Chile.

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Flaxseed is an ancient crop which has become important for human nutrition due to its valuable components. These are polyunsaturated oils (up to 68 % of alpha-linolenic acid), lignans, (cancer-preventing agents), proteins and soluble and insoluble dietary fibre. Flaxseed also contains some compounds that could impair nutrition, such as cyanogenic glycosides, which by hydrolysis can release hydrogen cyanide and phytic acid, which is responsible of reducing phosphorous and several minerals availability in animal and human diets.

In this work, a collection of 16 flaxseed varieties cultivated in Chile during 2006 season were evaluated in order to determine their content of total oil and fatty acid profile, as well as the protein, cyanogenic glucoside and phytic acid content. The oil content varied between 43 and 48% while protein varied from 21 to 24%. As expected, varieties with high oil content (over 46%) presented low protein content (less than 22%). Linolenic acid was found in a range from 55 to 60 %, being seven varieties over 57%. Cyanogenic glucosides varied from 0.8 to 3.44 mg/g and nine varieties presented values less than 2 mg/g. Phytate content don't varied so much, ranged from 0.8-1 %.

Genetic variability of the collection was analyzed using 10 polymorphic microsatellites. A high variability was found and three well differentiated groups were detected by clustering analysis using software PAUP 4.0 and the neighbor-joining method.

The chemical and genetic data here described will be used to design a molecular breeding strategy to develop new flaxseed varieties with high seed quality to support industrial production in Chile.

This work was supported by IICA-Canada as well as CGNA and INIA funds

Poster presentation 23: Biochemistry and Molecular Biology

Expression analysis of cell wall degrading genes in hybrids between *Fragaria chiloensis and F.* × *ananassa*.

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The Chilean strawberry breeding programme developed by University of Talca, has maintained different accessions of *Fragaria chiloensis*, collected from distinct edafoclimatic regions of Chile. A number of these have been used as a source of new genetic material for crosses with other *Fragaria* species. The hybrids generated have been characterized in terms of fruit quality by measuring fruit firmness, soluble solid content and titratable acidity. Among these parameters, fruit softening has a great relevance for postharvest life and quality of strawberry fruit. The process of softening is related to changes in metabolism of different cell wall components. The strawberry genotype "507" is a hybrid between *F. chiloensis* y *F.* × *ananassa* (cv. Chandler), which displayed an intermediate firmness value between the parents, and thus being a prospectively interesting genotype to analysis in terms of cell wall-degrading gene expression. By means of Northern-blot analysis, we detected differential levels of expression of several cell wall degrading genes (polygalacturonase, pectate lyase, pectin methylesterase, β-galactosidase, endoglucanase and expansin) between *Fragaria chiloensis* y *F.* ×*ananassa* (cv. Chandler) fruits. We now plan to carry out Real-Time PCR analysis to examine levels of gene expression in hybrid fruits during ripening.

Acknowledgements: We are grateful to the University of Talca via the Programa de Investigación "Frutilla Chilena Integral" and to the PBCT_CONICYT Anillo ACT-41 project for financial support.

Poster presentation 24: Biochemistry and Molecular Biology

Identification and characterization of xyloglucan endotransglucosylase/hydrolase (XTH) enzime in chilean strawberry (*Fragaria chiloensis*) fruit.

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Fruit ripening has gained the scientific attention due to its impact on fruit quality. During ripening of strawberry fruit, cell wall changes take place involving pectin and hemicelluloses content modification which reduce fruit firmness. The hemicellulose fraction is modified through the action of transglycosylase and hydrolase activity, both presented in XTHs enzymes. So far, XTH enzymes have been reported in kiwifruit, apple and pear. Two gene sequences with high homology to rosaceae XTHs have been identified within an ESTs database of *Fragaria chiloensis* fruit. By means of semiquantitative PCR we have detected a high transcript level of XTH at the large green stage of *F. chiloensis*. With the aim to isolate different XTH isoforms and to verify its gene expression pattern in strawberry fruit (*F. chiloensis* and *F.* × *ananassa*), we are now developing "Rapid Amplification of cDNAs Ends" assays and Northern-blots, respectively. In addition, the performance of XTH enzymatic assays and Western-blot analysis will provide complete evidence of XTH reported for the first time in strawberry fruit.

Acknowledgements: PBCT Project "Anillo de Ciencia y Tecnología" ACT-41.

Poster presentation 25: Biochemistry and Molecular Biology

bZIP transcription factors involved in response to salinity stress in tomato.

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As part of their responses to environmental stresses, plants have developed mechanisms that involve expression of genes with roles in conferring different degrees of tolerance. Among these genes, transcription factors are key regulatory end points of signal transduction cascades. Towards a better understanding of how crops respond to abiotic stress such as drought and salinity, we have previously isolated two bZIP transcription factors from tomato (*Solanum lycopersicum*), *SlAREB1* and *SlAREB2,* that share high homology with a group of transcription factors described in Arabidopsis and rice (ABI5/AREB/ABF). Their expression is induced in response to dehydration and high salinity, as well as by the phytohormone abscisic acid. The relative expression of both *SlAREBs* was evaluated through quantitative RT-PCR. Transcript levels were high in leaves, flowers and roots and less in mature seeds and mature fruits. The expression of *SlAREB1* and *SlAREB2* was examined in two-month-old tomato plants subjected to salinity stress for 6 hours to 30 days. Expression of both genes were affected by the treatment, however, that of *SlAREB1* showed most significant change with a induction peak at 6 hours in both shoots and roots. In order to evaluate their function in conferring stress tolerance, transgenic tomato plants over-expressing and down-regulating *SlAREB1* were generated. Transgenic sense and antisense lines were analyzed and selected to perform salinity tolerance assays by measuring physiological parameters such as relative water content and photosynthetic efficiency.

Supported by Fondecyt 1060843 and IFS C-4075

Poster presentation 26: Biochemistry and Molecular Biology

High affinity phosphate transporter promoters from Arabidopsis drive phosphate-modulated and environmental-dependent gene expression in transgenoc wheat.

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The root-specific and phosphate (Pi)-dependent modulation that characterize most high affinity Pi transporters (*Pht1*) genes makes their promoters as an ideal system to direct the regulated expression of Pi acquisition-related transgenes in plants. To investigate whether Arabidopsis *Pht1* would be functional in wheat, T_3 transgenic plants harboring the *AtPht1;2:GUS* and *AtPht1;4:GUS* constructs were functionally characterized, along with transgenic plants expressing the *GUS* reporter gene under the control of a 0.5 kb *TaPT2* promoter from wheat. Histochemical and biochemical GUS assays showed that the *AtPht1;2*, *AtPht1;4* and *TaPT2* promoters directed the reporter gene expression in roots of Pi-deficient hydroponics wheat albeit at low level. Gene expression was enhanced in Pi-deficient soil growing plants but only in those harboring the *AtPht1;4:GUS* construct, on which a 10-fold increased GUS activity was observed. Compared to hydroponics plants, GUS activity further increased by 30-fold in soil growing plants experiencing heat or heat-related stresses. Such conditions overrode the *AtPht1;4*-mediated response to Pi deficiency. GUS activity was almost undetectable in wheat leaves, which demonstrate that the three *Pht1* promoters direct transgene expression preferentially in the root system of this monocot. These results indicate that *Pht1;2* and *Pht1;4* promoters from Arabidopsis are functional in wheat though at low level, and that the *AtPht1;4* strength would potentially be enhanced in nature by stresses imposed by both the substrate itself and the aerial environment. The authors acknowledge Drs. Haroldo Salvo and Gastón Muñoz for their contribution to this work.

