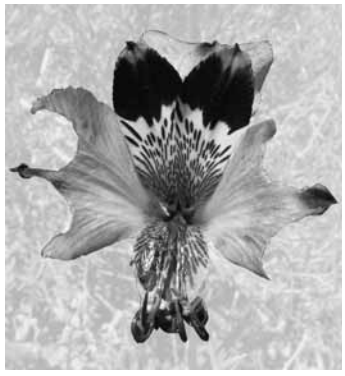

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MEETING PROGRAM

Wednesday December 1st

13:00-14:30	Registration and poster set up
15:00-15:30	**WELCOME** (Meeting Organizers- Lorena Norambuena)
15:30-16:30	**OPENING CONFERENCE** Chair: Manuel Paneque
	CONFERENCE 1. Enrique Rojo, A genetic switch for cell differentiation in *Arabidopsis*. Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología, CSIC (erojo@cnb.csic.es)
16:30-17:00	Coffee break
17:00-19:30	**ORAL SESSION 1** Chairs: Michael Handford and Lorena Norambuena
17:00	**CONFERENCE 2. Raúl Herrera, Studying wood formation in pine: combining omics approaches** Instituto de Biología Vegetal y Biotecnología, Universidad de Talca (raherre@utalca.cl).
18:00	**Ugalde J[1]**, Tejos R[2], Friml J[2] and León G. **The PIP5K1 and 2 enzymes are required for the normal reproductive development in *Arabidopsis thaliana***. [1] Laboratory of Plant Reproduction & Development, Center of Plant Biotechnology, Andrés Bello University, Chile.[2] Department of Plant Systems Biology, VIB Research Institute and Gent Universitiet, Belgium. (gleon@unab.cl)
18:30	Welcome Cocktail
19:00- 21:00	**Poster Session** (Odd number panels)
21:00	Dinner

Thursday December 2nd

7:45-8:45	Breakfast
9:00-11:00	**ORAL SESSION 2** Chairs: Claudio Pastenes and Alejandra Moya
9:00	**CONFERENCE 3. Erik H Murchie. Photoprotection and canopy photosynthesis in rice: prospects for improvement.**

Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, LE12 5RD, UK (erik.murchie@nottingham.ac.uk)

10:00 **Flores C,** Wegener G, Fuentes P and Stange P. **Expression and functional analysis of z-carotene desaturase 1 and 2 genes (zds1 y zds2) during *Daucus carota* development**. Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile (cstange@uchile.cl)

10:20 **Álvarez X** and Arce-Johnson P. **Morphological, molecular and ecophysiological characterization of transgenic citrus rootstocks developed to increase salinity tolerance.** Pontificia Universidad Católica de Chile. (parce@bio.puc.cl)

11:00 Coffee break

11:30-13:00 **ORAL SESSION 3**
Chairs: Claudia Stange and Manuel Paneque

11:30 **Salinas C[1]**, Huerta C[1], I Ramírez I[1], Freire M[1], Delatorre J[2] and Cardemil L[1] **The effect of water availability and temperature on Aloe barbadensis Miller (Aloe vera) physiology, gel production and composition** [1]Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile. [2]Departamento de Agricultura del Desierto, Universidad Arturo Prat. (lcardemi@uchile.cl)

11:50 **García-Rojas M[1]**, Gudenschwager O[2], Campos-Vargas R[1,3], Manríquez D[2], Defilippi B.G[2,3] and González-Agüero M[2,3]. **Internal browning development during cold storage of 'hass' avocados (*Persea americana* mill.): the role of acetyl-CoA carboxylase and stearoyl-ACP desaturase genes**.[1] Facultad de Ciencias Biológicas, Escuela de Ingeniería en Biotecnología, Universidad Andrés Bello.[2] Instituto de Investigaciones Agropecuarias (INIA) – CRI La Platina.
[3]The Plant Cell Biotechnology Millennium Nucleus (PCB-MN) (maugonzalez@inia.cl)

12:10 **Morales A[1,2]**, Zurita A[1] and Silva H[2,3] **Quinoa as Drought Tolerance Genes Source.** [1]Centro de Estudios Avanzados en Zonas Áridas, CEAZA. [2]Plant Functional Genomics & Bioinformatics Lab and Millennium Nucleus in Plant Cell Biotechnology (PCB), [3]Universidad San Sebastian (herman.silva@gmail.com)

12:30 **Moreno A**, Blanco F, Moreno I and Orellana A. **Characterization of the AtIRE1 / AtbZIP60 signalling pathway and the regulation of the AtbZIP60 mRNA splicing under different stress conditions in *Arabidopsis thaliana***. Núcleo Milenio en Biotecnología Celular Vegetal. Centro de Biotecnología Vegetal. Universidad Andrés Bello. Santiago, Chile. (aorellana@unab.cl)

13:00-14:30 Lunch

15:00-17:30 **ORAL SESSION 4**
Chairs: Herman Silva and Loreto Holuigue

15:00 **Villalobos L**, Ibáñez F, Pastenes C. **ABA mediated secondary metabolism in grape berries cv. *Carmenere*.** Laboratorio Fisiología del Estrés en Plantas. Facultad de Ciencias Agronómicas, Universidad de Chile (cpastene@uchile.cl)

15:20 **González AS**[1], Olea P[1], Bordeu E[1], Geny L[2], Alcalde J.A[1], Zuñiga A[1] **Effect of abscisic acid, (2-chloroethyl) phosphonic acid and indole-3- acetic acid on *Vitis vinifera* L. cvs *Cabernet sauvignon* and *Carménère* grapes and wine**. [1]Department of Fruit Production and Enology, Faculty of Agronomy and Forestry. Engineering, Pontificia Universidad Católica de Chile. Casilla 306-22, Santiago.[2]UMR 1219 Université Victor Ségalen Bordeaux2-INRA. Faculté d'Oenologie - ISVV 210 Chemin de Leysotte CS 50008. 33 882 Villenave d'Ornon. (ebordeu@uc.cl)

15:40 **Bastías A** and Casaretto J.A **Modulation of the metabolism in tomato fruits by an abscisic acid-regulated transcription factor**. Instituto de biología vegetal y biotecnología, Universidad de Talca, Talca (Chile). (jcasaretto@utalca.cl)

16:00 Coffee break

16:30 **CONFERENCE 4. Pablo Figueroa** [1] and John Browse . **MYC5 transcription factor is involved in jasmonate-dependent male reproductive development in *Arabidopsis***. Institute of Biological Chemistry, Washington State University, Pullman, Washington 99164-6340, USA [1]Current address: Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Alameda 3363, Santiago. (pablo.figueroa.l@usach.cl)

17:30 **Organizational Session –VI PLANT BIOLOGY MEETING**

19:00- 21:00 **Poster Session** (Even number panels)

21:00 Dinner

Friday December 3rd

8:00- 9:45 Breakfast

10:00-11:30 **ORAL SESSION 5**
Chairs: Andrés Zurita and Claudia Stange

10:00 **CONFERENCE 5. Sergio Svistoonoff[1]**, Leandro Imanishi[1,2], Hassen Gherbi[1], Valérie Hocher[1], Laurent Laplaze[1], Didier Bogusz[1] and Luis Wall[2] and Claudine Franche[1] **New insights in the molecular events underlying actinorhizal nodulation**.[1] Equipe Rhizogenèse, UMR DIAPC, IRD (Institut de Recherche pour le Développement), 911 avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France. http://www.mpl.ird.fr/rhizo. sergio.svistoonoff@ird.fr [2] Programa Interacciones Biológicas Departamento de Ciencia y Tecnología Universidad Nacional de Quilmes R. Sáenz Peña 352, B1876BXD Bernal, Argentina.

11:00 **Rojas P** [1], Caligari P.D.S[1], Sandoval C[2], Keller K[3] and Martin RR[3]
***Fragaria chiloensis* L. (Duch) viruses detected with Illumina sequencing** [1]Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Chile. [2]Facultad de Ciencias Agrarias, Universidad de Talca, Chile.3USDA- ARS, Horticultural Crops Research Laboratory, Corvallis, Oregon, USA. (projasb@utalca.cl)

11:20 Coffee break

12:00-13:00 **ORAL SESSION 6**
Chairs: Gabriel León and Claudio Pastenes

12:00 **Moyano TC[1,2]**, Vidal EA[1,2], Krouk G[3], Tanurdzic M[4], Coruzzi GM[3] and Gutiérrez RA[1,2,3] . **Identification of novel nitrate responsive small RNAs by Illumina high-throughput sequencing technology**.[1]Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile. [2]Núcleo Milenio en Genómica Funcional de plantas.[3]Department of Biology, New York University. [4] Cold Spring Harbor Laboratory. (tcmoyano@uc.cl)

12:20 **Armijo G**, Seguel A, García C, Salinas P, Leiva D and Holuigue L. **Functional analysis of LLP, a lectin like protein induced by salicylic acid in *Arabidopsis thaliana* and involved in the defense response against *Pseudomonas syringae*.** Departamento de Genética Molecular y Microbiología, Facultad Ciencias Biológicas, P. Universidad Católica de Chile, Santiago. (giarmijo@uc.cl)

12:40 Álvarez J M, **Riveras E**, Aceituno F, Gras D, Tamayo K P and Gutiérrez R A **TGA1 and TGA4 control root nitrate responses in *Arabidopsis thaliana*.** Núcleo Milenio en Genómica Funcional de Plantas. Departamento de Genética Molecular y Microbiología. Pontificia Universidad Católica de Chile. (rgutierrez@bio.puc.cl)

13:00-14:30 Lunch

15:00-17:00 **ORAL SESSION 7**
Chairs: Liliana Cardemil and Loreto Holuigue

15:00 **CONFERENCE 6. Ariel Orellana.The re-glucosylation of proteins during quality control in the Endoplasmic Reticulum in *Arabidopsis thaliana***. Center for Genome Regulation, Millennium Nucleus in Plant Cell Biotechnology, Center of Plant Biotechnology, Andres Bello University, Santiago, Chile. (aorellana@unab.cl)