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Poster presentation 27: Biochemistry and Molecular Biology

Boron transporter genes from *Vitis vinifera* and their association whit partenocarpic fruit development in Carménère cultivar.

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In the reproductive development of *Vitis vinifera* cv Carménère, seedless and seeded grapes of different size are produced in the same cluster. This phenomenon is caused by a parthenocarpic process which has been associated with several factors being boron deficiency one of the most important. .This micronutrient is required for several steps in reproductive development, including pollen germination and pollen tube growth.

In plants, boric acid enters to the root cells trough the influx transporter NIP5;1 and then loaded into the xylem by efflux transporter BOR1. The genes coding for the above mentioned proteins have been isolated and characterized in other plants species as *Arabidopsis thaliana* and *Oryza sativa*, where their function is restricted to root tissues. Here we describe the identification and transcriptional profile analysis of putative orthologous genes from *Vitis vinifera* cv Carménère (VvBOR1 and VvNIP5;1). They are expressed in aerial tissues including inflorescences, flowers and fruits at early developmental stages, indicating that the encoded transporters are also involved in the boron mobilization to sink tissues. The comparative transcription patterns between normal seeded and seedless grapes showed a strong repression of BOR1 in the last ones, suggesting that boron transport failure at the reproductive organs level is associated with the occurrence of the parthenocarpic phenomenon.

Poster presentation 28: Biochemistry and Molecular Biology

Comparison of gene expression profiles in the anthocyanin biosynthesis pathways of *Fragaria chiloensis* and *Fragaria x ananassa* cv. Camarosa fruit.

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Development and ripening process of native strawberry fruit, *Fragaria chiloensis*, is associated with a set of physical and chemical changes, such as softening, aroma, flavor and color. In the genus *Fragaria*, fruit color develops during ripening and is given by accumulation of anthocyanins. Production of these compounds is due to coordinated action of a group of enzymes codified by structural genes of the flavonoids biosynthesis pathway. To extend our knowledge of the ripening process of *F. chiloensis*, several suppression subtractive hybridization (SSH) libraries were generated, contrasting four developmental and ripening stages, from "small green" to "ripe" fruit. Between differentially expressed cDNAs, we found two related with the production of flavonoid compounds: Chalcone synthase (CHS) and Anthocyanidin synthase (ANS). A comparative study of the expression profiles of these genes was carried out between *F. chiloensis ssp. chiloensis f. chiloensis* and *F. x ananassa* fruits throughout their development process. The expression analyses of these genes revealed a correlation between their transcript levels and anthocyanin accumulation.

Financial support from the PBCT Anillo ACT-41 project is gratefully acknowledged as well as CONICYT for doctoral fellowship to P. Pimentel.

Poster presentation 29: Biochemistry and Molecular Biology

Superoxide dismutase activity as a response to water deficit in *Aloe barbadensis* Miller (Aloe vera).

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Aloe vera is a monocotyledonous CAM plant which is naturally adapted to water deficit. It is to be cultivated in arid zones of Chile, where the lack of water and high temperatures are conditions of the Chilean Desert and possible conditions in the future of our planet. Both stress conditions induce the accumulation of reactive oxygen species (ROS) as a consequence of photosynthetic inefficiency involving a loss in electron dissipation. The plant is protected from ROS by enzymatic and non-enzymatic mechanisms that transform ROS into non-reactive molecules. The objective of this work was to study the activity of the enzyme Superoxide Dismutase (SOD) wich transforms the superoxide radical into hydrogen peroxide and oxygen, protecting Aloe vera from oxidative stress.

Aloe vera plants were watered with a volume of 1.6 L/h/plant for 1 hour, 45 min, 30 min and 15 min (T1, T2, T3 and T4, respectively). The results showed that under water stress, the plant retains water in photosynthetic tissues, with a significant decrease in the tips of the leaves when this stress was extreme (T4). There was also a decrease in the quantity of proteins present in the leaves under T3 and T4 treatments.

The specific and total activity of SOD increased gradually with increasing water deficit. Independently of the water treatment, the activity was greater in the tips than in the bases of the leaves,. Electrophoretic analysis of the isoenzymes detected 5 Cu/Zn-SOD and one Mn-SOD isoforms in Aloe vera. Western blot analysis showed that the proteins of Mn-SOD do not increase in quantity with the increasing water stress. RT-PCR analyses showed that the expression of the Cu/Zn-SOD genes increased with water deficit. The results of this research allow us to conclude that the increase in SOD activity, especially of the Cu/Zn-SOD isoforms, protects Aloe vera from water deficit-induced oxidative stress.

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Poster presentation 30: Biochemistry and Molecular Biology

Multifactorial analysis of the fungal tolerance in genetically modified grapevine plants: defining prototypes for commercial purposes.

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Botrytis cinerea is a necrotrophic fungus that infects a high variety of horticultural crops producing the "grey mold" disease and significant economic damage. Chitinases are enzymes that catalyze the hydrolysis of sugars and in fungi have a role on the growth of hyphae and differentiation. In plants, which lack chitin, these enzymes form a defense system against fungal pathogens. A 42 kDa chitinase has been purified from *Trichoderma atroviride* (ex *T. harzianum*) and its gene has been widely used in genetic transformation of various plant species leading to the generation of genetically modified (GM) plants that show tolerance against *Botrytis´* infection.

We have previously developed several GM grapevine *Thompson Seedless* plants and released them into a biosafety field. After three seasons of evaluations (2006-8), the statistical analysis of the results in leaf-challenges by *Botrytis* allowed the identification of a select group of plants with a resistant phenotype. Within these lines, those bearing fruits were further analyzed for their ability as a botry-static agent in inhibition-of-fungal-growth experiments on potato-dextrose-agar (PDA) dishes. Inhibition of the radial growth and changes in the hyphae morphogenesis were observed, showing that berry juices from GM plants have an important ability inhibiting the regular radial fungal growth when compared to patterns obtained in control PDA dishes, PDA+ autoclaved GM berry juices and PDA + non-GM control juices. Results indicate the presence of a thermo-labile component in GM berry juices that determines an inhibited growth of *Botrytis*.

Poster presentation 31: Biochemistry and Molecular Biology

Inverse relation between endodormancy depth and mitochondrial respiratory capacity in buds of *Vitis vinifera.*

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Endodormant bud of deciduous fruit trees experienced a great delayed to initiate growth under favorable conditions of light and temperature. During ED, respiratory metabolism and other biological functions are repressed and remains at a low activity until the end of ED. In bud of apple (*Malus domestica*) and other fruit trees it has been reported enhancement in bud respiration before the occurrence of bud break.

In the present work, since April to August, ED depth and mitochondrial respiratory capacity (MRC) were monitored in bud of *V. vinifera* cv. Thompson Seedless in Santiago. The results showed an inverse relation between the deepness of ED and MRC. Additionally changes in the expression of alternative oxidase (AOX) and alternative NADH dehydrogenase transcripts were found during the ED period. These changes suggest a modification in the mitochondrial electrons transport chain (mCTE) during the ED period. Furthermore, mitochondria (Mit) isolated from endodormant buds respired only in the presence of NADH or NADPH, but not in the presence of Succinate. The use of NAD(P)H as the only respiratory substrate by bud Mit could indicate a possible *in vivo* relationship between tissue's redox state and mitochondrial respiratory activity.

Poster presentation 32: Biochemistry and Molecular Biology

A complex II iron-sulfur subunit has a stage-specific expression pattern during seed development and is regulated by embryogenesis-related transcription factors in *Arabidopsis thaliana*.

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Complex II or Succinate dehydrogenase catalyzes oxidation of succinate to fumarate and reduction of ubiquinone to ubiquinol. Three genes, *SDH2-1*, *SDH2-2*, *SDH2-3*, encode the iron-sulfur subunit in *Arabidopsis thaliana*. We have previously shown that *SDH2-1* and *SDH2-2* are ubiquitously expressed throughout the plant (flower, leaves, inflorescence and shoot), while *SDH2-3* transcript is only detected in seeds, and its expression is regulated by the B3-family transcription factors ABI3 and FUSCA3. Arabidopsis embryogenesis can be divided into three stages, namely morphogenesis, maturation and desiccation, with specific expression of a subset of genes at each stage, for example *At2S3* at maturation and *Em6* at desiccation. To determine the precise developmental stage of *SDH2-3* expression, we obtained seeds from siliques at different developmental stages, and show by RT-PCR that *SDH2-3* mRNA starts accumulating early in maturation, increases during desiccation and even further in dry seeds. We also provide evidence that LEC2, another B3-family transcription factor, is important for *SDH2-3* expression during the maturation phase, although it is not required for its expression during desiccation. In contrast, ABI3 and FUSCA3 are also required during desiccation.

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Poster presentation 33: Biochemistry and Molecular Biology

Releasing dormancy agents, Hydrogen Cyanamide and Sodium Azide, up-regulate class I β-1, 3-glucanases mRNAs expression in grapevine buds.

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The buds of temperate woody plants undergo a dormant and freezing-tolerant state prior to winter season arrival induced by decreasing photoperiod and/or temperatures. This state ends when chilling requirements are satisfied and plants are exposed to favorable growing conditions. There are evidences suggesting that dormancy induction and release be partially regulated by the deposition and degradation of β-1,3-glucans (callose) within the plasmodesmata, thus controlling the symplasmic cell-to-cell communication in bud tissue. Here we present results showing the effect of two dormancy breaking agents, Hydrogen cyanamide (HC) and sodium azide (NaN_3) on the expression of two class I β-1,3-glucanase transcripts (BGlu78 and BGlu22) in *V. vinifera*. We found that both chemicals increased the expression of the two β-glucanase transcripts, and that transcript accumulation in bud tissue was directly related with increases in the rate of bud-break, suggesting a possible role of these enzymes in grapevine buds endodormancy release.

Since both chemicals, HC and NaN_3 are inhibitors of mitochondrial respiratory activity, a possible "Pasteur effect" leading to glycolysis activation with concomitant glucose decrease could be the key signal in promoting the expression of β-1, 3-glucanase genes.

Poster presentation 34: Biochemistry and Molecular Biology

Determination of Leu1781 and Val1781 ACCase Inhibitor resistance alleles in L. *multiflorum* resistant biotypes, using Allelo-Specific and CAPs genotyping.

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The chloroplastic acetyl-CoA carboxylase (ACCase) is strongly inhibited by commercial ACCase inhibiting herbicides. To date, the literature describe 6 amino acid positions susceptible to confer resistance to ACCase inhibitors herbicides in ryegrass. Previous data revels that *L. multiflorum* resistant chilean biotypes presents frequently mutation in the nucleotide position 5341, from full-length chloroplastid ACCase of *A. myosuroides*, that led to known (Ile1781→Leu) and new (Ile1781→Val) amino acids substitutions. This mutation results from a substitution of an adenine (A) residue by either thymine (T) or guanine (G). Two genotyping methods (allele-specific PCR and CAPs) were setting and evaluated for determinate the A substitution at nucleotide position 5341 by T and G, respectively. Three *L. multiflorum* populations were obtained from fields with suspected resistance to ACCase inhibitors herbicides. Total DNA was extracted from leaf tissue from individual seedling plants of each population. For allelo-specific PCR assay two pairs of primers were designed to specifically prime *L. multiflorum* ACCase sequences containing T instead of A. For CAPs assay one pairs of primers were designed to PCR amplify a fragment including the targeted codon and detect a restriction site (RsaI) only in mutant-type sequences.

A total of 33 plants of LM-22 population were tested with the allelo-specific PCR assay of which 14 (42.5%) displayed the Leu allele. On the other hand, 40 plants of LM-16 and 40 plants of LM-19 populations were tested with the CAPs assay of which 24 (60 %) and 27 (67.5 %) displayed the Val allele, respectively. Our results confirm the high insensibility of these biotypes to ACCase inhibiting herbicides.

Poster presentation 35: Biochemistry and Molecular Biology

Molecular characterization of chalcone isomerase complex gene family on extreme plant *Deschampsia antarctica.*

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We describe the characterization of three genes for chalcone-flavone isomerase (CHI; EC 5.5.1.6) from *Deschampsia antarctica* (DaCHI 1 to 3 genes). PCR walking and RACE-PCR we carried out with *ad hoc* modifications. Sequence analysis showed that the DaCHI genes did not present introns, and that their structural organization seems to be complex. DaChi1 ORF was 696-bp long, coding for a 232-amino-acids peptide of 23,937 Da and isoelectrical point (IP) 4.9. DaChi2 ORF was 882-bp, coding for 294-amino-acids peptide of 30,663 Da and IP 6.32. Differences between these genes arose from two insertions in DaChi2, of 96-bp and 90-bp long. In spite of this, contiguous regions showed high identities (about 98%), leading to propose that there are some modified characteristics of active sites. DaChi3 showed to have the same structure depicted for DaChi2; however, its ORF was 357-pb, codifying for a 119-amino-acids peptide of 12,209 Da and IP 6.82. Changes in length of DaChi3 were caused because of four premature stop codons and one additional nucleotide insertion, located inside of the second one described in DaChi2. Then, DaChi3 is proposed to have a frameshifting that leads to propose it as a pseudogene (ψDaChi2).

mRNAs levels showed differential expression when high salinity was applied; ψDaChi2 was early induced up to 7-folds, with no response to light. On the contrary, DaChi1 and DaChi2 mRNAs showed response to luminosity and a late induction by salinity, although at lower levels than ψDaChi2. Differences in the proposed gene structure, deduced protein characteristics and differential mRNA expression patterns, allowed us to suggest possible new functions for DaChi genes, and the occurrence of a regulatory pseudogene in this species.