16:00 **Pérez P** and Norambuena L. **The compound Sortin2 mode of action is unique disrupting *Arabidopsis thaliana* endomembrane system** . Plant Molecular Biology Laboratory, Faculty of Science, University of Chile. Plant Cell Biotechnology Millennium Nucleus. (lnorambuena@uchile.cl)

16:20 **Miranda J P** and Handford M. **Morphological and biochemical characterization of plants with lower expression of *GONST3* and *GONST4*, nucleotide-sugar transporters of *Arabidopsis thaliana***. Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile 1 (mhandfor@uchile.cl)

16:40 Closing Ceremony

17:00 Coffee Break

OPENING CONFERENCE

INVITED SPEAKER

A GENETIC SWITCH FOR CELL DIFFERENTIATION IN ARABIDOPSIS

ENRIQUE ROJO

Departamento de Genética Molecular de Plantas,
Centro Nacional de Biotecnología, CSIC

Genetic networks for cell fate specification have been characterized in plants and animals, but the factors that first launch cells in the path to differentiation remain unknown. We have identified *MINIYO* (*IYO*), an Arabidopsis gene that is essential for initiating cell differentiation in all the plant meristems and during embryogenesis. IYO interacts with RNA polymerase II and is required for transcriptional reprogramming in differentiating tissues, where it supports transcriptional elongation activity on a genome-wide scale. IYO activity is restricted to cells initiating differentiation at the meristem periphery by means of transcriptional control and tightly regulated nuclear protein accumulation. Importantly, increasing IYO levels induces premature differentiation of meristematic cells. Our results identify IYO as the limiting factor for transcription of developmental programs in undifferentiated cells, and suggest that through targeted nuclear accumulation, IYO launches cell differentiation in Arabidopsis.

ORAL SESSION I

INVITED SPEAKER

STUDYING WOOD FORMATION IN PINE: COMBINING OMICS APPROACHES

RAÚL HERRERA
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Instituto de Biología Vegetal y Biotecnología, Universidad de Talca

An intricate molecular mechanism is involved in conifers wood formation. Particularly intriguing is the fact that the chemical and mechanical properties of wood vary within the stem position, which lead to spatially heterogeneous anatomical structure on the secondary xylem. This characteristic structure makes recognizable that within a growing season we could find six main type of wood named as: juvenile/mature, compression/opposite and early/late. This plasticity is due to ontogenic and environmental factors. The molecular mechanism triggering these phenotypes is still in its infancy. A combination of strategies has been used to elucidate the complex pathways. Molecular biology tools provided information of particular genes differentially expressed and proteomic approach revealed proteins involved in wood formation. A complex differential expression of key genes has been observed, particularly in those associated with cell wall biosynthesis, such lignin biosynthesis, cellulose synthesis, and genes involved in drought stress and also transcriptional factors. Genes from these functional assignations were validated by qRT-PCR. At the proteomic level, the functional categories “defense and stress related” and “cell wall” were over-expressed at the base of the trunk, but “gene and protein expression”, “secondary metabolism” and “transport” categories were over-expressed in crown wood. The study recognizes genes and proteins related to these functional groups which are differentially expressed along the seasonal gradient.

Support by Fondecyt Project 1071026 and ECOS-Conicyt C07B01.

THE PIP5K1 AND 2 ENZIMES ARE REQUIRED FOR THE NORMAL REPRODUCTIVE DEVELOPMENT IN *Arabidopsis thaliana*.

JOSÉ MANUEL UGALDE[1], Ricardo Tejos[2], Jiri Friml[2] and Gabriel León[1]
gleon@unab.cl

[1]*Laboratory of Plant Reproduction & Development, Center of Plant Biotechnology, Andrés Bello University, Chile*
[2]*Department of Plant Systems Biology, VIB Research Institute and Gent Universitiet, Belgium*

The Phosphatidyl Inositols (Ptdlns) are membrane lipids located on the cytoplasmic side of animal and vegetal cells, and they have an important role in the integration of several transduction signaling pathways. The enzymes PIP5K1 and 2 belong to the family of enzymes that phosphorilate the carbon 5 of the inositol ring, using preferentially as substrate the PI(3)P or PI(4)P to produce PI(3,5)P2 or PI(4,5)P2. These proteins have an 86% of identity on their sequences and had been identified as important in the transduction signaling pathway generated by the auxin hormone in *Arabidopsis thaliana.* Both genes are expressed preferentially on the reproductive tissues of Arabidopsis, and in single mutant plants show mild defects associated to reproductive development. In contrast, we've found that double mutants show several problems associated to the reproductive development, including production of dead grains of pollen, defects in the plant ovules, reduction in the number of seeds produced by the siliques and alterations in the early embryo development. Besides of alterations in the pollen tube elongation, suggesting an important role of this enzymes on its polar growth. Using biosensors of Ptdlns (GFP:PH-PLCγ and YFP:PH-FAPP) we'll study the dynamic of the PI(3,4)P2 and PI(4,5)P2 in the pollen tube tip of wild type and in double mutant plants. These results suggest an important role of auxins in the reproductive development trough the Ptdlns in Arabidopsis.

Funded by Fondecyt 11080037 and Odysseus program, FWO

ORAL SESSION 2

INVITED SPEAKER

PHOTOPROTECTION AND CANOPY PHOTOSYNTHESIS IN RICE: PROSPECTS FOR IMPROVEMENT

ERIK H MURCHIE
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Division of Plant and Crop Sciences, School of Biosciences,
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Rice is a major source of calories for people in many parts of the world and raising the rate of biomass production is an important target for the improvements in yield and yield potential. Tropical rice is frequently subjected to high temperatures and high irradiance levels and the saturation of photosynthesis is common. We have been analysing the regulation of photosynthesis in both optimal and sub- optimal conditions: in particular, we are interested in the dynamic efficiency of photosynthesis and the potential limitations placed on carbon assimilation by fluctuating environmental conditions and on the effect of photooxidative stress responses induced by high tropical irradiance levels. Field data shows sustained reduction in quantum yield and induction of photoprotective processes in rice under high irradiance in both optimal and suboptimal conditions. Models predict that slow relaxation of the quantum yield of CO_2 assimilation will reduce the rate of leaf photosynthesis during periods of rapid fluctuation between saturating light and light-limitation and this applies to canopies where light dynamics within canopies in time and space can be highly complex. We have generated transgenic plants which are altered in the expression of key genes involved in the photoprotection in rice, including psbS (short term induction and relaxation of non-photochemical quenching, NPQ) and β -carotene hydroxylase (regulation of kinetics of NPQ) and we are investigating the impact of such alterations to both leaf and canopy photosynthesis. Results to date will be presented and prospectives for future application of photoprotection in crop improvement discussed.

EXPRESSION AND FUNCTIONAL ANALYSIS OF Z-CAROTENE DESATURASE 1 AND 2 GENES (zds1 y zds2) DURING *Daucus carota* DEVELOPMENT.

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

Z-carotene desaturase (ZDS) enzyme participates in the carotenoid biosynthesis carrying out the z-carotene desaturation to produce lycopene. Carotenoids are pigments that, in plants, participate in photosynthesis, photoprotection and synthesis of abscisic acid (ABA) phytohormone. Carotenogenic pathway is regulated by light in both leaves and fruits during development. In *Daucus carota* (carrot), carotenoids are synthesized in opposite conditions; leaves exposed to light and in modified root grown in darkness. Otherwise other plants, carrot is the only plant in which two putative z-carotene desaturase genes have been described, termed zds1 and zds2. Aiming to elucidate the underlying regulation of carotenogenic pathway in carrot, analysis associated with zds1 and zds2 expression and functional analysis were fulfilled.

ZDSs protein abundance is higher in modified root compared to leaves in a full-grown plant. Moreover, despite zds1 and zds2 genes are expressed in both organs, the expression patterning is different during plant development. In leaves, zds1 transcript levels are higher at early developmental stage whereas zds2 transcript levels are higher in late developmental stages. In roots, the amount of zds2 transcript accumulation is higher than zds1, especially at late developmental stages. The ZDS protein amount during carrot development complements these results. Zds1 silenced-plants were not available to obtain, while zds2 silenced-plants had a wild type phenotype without changes in carotenoid composition. These results indicate that despite both genes are expressed, zds1 is the essential gen for plant development and zds2 could not have a function in carotenoid biosynthesis in carrot.

Acknowledgments IFS C4784-1and Fondecyt 11080066

MORPHOLOGICAL, MOLECULAR AND ECOPHYSIOLOGICAL CHARACTERIZATION OF TRANSGENIC CITRUS ROOTSTOCKS DEVELOPED TO INCREASE SALINITY TOLERANCE

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Pontificia Universidad Católica de Chile

Salinity adversely affects non-halophyte plant growth and, especially, crop yields. In Chile, salinity occurs mainly in soils and water from the valley of Copiapo to the north, which coincides with very favorable climate. Citrus species are an interesting alternative for commercial production under this climate, however, they have a very low salinity tolerance (10-15 mM NaCl), which restricts its production area.

The objetive of this reserach was to develop and evaluate transgenic plants of citrus rootstocks. Epicotils fron different citrus rootstocks were transformed with *Agrobacterium tumefaciens* with genes involved in the cascade of response to salinity, which have roles at a transcriptional control level and in direct mechanisms of stress response. We obtained transgenic lines of 'C-35' citrange 'Carrizo' citrange, *Citrus macrophylla* and trifoliate orange 'Rubidoux'.