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Poster presentation 36: Cell Biology

Characterization of *Arabidopsis thaliana* targets pathways of Sortin2, a synthetic compound that affects protein targeting to the vacuole.

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Endomembrane trafficking in eukaryotes is essential for the intracellular delivery of cargoes and membranes. In plants, the vacuole is one of the key compartments of the endomembrane system as the site of protein degradation, nutrient recycling and storage, of biological components. Sortin2, Sorting Inhibitor 2, is a synthetic compound that was identified using an assay in yeast for the aberrant delivery of a vacuolar marker protein. In *Arabidopsis thaliana* Sortin2 is able to alter seedling growth and vacuole morphology. Both phenotypes are dose-dependant and reversible in Arabidopsis seedlings, giving more research opportunities than the classical genetics. Using electron microscopy we have shown that Sortin2 treatment also results in the secretion of the endogenous plant homolog of CPY in Arabidopsis (AtCPY). Suggesting that there is cross-kingdom similarity in the mode of action of Sortin2 affecting the endomembrane system. In order to identify important features within the Sortin2 structure we have done Sortin2 structure-activity relationship (SAR) analyzing the bioactivity of Sortin2 analogs. To evaluate the impact of Sortin2 on different compartments within the plant endomembrane system we are analyzing the effect of Sortin2 by testing *A. thaliana* transgenic lines that express fluorescent protein markers for each compartment. We expect that Sortin2 would be a powerful and valuable tool for studying and dissecting pathways within the endomembrane system in plants.

Poster presentation 37: Cell Biology

Sortin2 is a novel auxin responsiveness inhibitor which specifically affects the response to indole-3-acetic acid in *Arabidopsis thaliana*.

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Plant endomembrane system is the site for synthesis of vacuolar and cell wall components, and this has been considered its more traditional function. Nevertheless, the endomembrane system has been increasingly implicated in plant-specific processes including hormones signaling, plant development, tropic responses, and pathogen defense. Using forward chemical genomics, a large-scale yeast screen identified Sortin2 within of a commercial compounds collection. A *Saccharomyces cerevisiae* genome-wide screen showed that Sortin2 treatment affects primarily components of the endomembrane system. In *Arabidopsis thaliana* Sortin2 affects the endomembrane system, altering the morphology of the vacuole and the delivery of a vacuole marker protein. Sortin2 also inhibits the gravitropic response of Arabidopsis seedlings. The gravitropic response is mainly regulated by auxin, a plant hormone that is synthesized in the apical part of the plant and transported from cell to cell in a polar fashion trough the vascular system which is essential for plant development and cell growth, affecting both cell division and cellular expansion. In order to examine the effect of Sortin2 on auxin signaling we have used a transgenic *A. thaliana* line expressing β-glucuronidase (GUS) as reporter protein under the control of the auxin-inducible promoter, DR5. Sortin2 inhibits the activity of DR5 when seedlings are treated with the auxin indole-3-acetic acid (IAA) indicating that Sortin2 blocks Auxin responsiveness. This effect is reversible. Interestingly Sortin2 does not inhibit the DR5 activation by 1-naphthaleneacetic acid (NAA) or 2,4-dichlorophenoxyacetic acid (2,4-D) which strongly suggest that Sortin2 is affecting auxin efflux in *Arabiopsis thaliana*.

Poster presentation 38: Cell Biology

Role of AIP2, an E3 ubiqutin ligase, in root development: Characterization of expression pattern and phenotype of mutant and overexpressor plants.

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Attachment of Ubiquitin to other proteins is a powerful mechanism that can regulate protein stability. AIP2 is an E3 ligase from *A. thaliana*, which control protein stability of ABI3 transcription factor during germination, by promoting its ubiquitination. However the pattern of expression of AIP2 gene in the whole plant, suggest that AIP2 is also involved in other biological process different than germination. In the present work we have analysed the putative role of AIP2 in root development using transgenic plants expressing AIP2 promoter:GUS fusion, with the aim of characterize the exact timing and localization of its expression in roots. Our results demonstrate that after 4 days post germination, when the main root has clearly emerged, AIP2 is express in the epidermis of the main root. Within the 5-6 days post-germination AIP2 transcript appears in the root hairs and the root-stem junction, and in later stages it appears in patches along the epidermic of the main root, always excluding the root tip. To test if this expression pattern correlates with a biological role of AIP2 in root development, we analysed root phenotypes of 35S:AIP2 and null *aip2* mutant plants, demonstrating that AIP2 is a positive regulator of lateral root elongation. Due to the relevance of auxin in root development, we also evaluated the effect this hormone in the root phenotype of AIP2 mutant and over-expressor plants, the results will be discussed. Finally, we performed two hybrid and TAP screenings, from which we identified several AIP2 interacting proteins. Two of these targets are currently under analysis and will be presented.

Fondecyt# 11060072

Poster presentation 39: Cell Biology

Identification of the molecular mechanisms involved in chloroplast avoidance movement in response to blue light using an in vivo motility assay in *Arabidopsis thaliana.*

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Photosynthetic organisms such as plants have developed diverse mechanisms in order to capture and utilize solar energy to realize complex metabolic processes. However, high intensity of certain wavelengths of light can cause irreversible damage to intracellular structures such as organelles. In order to avoid the damaging effects of high intense blue light (426-446 nm) chloroplasts have been shown to move away from this light source (avoidance response). In order to identify the molecular mechanisms involved in chloroplast avoidance movement in response to blue light, we have implemented an *in vivo* motility assay which permits us to visualize chloroplast movement in response to blue light in as little as 15 seconds. Analysis of Arabidopsis mutants which have been described previously as having alterations in chloroplast movement in response to blue light such as *chup1,* and *phot1* demonstrate that this assay may be used to identify mutants in chloroplast blue light avoidance response. Using this assay we demonstrate that this avoidance response is also altered in the *arp3* mutant, but not in *arp2* or *cry1*. We are continuing to use this assay to genetically identify the mechanisms associated with chloroplast blue-light avoidance response, such that these loci may also be analyzed for their participation directly or indirectly in chloroplast-actin interaction.

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Poster presentation 40: Genomics, Bioinformatics and Systems Biology.

Functional analysis of Botrytis cinerea hex-1 gene, encoding a putative "Woronin body" protein.

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Botrytis cinerea, also known as "gray mold fungus" causes serious pre- and post-harvest diseases in at least two hundred plant species, including agriculturally important crops and harvested commodities, such as grapes, tomatoes, strawberries, cucumbers, bulbs, flowers, cut flowers and ornamental plants. By means of a cDNA library from B. cinerea we were able to identify several genes putatively involved in early infection steps such as: adhesion, germination and hyphal growth with the purpose of developing novel approaches to prevent the natural course of the disease in the host. We identify a putative B. cinerea ortholog of hex-1 gene which is preferentially expressed during fungal growth. Hex-1 gene encodes the major protein of the Woronin body, a dense core vesicle whose function is to seal the septal pore in response to cellular damage. We have isolated the hex-1 gene of the Botrytis Bc05.10 isolate by PCR. The full-length cDNA obtained was about 1.6 kb long which included 700 bp of coding sequence. Expression analysis of hex-1 was carried out by RT-PCR at different growing times (30 min, 2, 4 and 24 hrs.) but no significant differences were found. To determine the specific role of hex-1 knockout mutant were generated by homologous recombination. Four mutants were obtained and confirmed; they showed morphological differences and a reduction in the rate of vegetative growth in comparison with the wild-type isolate. Analysis of the pathogenicity of the mutant on tomato leaves is under study.

Poster presentation 41: Genomics, Bioinformatics and Systems Biology.

The auxin receptor *AFB3* regulates root architecture in response to changes in nitrate availability in *Arabidopsis thaliana.*

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Nitrogen is an essential macronutrient for plant growth and development. Nitrate and other nitrogen nutrients and metabolites are able to act as signals that regulate gene expression at a global level in *Arabidopsis thaliana*. Root architecture regulation by nitrate is a well-described process but the molecular mechanism behind this developmental response is poorly understood. Root architecture is known to be regulated by hormone signalling pathways, principally by auxin and it has been shown that many auxin signalling components are regulated by nitrate. In particular, we have found that *AFB3*, one of the auxin receptors is induced rapidly and strongly by nitrate in Arabidopsis roots. This suggests that this receptor might have a specific function in root architecture regulation by nitrate. To address this hypothesis, we have analyzed how the number of initiating and emerging lateral roots are affected by nitrate treatments and if this is affected in an *AFB3* T-DNA mutant line (*afb3-1*). Our results show that nitrate treatments induce lateral root initiation and emergence and these responses are impaired in the *afb3-1* mutant, suggesting AFB3 is essential for lateral root development in response to changes in nitrate availability. Our current efforts are aimed at identifying components acting downstream of AFB3 that can be directly regulating lateral root development.

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Poster presentation 42: Genomics, Bioinformatics and Systems Biology.

Study of genes differentially expressed in late gravitropic response from Pinus radiata D. Don.

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In nature, conifer trees develop compression wood in response to gravitropic stimuli in the lower side of the stem. The genes involved and the molecular mechanisms under this phenomenon are still unknown. In this work we studied the genes expression at late times from the bending-induced radiata pine seedlings. We carry out a total RNA extraction at 24 hours and 1 month of induced gravitropic stimuli from the apical zone of stem that respond to bending. We took samples from the upper and lower half of the stem through a longitudinal cut. From those RNAs we built suppressive substractive libraries (SSH), containing a total of 1453 clones with a fragment size between 300 and 1500 pb. We estimated a redundancy value around of 40%. The BLAST analysis showed a differential gene expression involves in intracellular transport, metabolism, cell wall formation and hormonal signals transduction among others. This work is the first step to identify which genes are involved in the late modulation of gravitropic response from radiata pines and its relation with compression wood formation.

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Poster presentation 43: Genomics, Bioinformatics and Systems Biology.

Grapevine viruses in Chile: Detection of unreported and highly incidental viruses using metagenomic strategies.

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Fruit export is one of the main economic activities of Chile being grapevine among the most important species grown for table grapes and wine production. In this scenario, efficient and early detection of grapevine viral pathogens is critical to diminish losses by dissemination of infected material, which is the main cause of disease spreading in the field. Up to now, 60 viruses have been reported worldwide, affecting plant vigour and longevity, as well as the quality and quantity of the yield.

To determine the current status of virus infection in our country, we developed a multi-parallel viral detection oligonucleotide microarray containing 500 probes and able to screen 44 viruses simultaneously. After analyzing approximately 100 samples from different cultivars and geographic regions of Chile, we found a high rate of mixed infections of up to 6 viruses on the same sample. Moreover, we were able to detect for the first time in our country the presence of the *Closteroviridae* members GLRaV-4, GLRaV-7 and GLRaV-9 among others. These results were further confirmed by ELISA and RT-PCR, thus demonstrating the capabilities of this strategy for single highly parallel certification purposes and for virus discovery as well.

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Poster presentation 44: Genomics, Bioinformatics and Systems Biology.

Proteomic study of wood formation in maritime pine.

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Wood properties in maritime pine are highly variable at chemical, anatomical and mechanical levels. In this research, we tested the hypothesis that the observed variability at the phenotypic level, can be bound to the differential expression of proteins during the process of wood formation.

We use the tools of proteomics, Bidimensional electrophoresis and LC ESI MS/MS for the discovery of 165 proteins differentially expressed in a cambial age gradient, (from base wood to crown wood), an 93 overexpressed proteins in a seasonal gradient (from early wood collected at the beginning of the growing season, to late wood, collected at summer).

Complementary, chemical characterization of the samples was performed using analytical pyrolisis.

Our results showed that the secondary xylem formed at the beginning of the growing season, and the xylem formed by a young cambium, present a overexpression of proteins participating in the intense cell division, characteristical of those tissues, e.g. Biogenesis of cytoskeleton and hemicelluloses, RNA transcription, synthesis, folding and modification of proteins. In the xylem formed at the base of the trunk and at the end of the growing season we have found an over-expression of proteins from cell defense (they role will be to delay programmed cell death) and cell wall formation related proteins e.g. lignin biosynthesis. This study contributes to reinforce our knowledge over the molecular actors involved in the xylogenesis process. It opens, in another hand, research guides for the detection of genes involved in the genetic control of wood properties towards an objective of marker assisted selection.

Poster presentation 45: Genomics, Bioinformatics and Systems Biology.

Galactinol synthase genes: advances in their molecular characterization and expression in Lupin (*Lupinus luteus* L.*)*.

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Yellow Lupin (*Lupinus luteus* L.) is a promising protein source for fish nutrition. The superior seed protein content present in this species (45-58%), however, is accompanied by variable concentrations of Raffinose Family Oligosaccharides (RFOs), which cause flatulence in monogastric animals. RFOs concentration has been shown to increase in plants under stress conditions, suggesting a role on stress tolerance. Therefore, it is imperative to determine the exact roles that RFOs play on plant fitness before breeding for low RFOs content. In addition, the presence of multiple copies of GOLs genes in other species suggests the possibility to reduce RFOs only in seeds keeping intact their content in other plant tissues. Here, we report a study of Galactinol Synthase (GOLS) genes, a key enzyme involved in the RFOs synthesis. The phylogenetic study of GOLS genes from several plant species, obtained from the EST database of The Gene Index Project, allowed us grouping the leguminous species in eight subgroups. Eight sets of degenerated primers, developed in base to the subgroups alignments, amplified different GOLS genes in Lupin. The sequence characterization of these novel GOLS sequences will allow us to develop specific primers for the species, determine expression and possible roles of RFOs on plant fitness. We expect to find different copies of GOLS exhibiting tissue-specific-expression on Lupin plants under stress conditions.