For salinity tolerance assesment, transgenic plants were transfered to sand filled pots and irrigated with salinized solution (25% Hoagland solution suplemented with 50-75 mM NaCl) for 4-8 weeks, under greenhouse conditions. Transgenic lines with higher performance were selected for a molecular, morphological and ecophysiological characterization. We evaluated dry matter accumulation, mineral content, gas exchange parameters, chlorophyll fluorescence and water potentials, among others. Selected lines were propagated and are growing in order to be grafted with commercial varieties, to assess how transgenic rootstock performance influences commercial varieties salinity response. We are still evaluating new transgenic lines.

ORAL SESSION 3

THE EFFECT OF WATER AVAILABILITY AND TEMPERATURE ON *Aloe barbadensis* MILLER (ALOE VERA) PHYSIOLOGY, GEL PRODUCTION AND COMPOSITION

SALINAS C[1], Huerta C[1], Ramírez I[1], Freire M.[1], Delatorre J[2] and Cardemil L[1]
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[2]*Departamento de Agricultura del Desierto, Universidad Arturo Prat.*

Aloe Vera (*Aloe barbadensis* Miller) is a xerophytic CAM plant, original from Africa, naturally adapted to arid environments and cultivated in the IV Region of Chile since the year 2000. The objective of this work was to see if the conditions of the IV Region would affect the physiology of the plant and the production and quality of the gel and leaves which have a great commercial value due to medicinal, cosmetic and nutritional qualities. To study the physiological responses of Aloe vera to these conditions, we determined the growth and production of gel, quantifying the water use efficiency (WUE) of plants under different water regimes and temperatures. We also quantified the expression of genes encoding proteins associated with stress responses such as heat shock proteins (HSP), ubiquitin, and superoxide dismutase (SOD). The presence and concentration of sugars and polysaccharides responsible for the osmotic adjustment of the plant and for the gel economical qualities were also evaluated. Our results indicated that Aloe vera increases the WUE with increasing water deficit, due to a very efficient plant osmotic adjustment. Total sugar content increased significantly under high temperatures and water deficit. Analysis of partially methylated alditol acetates by GC-MS of reserve polysaccharides (fructans) and gel polysaccharides (acemannans) showed that the glycosidic linkages of these carbohydrates varied significantly during drought conditions. Semi-quantitative RT-PCR of heat stress genes (*hsp70*, *hsp100, ubiquitin* and *sod*) and western blot analyses for protein accumulation, indicated that both, gene expression and protein accumulation increase under water deficit and high temperatures. Our results lead us to the conclusion that heat stress and drought induce physiological and molecular changes in Aloe vera, changing the gel composition which could affect the commercial value of the gel.

Research supported by MULT 05/30-2 of the Dirección de Investigación, Universidad de Chile FONDECYT 1070899 and FONDECYT 7080094.

INTERNAL BROWNING DEVELOPMENT DURING COLD STORAGE OF 'HASS' AVOCADOS (*Persea americana* MILL.): THE ROLE OF ACETYL-COA CARBOXYLASE AND STEAROYL-ACP DESATURASE GENES

GARCÍA-ROJAS M[1], Gudenschwager O[2], Campos-Vargas R[1,3], Manríquez D[2], Defilippi B G[2,3] and González-Agüero M[2,3].
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Chile is one of the most important exporters worldwide of 'Hass' avocados (*Persea americana* Mill.). This variety is grown and exported from Chile to distant markets, especially Europe and EE.UU. During cold storage and shipment avocado fruit is affected by several processes, including internal browning, softening and water loss. Internal browning is caused by the interaction of maturity stage, senescence and temperature conditions during post-harvest life. Despite of the importance of this disorder, the biochemical and physiological processes involved remain unknown. In order to understand the causes of internal browning, a forward suppression subtractive hybridization (SSH) cDNA library was constructed. To date around 500 differentially expressed genes that codify for key enzymes involved in different biochemical pathways have been identified. From this group of genes, two of them showed a pattern of expression related to internal browning, i.e. acetyl-CoA carboxylase (ACCase) and stearoyl-ACP desaturase (SAD). RACE PCR assays were carried out to obtain full-length cDNA of two subunits of ACCase heteromeric form. Real-time quantitative PCR were performed to analyze transcription profiles for ACCase and SAD genes. In avocados with a high incidence of internal browning (stored for 40 days at 0°C) a decrease in the expression of ACCase subunits (*PamBC* and *PamBCCP*) during ripening was observed, and *PamBCCP* was expressed specifically in fruit. Moreover, *PamSAD* has a different response to the other analyzed genes, since it remains suppressed during storage at 0°C in fruit with of internal browning. The significance of these changes in expression will be discussed

Funded by Fondecyt 11080236.

QUINOA AS A DROUGHT TOLERANCE GENES SOURCE

ANDREA MORALES[1,2], Andres Zurita[1] and Herman Silva[2,3]

[1]*Centro de Estudios Avanzados en Zonas Áridas, CEAZA.*
[2]*Plant Functional Genomics & Bioinformatics Lab and Millennium Nucleus in Plant Cell Biotechnology (PCB),*
[3]*Universidad San Sebastian.*

Chenopodium quinoa is an important grain crop from the Andean region of South America. Recently, quinoa has gained international attention for its high nutritional value and tolerance to several abiotic stresses. Field determinations had shown that quinoa is tolerant to drought. We have assessed drought tolerance in several Chilean genotypes of quinoa in order to select the most tolerant. Through physiological parameters such as Fv/Fm, electrolyte leakage and relative water content we determined that the northern ecotype R49 is the most tolerant to drought. This ecotype can grow in a soil with -1.5MPa hydric potential. Our results shown that the R49 ecotype can survive in relative water content lower than 25%. Through the same screening strategy we are also developing a functional genomics platform to unravel the genes involved in drought tolerance. This strategy is based in the generation of a drought stressed quinoa full length cDNA library. This library is being used to transform *Arabidopsis thaliana* in a high throughput pipeline to screen for drought-tolerant transgenic plants. A second library, a subtraction cDNA library, is being constructed and a set of 1,000 differentially expressed transcripts will be sequenced. Based on the sequence analysis, metabolics pathways will be constructed by comparing these sequences to other reported plant sequences in response to drought stress.

This research is supported by: Project BioTecZA /Innova 06FC01IBC-71, Millennium Nucleus in Plant Cell Biotechnology ICM P06-065-F and CONICYT Fellowship D-21080654 to AM.

CHARACTERIZATION OF THE AtIRE1 / AtbZIP60 SIGNALLING PATHWAY AND THE REGULATION OF THE AtbZIP60 mRNA SPLICING UNDER DIFFERENT STRESS CONDITIONS IN *Arabidopsis Thaliana*

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Universidad Andrés Bello.
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The plant ortholog of IRE1, a sensor of the unfolded proteins inside of the endoplasmic reticulum, has been previously identified and characterized. Nevertheless, until now no substrate has been proved to be associated to the RNAse function of this sensor in plants. We used bioinformatics approaches and RT-PCR to identify a messenger that is spliced under ER stress conditions which processing depends on IRE1. Also to get information about the occurrence of this splicing event under physiological conditions, subsequent RT-PCR assays led us to analyze the presence of the spliced form of this messenger during plant development, abiotic stress and in treatments with hormones involved in the biotic stress (salicylic acid and methyl jasmonate). From our analysis we observed that the AtbZIP60 mRNA is spliced under ER stress conditions and this process is dependent of the presence of AtIRE1-1 and AtIRE1-2 because double mutant plants in these genes lack of the spliced form of the AtbZIP60 mRNA. Also the presence of this splicing form is detected in the plant buds, anthers and under treatment with salicylic acid. These results suggest that AtbZIP60 mRNA is processed under ER stress conditions and this process depends on IRE1 indicating that the IRE1 signaling pathway is also present in plants. Moreover our results explain the occurrence of two AtbZIP60 protein forms under ER stress conditions in a different way to the proteolically mechanism previously proposed.

Supported by MN-PCB ICM P06-065-F, Basal Project PFB-16 and Fondecyt No 3100036. *Moreno A. is supported by CONICYT-Doctoral fellowship.

ORAL SESSION 4

ABA MEDIATED SECONDARY METABOLISM IN GRAPE BERRIES CV. CARMENERE

LUIS VILLALOBOS, Freddy Ibáñez and Claudio Pastenes.

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The onset if ripening in winegrapes is mainly responsible for the final content of flavonoids. Recently, the phytohormone Abscisic Acid (ABA) has been proposed as one of the main signals triggering the onset of ripening in non-climacteric fruits, affecting secondary metabolism in berry skins. In order to better understand the mechanism by which ABA effects gene expression for the phenylpropanoid pathway and their corresponding metabolites, applications of exogenous ABA -few days before *veraison*- were made in the field in cv. Carmenere grape berries. ABA induced an increase of the *VvMYBA1, VvMYB4A, VvPAL, VvDFR, VvANS, VvUFGT* and *VvLAR2* transcripts, as well as the flavonoid content in grape berry skins from *veraison* to harvest. Coloring on ABA-treated fruits started earlier than control, accompanied by an increase in sugar content and a decrease in acidity. As for anthocyanin biosynthesis, the *VvPAL, VvDFR, VvANS, VvUFGT* and *VvMYBA1* transcripts followed a similar pattern than (+)-ABA content throughout the season reaching the highest level at *veraison*, while anthocyanins were maximum in concentration at 30 DAV, decreasing afterwards until harvest. ABA application resulted in a better quality and stability of anthocyanins. In relation to tannin biosynthesis, the levels of *VvLAR2* transcripts decrease from *veraison* to 30 DAV and then, increase again from 60 DAV onwards. Such pattern was paralleled by tannin concentration. Our results explain the strong relationship between drought and grape berry quality, as mediated by ABA.