Key words: Galactinol synthase – GOLS - Drought stress – Cold stress – Lupin – Lupinus luteus.

Poster presentation 46: Genomics, Bioinformatics and Systems Biology.

Targeted molecular dynamics study of potassium channels involved in salinity tolerance in *Arabidopsis thaliana.*

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Potassium (K+) is the most abundant cation in plants, being essential for many metabolic processes. Potassium channels dominate the K+ fluxes in plants cell types, participating in multiple events, which include stomatal movements and ions uptake from soil. Moreover, potassium channels contribute to salt tolerance in plants. These voltage-dependent channels (Kv) consist of four subunits with six transmembrane segments (S1-S6) each one. S4 segment acts as voltage sensor and S5-S6 segments constitute the pore. Kv channels switch between conducting and non-conducting states, as result of conformational changes in S4. It is still unknown how S4 is coupled with pore region to open and close the channel.

Interesting is the fact that K+ channels are functional at different voltage ranges. Despite their similarity, some of these channels conduct only inward currents (Kin) at negative voltages and other conducts only outward currents (Kout) at positive voltages.
This functional diversity of plants potassium channels increases the questions on molecular reasons behind these differences. In this context, KAT1 (Kin) and SKOR (Kout) channels, are model systems for structure-function analysis. Crystallographic structures of K+ channels solved to date suggest that they undergo large-scale motions during gating. This process is frequent on physiological time scales, but it is uncommon at molecular dynamics (MD) time scale. A MD approaches that solve this problem, is Targeted Molecular Dynamics (TMD), which guide a protein towards a conformation of interest, applying a force that superpose usual MD forces. In this study, TMD was used to accelerate the conformational transition between closed and open state in KAT1 and SKOR, allowing us to analyze at molecular level, their functional differences.

Thanks to: ACT/24

Poster presentation 47: Genomics, Bioinformatics and Systems Biology.

Molecular modelling and docking simulations of VpAAT1 from *Vasconcellea pubescens*.

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The aroma in fruits is an important attribute of quality that influences the consumer's acceptance, which is a complex character, determined by a set of low molecular weight volatile compounds. In *Vasconcellea pubescens* the aroma is formed mainly by esters, which are produced through an estherification reaction between alcohols and acyl-CoAs catalyzed by the enzyme acyl alcohol transferase (VpAAT). To gain insight about the mechanism of action of VpAAT1 at the molecular level, the comparative modeling methology was used to built the structure of enzyme, which was validated and refined with molecular dynamics simulation. The resulting model showed that the protein structure is composed of 15 β sheets and 14 α helix, the protein's active site is the segment HTMSD and is located in a loop between the sheet 7 and the helix 5. The molecular dynamic simulation of 2 ns showed that the residue H166 is part of the active site and exposed to the solvent channel, allowing the interaction with the substrates. Additionally, we explored the possibilities of interaction of a set of putative substrates with the AAT protein using molecular docking simulation. For the interaction with alcohols and Acyl-CoA, our results suggest than the stables conformation of the complex formed by these three molecules it is produced binding first Acyl-CoA to protein, and as result of the conformationals changes produced by the Acyl-CoA would enter the alcohol into the solvent channel. The model allowed us to clarify the enzymatic mechanism of VpAAT1.

Acknowledgements to Proyecto Anillo ACT-41.

Poster presentation 48: Genomics, Bioinformatics and Systems Biology.

Study of the aroma metabolic pathway in *Fragaria chiloensis.*

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Volatiles compounds from the native Chilean strawberry (*Fragaria chiloensis*) and cultivated strawberry (*Fragaria x ananassa*) were analyzed through gas chromatography-mass spectrometry and gas chromatography-olfatometry. A high concentration of 3-oxo-α-ionol and E-2-Z-6-nonadienol, were isolated only in fruits of the Chilean native strawberry. The 3-oxo-α-ionol compound contributes to floral and citric attributes of the native fruits and possesses interesting flavor aroma properties together with low aroma thresholds. The 3-oxo-α-ionol is a product of oxidative carotenoids cleavage. A potential carotenoid cleavage dioxygenase (FcCCD1) gene was identified in a 9,000 *Fragaria chiloensis* ESTs collection (MIFAB, Millennium Institute of Fundamental and Applied Biology). The open reading frame of the FcCCD1 consists of 1,565 bp encoding a 521 amino acid protein. The FcCCD1 is 78 % identical to LeCCD1A, an enzyme responsible for the formation of ß-ionone in tomato (*Lycopersicon esculentum*), that has been shown to be the second most important volatile compound contributing to the tomato fruit flavor. Expression of the gene was studied by RT-PCR at different fruit developmental stages from Chilean strawberry ecotypes. An induction of the expression of FcCCD1 was observed in the latest stage of ripening. On the other hand the E-2-Z-6-nonadienol compound contributes to green and cucumber-like aroma attributes of the native fruits and it is a product of the fatty acid catabolism. A potential hydroperoxide lyase (FcHPL) gene was identified in *Fragaria chiloensis* trougth PCR degenerated primers. This gene present a high homology to the hidroperoxide lyase identified in other rosaceas species like *Malus domestica* and *Prunus persica*.

This research was supported by ICM P06-065-F; Proyecto regular UNAB DI-51-06/R

Poster presentation 49: Genomics, Bioinformatics and Systems Biology.

Cloning and characterization of the *Botrytis cinerea cox-5* gene.

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Botrytis cinerea also known as "gray mold fungus" causes serious pre- and post-harvest diseases in at least two hundred plant species. Management of *B. cinerea* infection is mainly practiced by chemical control to prevent infection. There are several commercially available botryticides which have different modes of action, but in general their efficiency is not durable because of the rapid development of resistance caused by the selective pressure of the fungicides (Leroux, 2004). One approach to prevent the natural course of the disease in the host is to study early steps of infection such as germination. By means of a cDNA library from *B. cinerea* (previously described), we identified putative *B. cinerea* orthologs of *cox-5* gene which is preferentially expressed during conidial germination. The *cox-5* gene encodes the V chain of cytochrome oxidase involved in respiration. The full-length cDNA obtained was about 838 bases long which included 498 bases coding sequence and one intron. Through expression analyses we found no significant differences in the expression levels during germination stage. However, to examine the biological role of the *cox-5* gene we created disruption and knockout mutants, which were unable to complete germination in the presence of full nutrients. This result showed that *cox-5* gene has an important function in germination and could represent a new target to generate novel strategies of control to prevent the infection by this pathogen.

Poster presentation 50: Genomics, Bioinformatics and Systems Biology.