EFFECT OF ABSCISIC ACID, (2-CHLOROETHYL) PHOSPHONIC ACID AND INDOLE-3-ACETIC ACID ON *Vitis vinifera* L. CVS CABERNET SAUVIGNON AND CARMÉNÈRE GRAPES AND WINE

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The fruit of the grapevine (*Vitis vinifera L.*) is considered non-climacteric and because of this, its ripening would occur independently of ethylene, while abscisic acid is likely to be the key hormone during this period. However, even though this issue can greatly affect the quality of grapes and wine, recent research in this field show that the hormonal control of grape berry development remains controversial. In this work, changes in endogenous hormonal content following growth regulator applications were examined to determine their effects on grape skins and wine phenolic and general composition and quality of cultivars Cabernet Sauvignon and Carménère. Growth regulators applied were S-abscisic acid, (2-chloroethyl) phosphonic acid (CEPA) and indole-3-acetic acid (IAA). The treatments were made at veraison in commercial concentrations (400; 480; and 265 mg L^{-1}, respectively) during 2008 and 2009 seasons in a commercial vineyard in Cachapoal valley and in plants in pots in Maipo valley. Grape skin endogenous ABA and IAA levels were measured by HPLC coupled to DAD and fluorescence detectors. The chemical composition of grape skins and red wine were measured by HPLC coupled to a DAD detector for the analysis of anthocyanins and by spectrophotometry for phenolic compounds. Results show that applied ABA have a significant effect on increasing endogenous ABA and applied CEPA have a significant effect in reducing endogenous IAA grape skin content. CEPA and ABA treatments improved phenolic composition of grapes skins and wine without an increment on wine alcohol content. The relevance and projections of this work in relation to the hormonal control of grape berry ripening and possible future industrial applications to improve red wine quality are discussed.

MODULATION OF THE METABOLISM IN TOMATO FRUITS BY AN ABSCISIC ACID-REGULATED TRANSCRIPTION FACTOR

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Fruit development is a complex process that involves several coordinated physiological and metabolic changes, most regulated by plant hormones. Whereas ethylene can function at later stages during ripening of climacteric fruits such as tomato, new evidences point to a possible role of the phytohormone abscisic acid (ABA) in earlier stages of fruit ripening. We have previously shown that a particular group of bZIP transcription factors that mediate ABA- and stress-regulated gene expression in seeds and vegetative tissues in tomato are also expressed in fruits. In order to test the hypothesis of a possible function of these transcription factors during the development and ripening of the tomato fruit, transgenic plants that over-express and down-regulate one of these transcription factors, *SlAREB1*, have been used to analyze changes in gene expression and in the content of metabolites. Immature green, mature green and red ripe fruits of wild type and trangenic plants were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Metabolic profiles were generated and the results indicated that over-expression of *SlAREB1* caused an increment of several amino acids. Besides, other compounds such as organic acids and sugar derivatives were also altered. Moreover, down-regulation of *SlAREB1* resulted in lower levels of those metabolites. The higher content of some compounds correlated with increased expression of genes encoding proteins involved in their synthesis. Our data suggest that ABA signaling may be relevant for normal metabolism that takes place during fruit maturation.

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INVITED SPEAKER

MYC5 TRANSCRIPTION FACTOR IS INVOLVED IN JASMONATE-DEPENDENT MALE REPRODUCTIVE DEVELOPMENT IN ARABIDOPSIS

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Jasmonates play a critical role in both plant defense and male reproductive development. The transcription factor MYC2 has been described as a major player acting early in the JA signaling pathway in *Arabidopsis* vegetative tissue. Nevertheless, the identity of the transcription factor(s) involved in early JA-dependent responses in *Arabidopsis* male-reproductive tissue (e.g. stamens) is unknown. We assayed whether the closely related bHLH transcription factors MYC2, MYC3, MYC4 and MYC5 are involved in JA responses in male reproductive tissue by overexpressing each protein fused to an EAR repression motif. All the mentioned transcription factors evaluated with the exception of MYC5 were unable to block JA responses in male reproductive tissue. *MYC5-EAR* overexpression plants were male sterile with reduced stamen filament elongation, delayed dehiscence during pollination, reduced pollen viability, impaired pollen tube growth, and hyposensitive to JA-mediated root growth inhibition. Each of the phenotypes observed in *MYC5-EAR* plants are also observed in *coi1-1*, which is completely impaired in JA signaling. Although loss-of-function *MYC5* mutants did not show any JA-related phenotype, overexpression of *MYC5* resulted in stunted plants which were hypersensitive to JA-mediated root growth inhibition and overexpressing seedlings have higher content of anthocyanin than WT counterpart. *MYB* genes (e.g. *MYB21*, *MYB24*, *MYB57* and *MYB108*) which are up-regulated by JA in stamen from stage 12 flowers, are down-regulated in *MYC5-EAR* stamens, which suggest that MYC5-EAR blocks JA responses in male-reproductive tissue by down-regulating *MYB* genes. MYC5 transactivates JA-responsive promoters from vegetative and male-reproductive tissue (i.e. *JAZ2* and *MYB21*) in carrot protoplasts. In summary, our results are consistent with MYC5 being a positive and early regulator involved in JA-dependent male-reproductive development in *Arabidopsis*.

This work was supported by funding from the US Department of Energy and the Agricultural Research Center at WSU.

ORAL SESSION 5

INVITED SPEAKER

NEW INSIGHTS IN THE MOLECULAR EVENTS UNDERLYING ACTINORHIZAL NODULATION

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More than 200 species of dicotyledonous plants, mostly trees and schrubs, belonging to eight different families can enter actinorhizal symbioses with the nitrogen-fixing soil actinomycete *Frankia*. The establishment of the symbiosis involves both specific recognition of symbiotic partners and developmental adaptations of the host plant leading to the nitrogen-fixing nodule. Nevertheless, the key molecules that control specific recognition of the actinomycetal partner by the plant have not been characterized. On the plant side, molecular approaches and genomics have been developed to understand changes in gene expression resulting from the interaction with the actinomycete.
Great efforts are currently underway in our team to identify the changes in the global patterns of gene expression occurring during different stages of the symbiotic interaction by using microarray technologies in the actinorhizal plant *Casuarina glauca*. The goal is to identify key genes involved in the early steps of the symbiotic process, and to perform a comparative analysis in actinorhizal plants and legumes. ESTs that exhibit homology with the early symbiotic genes involved in the Nod factor transduction pathway of Legumes are currently being characterized. A functional analysis of *CgSymRK*, a gene from *Casuarina* homologous to the receptor-like kinase gene *SymRK/DMI2* from legumes, has been previously reported (PNAS, 2008, 105 :4928-4932). Silencing experiments based on RNA interference technology established that *CgSYMRK* was necessary for both nodule and mycorhiza (AM) formation in *Casuarinacea*e. Similar experiments are currently elaborated with *CgCCaMK*, a calcium-dependant calmodulin kinase-like gene from *C. glauca*, and with *CgNIN*, a gene encoding a putative transcriptional regulator necessary for bacterial invasion and nodule organogenesis in legumes. Using two other actinorhizal plants, *Alnus glutinosa* and *Discaria trinervis* we are also studying the conservation of molecular mechanisms leading to actinorhizal development.

Fragaria chiloensis L. (DUCH) VIRUSES DETECTED WITH ILLUMINA SEQUENCING

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Fragaria chiloensis ssp chiloensis (L.) Duch, the mother of the current commercial strawberry (*F x ananassa*), produces fruits with unique quality characters and has the potential to become a new alternative fruit in the market. However, several problems affect fruit yields including poor husbandry practices, low input production, short flowering period and viral diseases. With this latter problem in mind, wild and cultivated *F. chiloensis* plants were collected at different locations in southern Chile, in order to determine the viral status of the species. Aphid borne viruses have been found in wild and cultivated ecotypes mostly in mixed infections and it has been reported that these plants do not show symptoms. We used a high-throughput parallel sequencing system (Illumina) of double stranded RNA in order to determine the virological status of *F. chiloensis*. 75% of the contigs homologues to strawberry viruses corresponded to SMoV, 19% to SMYEV, 5% to SVBV and 1% to SCV. No evidence of new viruses affecting the species was found.

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ORAL SESSION 6

IDENTIFICATION OF NOVEL NITRATE RESPONSIVE SMALL RNAS BY ILLUMINA HIGH-THROUGHPUT SEQUENCING TECHNOLOGY.

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Nitrogen (N) is an essential macronutrient for plant growth and development and is available mainly as nitrate in agricultural soils. Nitrate can act as a potent signal to regulate genome-wide gene expression in plants. Previous studies have shown on a case-by-case basis that post-transcriptional regulation by small RNAs (sRNAs) is an important mechanism for nitrate responses in Arabidopsis roots. sRNAs are 21-24 nt non-coding RNAs that have emerged as key regulators of gene networks in plants and other organisms. The development of new technologies for high-throughput sequencing such as 454 and Illumina, has facilitated the discovery of many sRNAs in Arabidopsis and in other species.

In this study we identified novel nitrate responsive sRNA/target modules in Arabidopsis roots using the Illumina sequencing technology. We sequenced the sRNA fraction of nitrate- and control-treated Arabidopsis roots and obtained approximately 6 million reads from each sample. These sequences were analyzed using public and custom made bioinformatics tools. Using algorithms for predicting new miRNAs, we identified small RNA molecules corresponding to potentially novel miRNAs. We were able to validate some of these novel miRNAs using several independent lines of evidence. In order to complement our analysis, we also used a sequencing approach to identify new nitrate regulated genes using Illumina sequencing of the mRNA fraction. We found several genes regulated by nitrate that are not present in the ATH1 Affymetrix chip. We independently validated these new nitrate regulated genes by reverse transcription and real time quantitative polymerase chain reaction.

We identified and experimentally validate a novel nitrate-regulated miRNA/target module that might regulate carbon skeleton availability for nitrate assimilation in Arabidopsis roots. Our results show that sequencing approaches are powerful tools for the discovery of new genes and their regulation.

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FUNCTIONAL ANALYSIS OF LLP, A LECTIN LIKE PROTEIN INDUCED BY SALICYLIC ACID IN *Arabidopsis thaliana* AND INVOLVED IN THE DEFENSE RESPONSE AGAINST *Pseudomonas syringae*.

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Plants have evolved complex systems to respond and adapt to stressful conditions. The control of these mechanisms is mainly mediated by plant hormones, such as salicylic acid (SA). The role of SA has been mainly characterized in the defense response induced by biotrophic pathogens that are specifically recognized by the plant. Gene activation mediated by this hormone is essential for the local response of the plant and the subsequent systemic immunization. Previously in our laboratory, using *Arabidopsis thaliana* as a model, we identified a group of genes early-activated by SA, in which the LLP gene (from lectin-like-protein) has the highest level of activation. This gene codes for a protein with similarity to proteins of the legume lectin family that has not an associated biological function. LLP is activated by inoculation with the avirulent bacteria *Pseudomonas syringae* pv. tomato AvrRpm1 (Pst AvrRpm1) and this activation is SA-dependent. The purpose of this work is to evaluate the role of LLP in the defense response to pathogens in Arabidopsis, specifically in the plant-Pseudomonas interaction. For this, we isolated and characterized a homozygous mutant line null for *LLP*. In parallel, we developed transgenic lines overexpressing LLP fused to c-Myc epitope or to GFP protein. Our results of subcellular localization, by using confocal microscopy, indicate that LLP-GFP is located in the plasma membrane of the plant cell. Then we made a loss or gain of function analysis, by evaluating the proliferation of different strains of *Pseudomonas* in the null and overexpressor lines. We determined that LLP participates in the defense response to PstAvrRpm1, reducing bacterial proliferation. Currently we are investigating the specific role of this gene in the defense response. The results of this work will contribute to reveal the role of SA in plant-pathogen interactions, which can be used for future biotechnological applications.

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TGA1 AND TGA4 CONTROL ROOT NITRATE RESPONSES IN *Arabidopsis thaliana*

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Nitrate is one of the most important sources of nitrogen (N) in agricultural soils. Nitrate regulates plant root morphology and acts as a potent signal to control global gene expression in Arabidopsis. However, the mechanisms involved in regulating gene expression in response to nitrate in plants are still mostly unknown. We used a bioinformatics approach to identify regulatory factors that mediate nitrate responses in Arabidopsis. Our bioinformatics strategy produced a ranking of genes based on their "nitrate regulatory potential". The top candidate of our ranking was TGA1, a bZIP transcription factor. TGA4, is a closely related member of the bZIP family that was also found in the ranking. Both *TGA1* and *TGA4* mRNAs accumulated strongly and quickly after nitrate treatments. To evaluate the function of these transcription factors, we analyzed *tga1/tga4* double mutant phenotypes under different N-nutrient conditions. The *tga1/tga4* double mutant showed a shorter primary root than *wild-type* plants grown in a medium containing a sufficient amount of nitrate. To understand the molecular basis of this phenotype, we performed transcriptomic analysis to evaluate the effect of nitrate in the wild type and *tga1/tga4* double mutant plants using the Affymetrix ATH1 chip. Interestingly, 97% of the genes that depend on TGA1/TGA4 for normal expression are regulated by nitrate treatments. Among the nitrate-responsive genes that depend on TGA1/TGA4, we found the nitrate transporters *NRT2.1*, *NRT2.2* and the nitrite reductase genes. These genes are key for nitrate uptake and reduction. Using chromatin immunoprecipitation assays we discovered that TGA1 is bound to NRT2.1 promoter in a nitrate-dependent manner. These results indicate that TGA1 and TGA4 are important regulatory factors mediating the nitrate response of Arabidopsis root organs.

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ORAL SESSION 7

INVITED SPEAKER

THE RE-GLUCOSYLATION OF PROTEINS DURING QUALITY CONTROL IN THE ENDOPLASMIC RETICULUM IN *Arabidopsis thaliana*.

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Glycoproteins synthesized in the endoplasmic reticulum (ER) are subjected to a quality control to ensure the folding of proteins going through the secretory pathway. This process is based in the retention of proteins through the action of chaperones such as calnexin and calreticulin. These proteins bind the glucose residue present in monoglucosylated N-linked oligosaccharides of newly synthesized polypeptides, retaining the glycoproteins in the ER. Once the glucose linked to the oligosaccharide is cleaved off, the protein can migrate through the secretory pathway. However, if the protein is not completely folded, it is necessary to retain it until reaches the final conformation. A mechanism to retain these proteins in the ER is based in the reglucosylation of the N-oligosaccharide bound to the protein such that calnexin and calreticulin can bind the protein again. This mechanism requires a glucosyltransferase and UDP-glucose that is imported from the cytosol through a nucleotide sugar transporter. We have identified and characterized a glucosyltransferase (UGGT) and the transporters (AtUTr1 and AtUTr3) that seem to be involved in the reglucosylation of proteins in the ER from *Arabidopsis thaliana*. Mutants in UGGT are viable; however, they have shorter roots and their root hairs are abnormal. They are also less tolerant to stresses. Mutants in AtUTr1 or AtUTr3 show no obvious phenotype; although, they have a decrease in the uptake of UDP-glucose into the ER and show activation of the unfolded protein response (UPR). Attempts to obtain the double mutants were unsuccessful. Further analysis indicated that mutations in both genes lead to alterations in pollen and ovules.

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THE COMPOUND SORTIN2 MODE OF ACTION IS UNIQUE DISRUPTING *Arabidopsis thaliana* ENDOMEMBRANE SYSTEM

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The Endomembrane system (ES) is essential to a fully functionality of eukaryotic cells. Its complexity and redundancy have been overcome by the means of ES disrupting drugs. Sortin2 is a synthetic chemical compound isolated due to its effects over localization of vacuole soluble protein CPY in yeast and plants. It also alters vacuole morphology in plants. This shows that Sortin2 is disrupting the secretory pathway in both systems. Given the particular Sortin2 bioactivity it would be useful to characterize it in order to study and dissect new ES pathways. Since the secretory and endocytic route within the ES are highly connected our aim is to analyze the Sortin2 effect over the *Arabidopsis* endocytic pathway to fully understand its action mechanism. Using different fluorescent markers of the *Arabidopsis* ES we evaluated the behavior of the main endocytic compartments and specific protein trafficking under Sortin2 treatments using confocal microscopy. We found that Sortin2 alters endocytic route components such as endosomes and pre-vacuolar compartment (PVC). Consistently, Sortin2 provokes unusual trafficking of the auxin transporters PIN:GFP proteins from plasma membrane towards vacuole. Using characterized drugs such as Latrunculin B and wortmannin we found that this Sortin2-induced protein trafficking requires cytoskeleton functionality and PVCmediated trafficking. Sortin2 antagonize the exocitosis inhibitory effect of BFA since it stimulates the protein trafficking towards vacuole through PVC as it also does with the endocytic marker FM4-64 Sortin2. Altogether our result shows that Sortin2 mode of action is unique within the already known drugs. PIN protein differential degradation is the process allowing auxin to flow distinctly commanding root bending under gravitropic stimulus. The endocytic-emphasized effect of Sortin2 is sufficient to disrupt the gravitropic response due to the impairment of the required PIN2 differential degradation. Overall these results link a cellular disruption with a phenotype at a whole plant level.

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MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISATION OF PLANTS WITH LOWER EXPRESSION OF *GONST3* AND *GONST4*, NUCLEOTIDE-SUGAR TRANSPORTERS OF *Arabidopsis thaliana*

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For the synthesis and/or modification of glycoconjugates in the lumen of the Golgi apparatus, the presence of specific nucleotide-sugar transporters (NSTs) is required, importing the cytosolic nucleotide-sugars. We are characterising GONST3 (At1g76340) and GONST4 (At5g19980), putative NSTs in *Arabidopsis thaliana*. In our laboratory, we determined that GONST3 transports GDP-fucose and GDP-mannose, while GONST4 transports GDP-fucose and is the only NST described to date capable of transporting GDP-L-galactose.
In this work, we describe the morphological and biochemical effects of decreasing the expression levels of *GONST3* or *GONST4*. To achieve this goal, we performed semiquantitative RT-PCR and determined that *gonst3* lines (obtained by cosuppressive post-transcriptional gene silencing) have 40-50% expression compared to wild-type plants, while *gonst4* lines (from AGRIKOLA) have 50-60% expression.
When we studied the transport capacity in vivo with radioactive nucleotide-sugars, one of the *gonst4* lines was able to transport more GDP-mannose, although the transport capacity of GDP-fucose or GDP-L-galactose remained unchanged compared to wild-type plants. No differences were found between wild-type, *gonst3* and *gonst4* lines when the cell wall composition was analysed by HPLC.
However, at the morphological level, substantial differences in both *gonst3* and *gonst4* mutants were observed. We found that both have a conditional phenotype when grown in MS supplemented with 3% sucrose, but not in 1% sucrose or 3% sorbitol, corresponding to a greater number of lateral roots and modified root hairs. This phenotype was maintained by adding 10 mM fucose in the media, but was reversed with 0.025% butanol, which has been described as a specific inhibitor of Golgi-localised phospholipases. These results suggest that GONST3 and GONST4 might be closely linked to a phospholipase (ie. PLDζ2), which may mediate an alteration in the plasma membrane.

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POSTER SESSION

1.

CHARACTERIZATION OF THE ASCORBATE PEROXIDASE ENZYMATIC ACTIVITY IN A*loe barbadensis* MILLER (ALOE VERA) LEAVES DURING DROUGHT AND HEAT STRESS.