The role of cytokinin signalling in the nitrate response of *Arabidopsis thaliana.*

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Nitrogen is an essential macronutrient for plant growth and development. Nitrate is the main source of nitrogen available in agricultural soils and can act as a signal to modulate global gene expression in *Arabidopsis thaliana,* but the molecular mechanisms involved in the plant response to nitrate are unknown. Research from our group and others suggest that plant hormones, and especially cytokinins, play a key role in the nitrogen response. For example, cytokinin levels increase rapidly after nitrate treatment of N-starved plants. The expression of genes involved in cytokinin biosynthesis and signal transduction is induced by nitrate treatments. As a first step to understand the role of cytokinin in the nitrogen response, we evaluated the response to nitrate treatments of the hyposensitive cytokinin receptor mutants *ahk2, ahk3, ahk4,* as well as combinations of double mutants of these receptors. Phenotypic analysis of the double mutants under different conditions of carbon and nitrogen indicate that cytokinin signal transduction is required for the primary root growth induced by nitrate. This growth phenotype is not a general root growth defect in the mutant, but it is manifested in the presence of nitrate. This result suggests that cytokinin act as a signal downstream of nitrate. Interestingly, none of the individual mutants showed phenotypic differences as compared to wild type plants. This result indicates that the nitrate effect is not mediated by a specific receptor, but is a consequence of a general defect in cytokinin signalling. Current studies aim to better understand the nitrate/cytokinin interaction and the mechanisms underlying the cytokinin mutant phenotype.

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Poster presentation 51: Genomics, Bioinformatics and Systems Biology.

Differential expression of genes involves in ethylene biosynthesis during gravitropic response in radiata pine (*P. radiata* D. Don).

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Gravistimulation of conifer tree stems affects the normal wood development by unilaterally inducing wood with modified chemical and physical properties, called compression wood. The response mechanism is unknown but it is suggested the involvement of the phytohormone ethylene, nevertheless, the pattern expression of genes involved in the biosynthesis of ethylene during the gravitropic response in gymnosperm trees is still unknown. Using Real time PCR technique we evaluated the genes expression involves in the biosynthesis of ethylene during the gravitropic response in the stem, separated as upper and lower half which generate opposite and compression wood, respectively. 1-year old radiata pine seedlings (*Pinus radiata* D. Don) were exposed to gravitropic stimuli and harvested at both early times response 2,5h and 10h, and late times at 24h and 1 month. The genes encoding 1-aminocyclopropane-1-carboxylate oxidase (ACO) were expressed differentially across the tissues and, most importantly showing a differential expression between both sides of inclined stems whereas the previous gene in the pathway 1-aminocyclopropane-1-carboxylate sintetase ACS showed the same patterns.

Finantial support from Ecos-Conicyt C07-B01, Fondecyt 1071026 and Conicyt doctoral scholarship to PR.

Poster presentation 52: Genomics, Bioinformatics and Systems Biology.

Identification of molecular markers in *Prunus persica* candidate genes that are co-regulated under different post-harvest conditions.

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In order to identify candidate genes that may be incorporated into a peach marker assisted breeding program for 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 genes under different post-harvest conditions. These clusters of co-regulated genes include clusters of genes that show induction/repression in ripe fruits, induction/repression during cold-storage, or induction/repression in woolly fruits.

Members of each cluster of co-regulated genes have been analyzed bioinformatically to identify Single Nucleotide Polymorphisms (SNPs) and/or Expressed Sequence Tag - Simple Sequence Repeats (EST-SSR). Analyses using sequence information from 11 different varieties of *Prunus persica* have revealed 20 putative SSRs and 555 putative SNPs in these genes.

Further analyses of these co-regulated genes, the polymorphisms detected in these genes and the transcriptional networks that regulate the expression of these genes may provide insight into the divergent phenotypes detected in these varieties. Additionally, the molecular markers detected in these genes may serve as useful tools in marker assisted breeding programs to improve post-harvest fruit quality in peaches and nectarines.

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Poster presentation 53: Plant Physiology.

Tolerance strategies of Quinoa plants under salt stress.

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Salinity is an ever-present threat to crop productivity, especially in countries where irrigation is an essential support of agriculture. Unfortunately, there is a strong link between irrigation and salinization, which questioned the sustainability of this strategy to increase food production. Thus, basic and applied research on crop salt tolerance has become a main target for plant breeding. The quinoa (*Chenopodium quinoa* Willd.) is considered one of the few crops naturally adapted to extreme climatic and geographical conditions typical of arid or semi-arid. Quinoa can also grow at sea level near the coast, in the presence of high levels of salinity and scarce precipitation. Quinoa grain represents an important protein source for Andean Altiplano population (3600-4200 m.o.s.l.).

Ten different Chilean ecotypes of quinoa collected along a latitudinal gradient of ca. 2700 kilometers are currently being characterized by monitoring growth and development throughout ontogeny.

Aim of this research is to analyze salt-induced tolerance mechanisms in quinoa. We investigated salt-induced modifications on a) growth and development, in particular on transition from the vegetative to the reproductive phase, and b) biochemical parameters and expression of genes associated with salt stress.

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

Poster presentation 54: Plant Physiology

Role of Raffinose Family Oligosaccharides (RFOs) in Lupin Seed.

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Lupinus luteus seeds are one of the best sources of protein for salmon industry, but unfortunately they contain high content of raffinose family oligosaccharides (RFOs). In monogastric diets, these sugars are considered antinutritionals since they are not digested, producing flatulence when metabolized by the intestinal microbiota. RFOs, however, seem to be important for plant fitness. Many roles for RFOs in plants have been suggested, including: phloem loading, carbon storage, desiccation tolerance, cold and drought acclimation, seed longevity and germination. This study has been initiated to understand the importance of RFOs in lupin seed germination process and symbiosis establishment with nitrogen-fixing bacteria. In order to fulfil these objectives, first we will determine RFOs localization in seed by analyzing by HPLC the sugar content of different parts of the seed. Secondly, the expression of the key enzyme of RFOs synthesis will be followed during seed maturation using northern-blot. Thirdly, we will test the presence of RFOs in exudates of germinating seed under different environmental conditions. Finally, some chemotaxis experiments using a microcapillary technique will be conducted on Bradyrhizobium sp., which is a lupine nitrogen-fixing bacteria symbiont. This knowledge is necessary to develop biotechnological strategies and generate new lupin varieties with low levels of RFOs in seeds.

This work is supported by FONDECYT.

Poster presentation 55: Plant Physiology

Water stress induced thermotolerance in bean plants photosynthetic apparatus.

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Higher plants are constantly exposed to environmental stresses against which they must develop protective mechanisms. It has been commonly observed that exposure to a particular stressing factor would induce protection against a different one, phenomenon known as crossed tolerance. In bean plants we observed water stress induced termotolerance of the photosynthetic apparatus. We have found that the temperature threshold for non reversible damage in bean plants would be significantly increased under drought conditions as observed by the temperature dependent minimal fluorescence (Fo) kinetics. Also, oxygen evolution under high CO_2 air is negatively affected in water stressed plants up to a lesser extent compared to well watered plants. The basis for the observed termotolerance in bean plants grown under water stress conditions are discussed comparing their pigments content, lipids and fatty acids composition to well watered plants in two bean varieties, which have some differences in tolerance to abiotic stress. Here we report that Orfeo INIA variety reduce the proportion of monogalactosyldiacylglicerol and increase the satured acid content into thylacoid membranes under drought conditions, compared to Arroz Tuscola variety. Our results suggest a possible role for this lipids rearangement in the thermotolerance phenomenom.