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Formed as natural byproducts of aerobic metabolism, reactive oxygen species (ROS) can rapidly oxidize and irreversibly damage proteins, lipids and DNA. Plants have diverse enzymatic and non-enzymatic mechanisms to prevent this damage, and CAM plants (like *Aloe barbadensis* Miller) are fully adapted to live continually exposed to abiotic stress. Ascorbate Peroxidase (APX) is a key enzyme regulating H_2O_2 levels and signaling in plant cells, protecting them from oxidative stress induced by the accumulation of this particular type of ROS. Our objective was to study the changes in specific activity of APX in Aloe Vera leaves under drought and heat stress conditions. Plants in drought conditions were watered according to the field capacity of the soil: 25%, 50%, 75% and 100% (T1, T2, T3 and T4 respectively). High temperature treatment was applied introducing the whole plants into an incubator at 25°, 35°, 40° and 45°C. Also combined treatments were made exposing drought stressed plants of T3 and T4 to 40° and 45°C. Results showed that APX specific activity remained relatively stable in the bases of the leaves in all stress treatments, but in the leaf tips of T2 and T3 the activity was higher than in control and bases samples, descending again in T4. In combined experiments of water stress and high temperature, the high levels of specific activity in T3 only decreased when plants were exposed to 45°. There wasn't any significant difference of activity in the plants subjected to the heat treatment alone, but native electrophoresis showed a new slow-migrating isoform of APX only in leaf bases under 40° and 45° treatments, as well as in the T3-40° combined treatment. Measurements of ascorbate and its oxidized form showed a decrease of this antioxidant in the most severe drought and heat treatments.

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2.

MOLECULAR CHARACTERIZATION THE GENE GERMIN OBTAINED OF *Deschampsia antarctica* DESV., INDUCED BY SALINE STRESS.

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Plant species are exposed to various environmental conditions, many of which might be adverse and affect their normal development and productivity. *Deschampsia antarctica* Desv., is the only plant grass inhabiting the Antarctic maritime. In extreme weather conditions characterized by conditions including high salinity. Its unique response to this stress condition, apparently due to the coordinated expression of genes, the product of millions of years of evolution and adaptation to their habitat, interesting to be identified and characterized, for future use in crop improvement agricultural.

Previous studies indicate that gene expression would be regulated by Germin salt and other abiotic factors. Besides being an oxalate oxidase has an important role in plant physiological development and homeostasis.

Our research group identified a group of genes differentially expressed in Deschampsia under natural conditions. BLAST sequence analysis, determined a 98% similarity to a transcript encoding a Germin of *Hordeum vulgare*.

To analyze the expression of this gene,tests were conducted in hidroponic culture of plants *Deschampsia antarctica* Desv. with different concentrations of NaC1.

The results of RT-PCR analysis to confirm the germin gene expression, the realtime PCR determined differences in expression levels and tissue-specific expression of the gene.

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3.

DEVELOPING STRATEGIES FOR IMPROVING TOLERANCE TO DROUGHT AND SALINITY IN CROPS.

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Soil salinity is one of the major environmental factors limiting agricultural productivity in many regions of the world, because most crops are glycophytes and usually salt sensitive. Salinity imposes two types of stress on plant tissues, which sequentially affect plant on many aspects, including mineral and water uptake, enzymes activities, photosynthesis and metabolism. One of these stress is the water deficit resulting from the relatively high solute concentrations of the soil, the other is ion-specific stresses resulting from altered K^+/Na^+ ratios, Na^+ and Cl^- concentrations that are unfavorable to plants. Grapevine (*Vitis* spp.) is the most economically important fruit species worldwide and is classified as having medium salt tolerance and high drought tolerance. The aim of our work is to design strategies for enhancing the adaptability of grapevine to saline soil, by using genetic engineering. For this purpose we constructed a series of gene cassettes that allow expressing genes conferring tolerance under the control of stress-inducible and tissue-specific promoters. Tolerance to stress conferred by these constructs is being assayed in Arabidopsis.

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4.

ROLE OF GLUTAREDOXIN GRXS13 IN PROTECTION TO OXIDATIVE STRESS IN ARABIDOPSIS

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Salicylic acid (SA) and reactive oxygen species (ROS) play a key role in cellular responses to different stress conditions. Our group has previously reported the identification of a set of early SA-induced genes (SAIGs); one of these genes codes for the glutaredoxin GRXS13. Glutaredoxins are small oxidoreductases involved in the maintenance of cellular redox homeostasis. We showed that GRXS13 gene is activated in response to SA, high light (HL) and methyl viologen treatments in Arabidopsis plants. We studied the subcellular localization of this protein, by expressing GRXS13 fused to GPF in transient transformation assays in tobacco plants. We detected that GRXS13-GFP fusion protein is located in the nucleus and the cytoplasm under basal conditions, and re-located to the chloroplasts under stress conditions. To determine the role of GRXS13 in oxidative stress we have obtained different transgenic lines that silence and over-express this gene. Gain and loss of function analysis indicate that GRXS13 is involved in tolerance to abiotic stress producing oxidative damage, such as high light and UV-B stress. Accordingly, the silenced plants showed retarded growth under short day conditions. To assess whether the role of GRXS13 in conferring abiotic stress tolerance is due to its capacity to protect cells from the oxidative damage, we are evaluating oxidative damage of proteins (carbonylation and glutathionylation). Our results indicate that over-expressor lines for *GRXS13* exhibit low levels of protein glutathionylation, while the silenced lines showed an increase in protein glutathionylation compared to WT. These results indicate that GRXS13 play an important role in redox homeostasis maintenance under oxidative stress conditions in Arabidopsis.

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5.

FUNCTIONAL ANALYSIS OF LLP *(lectin-like-protein)* PROMOTER OF *Arabidopsis thaliana* IN RESPONSE TO SALICYLIC ACID AND BIOTROPHIC PATHOGENS.

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LLP (*lectin-like-protein*) gene from *Arabidopsis thaliana* was identified in our group as a gene activated by salicylic acid (SA), a plant hormone that plays a fundamental role in the defense response against different types of stress, particularly biotrophic pathogens. This gene codes for a protein with similarity to proteins of the legume lectin family that has not an associated biological function. LLP is activated by inoculation with the avirulent bacteria *Pseudomonas syringae* pv. tomato AvrRpm1 (Pst AvrRpm1) and this activation is SA-dependent.
LLP activation by SA requires the co-activator NPR1 and TGA class II transcription factors (TGA2/5/6). These results guide us to study the *LLP* promoter as a model to understand the activation mechanism of genes by SA and pathogens. With this purpose, we identify over-represented motifs in the upstream sequence of *LLP* and other co-expressed SA-induced genes, by using a motif sampler analysis. In parallel, we made genetic constructs using the *LLP* promoter fused to *GUS* and *GFP* as reporters in the binary vector pKGWFS7, to obtain transient expression in *Nicotiana benthamiana*, and stable transformation in *Arabidopsis thaliana*. This approach will allow us to document the spatial and temporal promoter activity under different treatments with SA and virulent and avirulent strains of *P. syringae*, as well as to elucidate the promoter elements responsible for SA-mediated activation.

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6.

IDENTIFICATION OF TRANSCRIPTION FACTORS THAT ARE INDUCED IN RESPONSE TO HIGH SALINITY IN ROOTS OF *Arabidopsis thaliana*.

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Plants are constantly exposed to different conditions of biotic and abiotic stress. There is a partial overlap in the physiological responses of plants to these different stressful conditions. This overlap is also evidenced at the genetic level, which allows to identify genes that are activated by different or specific stress conditions. Among abiotic stress conditions, high salinity becomes very important because of its wide distribution and the negative effects on the plant growth. With the objective to identify transcription factors that are induced specifically in roots under salt treatments an *in silico* analysis of the Arabidopsis transcriptome was first realized, using public available microarray data. The microarray data were normalized in R through gcRMA, and to identify differentially expressed genes a two way anova test was used. To identify the key transcription factors and pathways that are differentially expressed specifically in response to high salinity, the lists of genes induced by different abiotic stresses were intersected by using the sungear tool. With this new list of genes a hierarchical clustering was performed. We selected genes that are specifically induced in roots during salinity conditions. The promoter sequences of this group of genes were analyzed to identify overrepresented motifs. We also performed a gene network analysis with this group of genes, and selected TFs that showed the greatest number of interactions in the network and that were induced in roots under salt treatments. Among these factors, we selected an AP2 type factor, which might regulate an important group of genes that have overrepresented the gene ontology terms *protein binding, cell motility* and *motor activity*. Preliminary results show that this TF is induced in roots of Arabidopsis treated with 150 mM NaCl. Future experiments will assess the role of this gene in response to high salinity using mutant Arabidopsis plants in this TF.

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7.

TGA TRANSCRIPTION FACTORS MEDIATE GLUTAREDOXIN *GRXC9* GENE ACTIVATION BY SALICYLIC ACID IN *Arabidopsis thaliana*

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Salicylic acid (SA) is a plant signaling molecule that triggers the activation of genes which are essential for defense against different biotic and abiotic stresses. *GRXC9* gene, coding for a glutaredoxin with putative antioxidant function, is one of the genes transiently activated by SA we identified in Arabidopsis. We are interested in elucidate the mechanism of transcriptional activation of this gene by SA. *In silico* promoter analysis of *GRXC9* gene identified two putative SA-responsive *as-1*-like elements in its proximal region. These elements have been previously described as targets for bZIP factors from the TGA family in promoters of model defense genes. To elucidate whether factors from the TGA family were required for *GRXC9* activation by SA, *tga* mutants were used. Our results showed that SA-mediated transcription of *GRXC9* is abolished in *tga2/5/6* and *tga2/3/5/6* mutants, while is not altered in *tga1/4, tga3* and *tga7* mutants. These results indicate that subclass II of TGA factors (TGA2/5/6) are esential for *GRXC9* expression activated by SA. Furthermore, by using chromatin immunoprecipitation (ChIP) assays we detected that TGA2 and TGA3, but not TGA1, are constitutively bound to *GRXC9* proximal promoter in the presence or absence of SA. The transactivation and dimerization ability of these TGA factors were evaluated by using yeast two- and one-hybrid assays. According to our results, we propose that SA activates GRXC9 transcription via a co-regulator that interacts with the TGA 2/3 factors bound to the *as-1* elements of *GRXC9* promoter.