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Poster presentation 56: Plant Physiology

Inhibition of ethylene in apricot fruit: action versus byosinthesis.

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Apricot (*Prunus armeniaca* L.) fruit is highly susceptible to flesh softening and loss of flavor, particularly during postharvest storage. Most of these changes are under ethylene regulation. During the last two seasons we have studied the effect of 1-methylcyclopropene and aminoethoxyvinylglycine (an ethylene action and an ethylene synthesis inhibitor, respectively) on quality attributes of Modesto and Patterson apricot cultivars. Both ethylene inhibitors have been effective in reducing ethylene production, fruit softening and color development. On the other hand, soluble solids concentration and titratable acidity have shown an ethylene-independent pattern. In order to understand the effect of both inhibitors in the ethylene biosynthetic pathway, we identified, cloned and characterized the expression pattern of the key genes involved in ethylene synthesis and perception (ACS, ACO, ETR, ERS and EIL). The expression profile was characterized by qPCR as ripening progressed at 20 °C after harvest. The significance of the changes measured during apricot ripening is discussed. (Fondecyt 1060179).

Poster presentation 57: Plant Physiology

Modulation of *Pao* gene expression and chlorophyll concentration in leaves of *Vitis vinífera* cv Carménère by water availability.

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Chlorophylls are the centrepiece for light harvesting in higher plants and other photosynthetic organisms. Under stressing conditions, chlorophylls are degraded as a protective strategy as well as the result of leaf senescence. Even though leaf senescence starts with chlorophyll degradation, human visual perception of such event is possible only after at least two weeks from triggered. The precise knowledge of its occurrence is, in turn, crucial for plant nutrition practices. In the path for degradation, chlorophyll is converted to colourless nonfluorescent chl catabolites by the enzymes pheide a oxygenase (PAO) and red chl catabolite reductasa (RCCR). In this study we report *Pao* gene expression in leaves of *Vitis vinífera* cv Carménère under different irrigation regimes along the season. To determine the presence of this gene in *Vitis vinífera* and because the cultivar Carménère has not been sequenced, we determined *in-silico* the presence of the gene *Pao* in the database of GENOSCOPE. This allowed us to generate a likely sequence corresponding to *Pao* of *Vitis vinifera* and helped us in the design of primer pairs, sequencing its open reading frame (ORF). Subsequently, it was determined the concentration of total chlorophylls in leaves and the *Pao* gene expression by means of Real-time PCR. From March on, expression of *Pao* increased with the subsequent decrease of chlorophyll content. Increasing water stress conditions increased the expression of Pao, reducing the chlorophyll content of leaves as a result of a senescing process.

Poster presentation 58: Plant Physiology

Flavonoid compounds in Carménère (*Vitis vinifera* L.) berry grapes exposed to different light intensities.

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Flavonoids are, among others, important compounds for organoleptic attributes of wine. Anthocyanins, flavanols and flavonols confer color, astringency and bitterness to red wine and, also, determine the potential for aging. Their concentration results from a various combinations of factors from soil, weather and cultural practices. As for the latter, leaf removal exposing clusters to either direct or diffuse light are a common practice in cold climates viticulture. As for warmer climates, the effect of light exposure is still controversial. In the present study, the effect of light intensity on sugar content and pH, total phenols, tannins and anthocyanins content, as well as gene expression for enzymes of the phenylpropanoid pathway were assessed in Carménère berry grapes from a Maipo vineyard. Also, determination of the anthocyanin profile of berry skins was determined. As expected, in the Maipo Valley, light exposure severely affects berry skin and flesh temperature. However, no effect is observed on berry size and volume, as well as sugar concentration, acid content and pH. From the three genes assessed, only DFR (dehydroxy-flavonol reductase) increases its expression upon light, but contrary to what has been reported so far, low light intensities increases expression. Low light intensity results in high anthocyanin content from veraison to harvest, particularly the glucosilated and acetyl-glucosilated forms compared to the cumaryl- glucosid forms. This is important for wine making since the former are less stable and, therefore, more extractable compared to the latter.

Poster presentation 59: Plant Physiology

Stem carbohydrate in recombinants chromosomes substitution lines (RCSLs) of barley (*Hordeum vulgare* L.) and its relation with drought tolerance.

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The stem carbohydrate (CHO) content of 24 recombinants chromosomes substitution lines (RCSLs) of barley (*Hordeum vulgare* L.) were evaluated at two environments, Cauquenes (without irrigation) and Santa Rosa, Chillán (with irrigation), during 2007/2008 growing season. The RCSLs were derived from the crossing of the accession of *H. vulgare* subsp. *spontaneum* (Caeserea 26-24 from Israel), as a donor, and *H. vulgare* subsp. *vulgare* cv. Harrington (the North American malting quality standard) as the recurrent parent. The cv. Harrington was used as reference in all environments. The experimental design was an α-lattice with four replicates and five incomplete blocks for each replicate. CHO was determined using the Anthrone method and spectrophotometer at 620 nm, at ear emergence and physiological maturity. In addition, grain yield, harves index and yield components were measured at the end of the growing season. The ANOVAs showed a strong and significant effect of the environments in all variables, indicating that CHO were higher in the driest environment (Cauquenes), but grain yield, 1000 kernel weight and harvest index were higher in the more favourable environment (Santa Rosa). Also, the genotypic effect was significant for all the variables. The correlation matrix showed no significant correlations between CHO and grain yield, harvest index or yield components in Cauquenes, but a positive and significant correlation between CHO at ear emergence and 1000 kernel weight ($r = 0.44$; $P < 0.05$) in Santa Rosa. However, the correlation between CHO and grain yield, harvest index and number of kernel per spike were negative in Santa Rosa.

Poster presentation 60: Plant Physiology

Influence of ethylene in aroma formation in packham´s triumph pears.

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Pear is a climacteric fruit and its ripening is regulated by ethylene. As the fruit ripens there is a significant increase in ethylene production and also in aroma formation, which is essential for the organoleptic quality. In pear the aroma is provided mainly by esters, which are synthesized by the enzyme alcohol acyl transferase (AAT). To elucidate the role of ethylene on the production of aroma and the AAT enzyme, the effect of 1-MCP, a blocker of the ethylene receptor, was tested. For that, 72 Packham´s Triumph pear fruits were divided into two groups: the first was treated with 1-MCP (200 ppb, for 12 h at 20°C), and the second with Ethrel (2000 ppm). After treatment the fruit was maintained at 20°C for up to 13 days, and assessments were conducted every 2-3 days (firmness, respiration rate, ethylene and volatiles production rate, AAT activity and AAT gene expression level). The 1-MCP treated fruit displayed a lower ethylene production rate than that of Ethrel treated fruit. Also a lower respiration rate during the first 5 days was observed in 1-MCP treated fruit, although no significant differences were found in other quality parameters (firmness, SS, acidity). It was also noted a lower rate of production of volatile compounds in 1-MCP treated fruit, which is coincident with a lower activity of AAT. A higher AAT activity was observed in pear skin compared to pulp tissue. These data confirm that ethylene has an important role on AAT and aroma formation in pear fruit.

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