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8.

UPR SIGNALLING PATHWAY IS IMPORTANT IN THE STABLISHMENT OF DEFENSE RESPONSE IN *Arabidopsis thaliana*.

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Introduction: Salicylic acid (SA) is a key hormone in the defense response in plants and such response requires the synthesis of several glycosylated proteins (PRs). The transcriptional activation of *PR* genes depends of the interaction between the coactivator NPR1 and TGAs bZIP transcription factors. Moreover it has been described that the exogenous application of SA activates the transcription of genes related to endoplasmic reticulum stress (UPR, unfolded protein response) and this requires the NPR1 coactivator. Interestingly the transcription factors involved are not known. There are two transcription factors related to ER stress in plants, bZIP28 and bZIP60.
Based on this we proposed that the transcriptional activation of UPR genes required during the defense response is regulated by the interaction between bZIP28 and bZIP60 with NPR1.

Methods: We evaluate the involvement of bZIP28 and bZIP60 and the importance of NPR1 in the expression of UPR genes this in response to SA and tunicamycin treatments. Using qRT-PCR we analyzed the expression of several UPR marker genes *(BiP1/2, BiP3, UGGT, CNX, CRT2 and PDIL)* in WT and mutants plants *(bzip60, bzip28 and npr1-1)*. Also we analyzed the phenotype of *bZIP60 and bZIP28* mutant plants during the infection with pathogens related to the basal and induced defense responses.

Results: We achieve to identify different group of genes considering the dependence of the transcription factors analyzed. Interestingly the absence of the bZIP60 and bZIP28 genes renders the plants more susceptible to biotic stress comparing with wild type plants.

Conclusions: These results suggest a complex regulatory crosstalk between the classic components described for defense response and the UPR signaling pathways. Interestingly this represents the discovery of new components of the defense response in *Arabidopsis thaliana.*

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9.

CHARACTERIZATION OF *SLNAP1* AND *SLNAP2*, NAC-LIKE TRANSCRIPTION FACTORS ENCODING GENES IN *Solanum lycopersicum*.

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The NAC family of transcription factors is involved in different processes during plant development, including embryo and shoot meristem development, lateral root formation and auxin signaling. In addition, the role of these transcription factors has been associated with senescence, defense and plant response to abiotic stresses. Although the NAC family is widely present in plants but not in other eukaryotes, comprising an abundant group of transcription factors described in herbaceous plants, few *NAC* genes have been identified and characterized in commercial crops. In this study, we identified two members of this family in tomato (*Solanum lycopersicum*), showing gene expression profiles related to senescence and stress response from available global gene expression data sets. These *NAC*-like transcription factors genes *SlNAP1* and *SlNAP2* have putative orthology with NAP (NAC like activated by PI/AP3) from *Arabidopsis thaliana*. This observation is based on the presence of NAC family conserved domains in the predicted proteins, as well as phylogenetic analysis. Therefore, we analyzed the expression levels of these genes in different tomato tissues during plant development by RT-qPCR. The obtained results suggest a role in senescence, as it has been previous described in other plant models. Additionally, changes in gene expression in response to biotic and abiotic stresses were also analyzed. All these data indicate that these transcription factors may have a putative role in development and stress response. Ongoing experiments are being conducted to determine their function and effects when ectopically over-expressed in tomato and other model species.

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10.

EVALUATION OF THE FUNCTIONALITY OF *Daucus carota* PHYTOENE SYNTHASE GENES (psy1 and psy2) BY MEANS OF HETEROLOGOUS COMPLEMENTATION.

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Carotenoids are isoprenoid pigments synthesized by all photosynthetic organisms and some non-photosynthetic bacteria and fungi. In plants, carotenoids are synthesized in plastids, where they are constituents of light-harvesting complexes and photosynthetic reaction centers.
Several enzymes are involved in their biosynthesis such as phytoene synthase (PSY) which catalyzes the head-to-head condensation of two geranylgeranyl diphosphate (GGPP) molecules to yield phytoene, the first committed reaction in carotenogenesis, representing a key step in this pathway.
In carrot (*Daucus carota*), two *psy* genes (*psy1* and *psy2*) have been reported. Both genes are expressed differentially in leaves and roots during plant development, but their functionality remains to be elucidated.
In this study the functionality of psy1 and psy2 genes from *D. carota* was evaluated through heterologous complementation in *Escherichia coli*. The heterologous expression system using *E. coli* have been used in the last years to express and evaluate the functionality and activity of several carotenogenic genes. The strain BL-21 pBAD/ΔCrtB, containing the *Erwinia uredovora* carotenogenic pathway and a mutated version of the *crtB* gene (that corresponds to *psy*), was complemented with the constructions pETBlue1/psy1 and pET28a/psy2. In both cases, orange colonies were obtained indicating carotenoid synthesis; in contrast to the BL-21 pBAD/ΔCrtB used as negative control (white colonies) where no carotenoids were synthesized. These results were corroborated by HPLC analysis, indicating differential capability between both genes to produce carotenoids in bacteria. We prove in this way that both psy genes of *Daucus carota* code for functional PSY enzymes. This study can be focused in nutritional improvemet of fruits and plants with commercial relevance by means of increasing carotenoids which are known to be beneficial to human health.

11.

IDENTIFICATION AND STRUCTURAL-FUNCTIONAL ANALYSIS OF THE PROMOTERS OF THE CAROTENOGENIC GENES *psy2* AND *lcyb1* FROM CARROT (*Daucus carota* L.)

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Carotenoids are widespread isoprenoid molecules essential for animal health and plant viability. The biosynthetic pathway of these compounds has been well established, although the regulatory mechanisms of their biosynthesis still remain to be completely understood. It has been proven that the regulation occurs mainly at the transcriptional level and is mediated by light and development. In *Daucus carota* (carrot), carotenoids, particularly β-carotene, are synthesized at very high levels in the modified root, which develops in darkness. Among the carotenogenic genes that lead to β-carotene production in carrot, *psy2* and *lcyb1* present the highest increase in their transcripts during development of leaves and modified root. Furthermore, both genes, especially *psy*, have been proposed as key regulatory steps for this pathway in plants.

To further investigate the regulatory mechanisms that control the carotenogenic pathway in carrot, we identified by means of GenomeWalker two promoter regions of 769 and 1057 bp of *psy2* and *lcyb1*, respectively. Bioinformatic analysis of these promoters showed many regulatory boxes related to light and to phytohormones such as ABA, auxins, gibberellins, ethylene and methyl jasmonate.

To evaluate the expression of each promoter, the complete fragment and a minimum promoter, comprising the transcriptional start site, of each gene were fused to GFP. The minimum promoter of *psy2* also included most of the hormone *cis* elements. GFP expression in transient transformed tobacco leaves and tomato and pepper fruits, showed that all constructs were functional in these organs.

These results indicate that the upstream regions amplified from *psy2* and *lcyb1* genes of *D. carota* are functional promoters, driving the heterologous expression of the reporter gene in photosynthetic organs (leaves) and fruits. The importance of the regulatory *cis* elements will be determined in leaves and roots of stably-transformed *A. thaliana* and *D. carota* plants, subjected to hormonal and light treatments.

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12.

SELECTION OF ENDOGENOUS PSY GENES FOR METABOLIC ENGINEERING OF CAROTENOID CONTENT IN *Brassica napus* SEEDS

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In plants, the first committed step of the carotenoid biosynthethic pathway is catalyzed by the enzyme phytoene synthase (PSY). PSY catalyzes the condensation of two molecules of geranylgeranyl diphosphate (GGPP) to produce C40 phytoene, a colorless carotenoid. Studies in several plant species demonstrated that it is possible to increase the carotenoid content in seeds by overexpression of bacterial (*CrtB*) or plant PSY genes. In many instances, the choice of transgenes has proven crucial in achieving higher carotenoid levels in transgenic plants. In Golden Rice, for example, overexpression of a seed-specific maize PSY outperformed a former version overexpressing a petal-specific daffodil PSY. In *Brassica napus*, overexpression of *CrtB* increased seed carotenoid levels 50 times but the overexpression of a plant or endogenous PSY has not been reported to date. Our laboratory has identified and cloned 5 *B. napus* PSY genes. In this study, we report the screening and selection of these endogenous BnaX.PSY genes based on their tissue-specific expression, protein sequence and functionality, for metabolic engineering of carotenoid content in *B. napus*. First, we designed PSY paralog-specific PCR primers and performed rapid amplification of cDNA ends (RACE) to determine their full-length cDNA sequence.

Secondly, we characterized their tissue-specific expression using RT-PCR followed by cDNA-SSCP analysis. As a result, one BnaX.PSY preferentially expressed in petals and another found to be expressed in seeds were selected for further studies. We performed in silico analyses including structural modeling and molecular dynamics simulations to evaluate protein sequence differences and their putative effects on protein activity. In addition, a heterologous complementation test in *E.coli* BL-21/•CrtB is underway to ensure that both genes are funtional. The selected endogenous BnaX.PSY genes together with other bacterial genes for ketocarotenoid biosynthesis and carotenoid accumulation will be introduced in *B. napus* to generate oilseeds with added value.

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13.

HIGH THROUGHPUT SNP GENOTYPING IN *Brassica napus* L.: SEQUENCE CAPTURE MICROARRAY DESIGN

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The advent of next generation sequencing (NGS) technologies has facilitated the access to genomic sequence information due to their fast, systematic and cost-effective way of operation. However, a limiting step at applying NGS technologies resides in the specific enrichment of genomic DNA. Recently, a microarray-based genomic selection (MGS) method, known as sequence capture, has addressed this limitation allowing for the isolation of user-defined genomic sequences in one simple step. Targeted enrichment of specific genomic regions allows for large-scale resequencing and characterization of natural genetic variation in crop species with large and complex genomes. The assessment of this genetic variation among individuals or cultivars can result in the identification of polymorphisms leading to the development of molecular markers that could be used in advanced plant breeding programs. In this study, we combined Roche NimbleGen sequence capture microarray technologies with NGS to discover single nucleotide polymorphisms (SNPs) in specific captured areas of the allopolyploid *Brassica napus* L. (AACC, 2n=38) genome associated to traits of agronomical and nutritional importance. Briefly, we designed a 2.1 Mega-base custom array containing target sequences of interest. These sequences correspond to 22 regions of the A and 17 regions of the C haploid genomes that have been previously associated with quantitative trait loci (QTL) explaining seed oil quality and content, seedling vigor, seed yield and black leg resistance. This microarray will be used to obtain cultivar-specific sequence information and assess sequence variation from ten diverse cultivars (6 winter, 4 spring) which exhibit contrasting phenotypes for these traits. In addition, this sequence capture microarray has the potential of becoming a high-throughput SNP genotyping platform for the species.

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14.

EXPRESSION ANALYSIS OF *Vitis Vinifera L.* EPIGENETIC REPRESSOR-LIKE GENES DURING GRAPEVINE SEASONAL FLOWERING

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In temperate regions, grapevine (*Vitis vinifera L.*) sexual reproduction occurs over two growing seasons separated by a dormancy period. During the first growing season, one to three inflorescence primordia arise within latent buds around spring and summer (flowering induction). Then, the onset of cold season (decreasing photoperiods and temperatures) promotes the grapevine plant dormancy. During dormancy, a pause in the flowering process and growth apparently takes place. The bud burst occurs in the following spring and then the immature inflorescences continué differentiation to produce flowers. Thus, a seasonal separation between inflorescence primordia and flower development occurs in grapevine. We hypothesized that a repression mechanism involving epigenetic changes could be at the basis of seasonal separation between flowering induction and flower develop in grapevine. Therefore, we analyzed the expression behavior of four grapevine genes with homology to the Arabidopsis epigenetic repressor genes *FERTILIZATION INDEPENDENT ENDOSPERM (FIE), EMBRYONIC FLOWER 2 (EMF2),CURLY LEAF (CLF)* and *EARLY BOLTING IN SHORT DAYS (EBS)* during the *V. vinifera* bud development. We observed that the putative grapevine epigenetic repressor genes (*VvCLF-like, VvEMF2-like,VvEMF2-like and VvEBS-like)* were mainly expressed in latent buds. In *Arabidopsis*, CLF, EMF2 and EBS mediáte vegetative development by transcriptionally silencing various flower MADS box genes (*AG, PI, SEP3 and AP3*). Therefore, the overlapping expression patterns of *VvEBS-like, VvEMF2-like* and *VvCLF-like* in grapevine latent buds suggest that their proteins could have been recruited for epigenetically repressing the flower MADS-box genes (*e.g. VvAG, VvPI, VvSEP*) during the first growing season, thus, contributing to the seasonal separation between inflorescence primordia and flower development in this species.

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15.

Agrobacterium tumefaciens-MEDIATED GENETIC TRANSFORMATION OF *Eucalyptus globulus*

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Eucalyptus globulus Labill. (Tasmanian Blue Gum; sensu Brooker) is native to South-Eastern Australia and is one of the most important pulpwood plantation species in temperate regions of the world. Considerable plantation areas are grown for this purpose in Australia, Chile, Portugal and Spain, due to its versatility, fast growth and fiber characteristics.
The propagation of this species has been carried out mainly from seeds. In that way, use of somatic embryogenesis would be potentially useful for large scale propagation strategies and genetic transformation of desirable genotypes to assist tree breeding and plantation establishment. However, this is a difficult genus reported as extremely recalcitrant to these techniques.
The purpose of this study is the establishment of a somatic embryogenesis procedure in order to produce transformed tissues able to regenerate into whole plants. Transformation was achieved by co-cultivation of induced mature zygotic embryos with *Agrobacterium tumefaciens* EHA 105 strain containing the pBIN m-gfp5-ER plasmid carrying green fluorescent protein (GFP) and neomycin phosphotransferase (NPTII) genes under the control of CaMV 35S promoter. Somatic embryogenesis induction was obtained evaluating three media (MS, B5 and modified DKW) and supplements of different growth regulator combinations (ANA, AIA and Kinetin). Callus was formed at explant´s surface in all tested media and embryogenic calli were observed after 45 days of cultivation. For transformation assays, GFP expression was observed in calli after six weeks of treatments. The integration of the NPT II marker gene was detected by PCR in leaves of the regenerated whole plants.

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16.

ESTABLISHMENT OF REGENERATION AND TRANSFORMATION SYSTEMS FOR GRAPEVINE ROOTSTOKS

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Most table grape varieties are currently grafted over disease or stress resistant rootstocks. These rootstocks are hybrids from crosses between different grapevine species (*Vitis sp.*). However, even with this agronomic practice there are several pathogens that attack grapevine causing severe damage on fruit quality and production. Among them, more than ten different viruses can infect grapevine and there is not a treatment against this pathogen. The aim of this work is to obtain grapevine resistant rootstocks for five different grape viruses by means of genetic transformation. Our strategy is to use gene silencing to induce virus resistance not only in the rootstocks but also in the whole plant. The first step is to establish an efficient regeneration system to obtain transgenic plants. Two strategies for rootstock regeneration were assayed in this work: somatic embryogenesis and induction of meristematic tissues. Somatic embryogenesis was induced in Harmony, Freedom and 110 Richter rootstocks culturing anthers and ovaries of immature flowers in PIV media (Nitch salts, 2-4D and BAP). After 6 months a least 4% of anthers and 15.8 % of ovaries initiate embryogenic growing. Secondary embryogenesis was also obtained and studied under electron microscope. Meristematic tissues have been induced culturing nodal segments of Harmony, Kober and C1613 rootstocks in MS medium with Bencylaminopurine at 3mg/l. Explants were cut and subcultured every three weeks to obtain the meristematic tissues of appropriate size for transformation experiments. Besides, a virus silencing construction was made and transformed on *Agrobacterium tumefaciens* GV3101. This construction contains in tandem, partial genomic sequences of five viruses that infect grapevines separated by an intron from the same sequence in the opposite sense, to induce gene silencing during transcription. The construction includes nptII for kanamycin selection of transformed tissues. Transformation experiments and evaluation of selected plants are ongoing.

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17.

LIGHT PERCEPTION AND GENE EXPRESSION: WHY AND HOW DOES THE NECROTROPHIC FUNGUS *Botrytis cinerea* RESPOND TO BLUE LIGHT?

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Light has a profound influence on the physiology of different organisms. Despite the considerable interest that the connection between light and diseases has attracted in the last few years, little is known about the molecular mechanisms behind this association.

Recent studies have reported daily variations in host susceptibility and in the virulence of certain pathogens. These alterations then, appear to be modulated by the 24-hour light-dark oscillations observed within a day. This suggests that circadian clocks, endogenous cellular timekeepers, could play a regulatory role in these processes. Interestingly, the molecular mechanism of light regulation of virulence among fungi has not been described yet.

How light or circadian rhythms per se modulate *Botrytis*-plant interactions has not been well documented. While among fungi, *Neurospora crassa* is probably one of the most well characterized models regarding photobiology and circadian regulation, little is known in other ascomycetes. In *Neurospora*, White Collar-1 (a photoreceptor) and White Collar-2 compose a transcriptional complex (WCC), that integrates light signals and also regulates the expression of the gene frequency (*frq*) setting the bases of a negative transcriptional-translational feedback loop that constitute the circadian oscillator. Thus, WC-1 not only plays a major role as a component of the circadian machinery, but also as the central blue-light photoreceptor in the cell.

In this work, we have characterized some of the *Botrytis* phenotypic responses to light, in cultures grown in constant darkness, light or light/dark cycles. Also, based on a comparative genomics-approach we have started to validate the expression of *Botrytis* putative circadian oscillator components. Moreover, we have confirmed light-inducible expression of several genes and also daily oscillations in *Bc-frq* under constant dark conditions. Interestingly, transcription factors were identified among the target genes responding to light. Finally, we will discuss the potential impact of light-regulated processes in determining the plant-fungal pathogenic interaction.

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18.

Dfg10 ORTHOLOGS GENES IMPAIRMENT HAS EFFECT ON *Arabidopsis thaliana* DEVELOPMENT.

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The chemical compound Sortin2 alters endomembrane system in *Saccharomyces cerevisiae* and *Arabidopsis thaliana* affecting vacuolar targeting and also endocytosis. A genome-wide analysis showed that dfg10 gene is important for Sortin2 action in *Saccharomyces* due that its deletion confers resistance to this chemical compound. Since the conservative effect of Sortin2 in yeast and plants we hypothesize that Arabidopsis ortholog dfg10 gene may be also involved in Sortin2 action in endomembrane system. To identify possible dfg10 ortholog genes in *Arabidopsis* we have used bioinformatics tools. We found three genes, At1g72590, At2g16530 and At5g16010 which function are unknown however they share a steroid 5-a-reductase motive. To study their functionality we identified T-DNA insertional mutant lines of two ortholog candidate genes. The insertions were confirmed and we isolated heterozygous and homozygous lines genotyping them. We obtained two T-DNA homozygous mutant lines, which insertions are localized on the promoter region of At5g16010. For At2g16530 locus, we isolated a homozygous line which insert interrupts the promoter region. However, we could not obtain a homozygous At2g16530 exon-interrupted mutant line suggesting a fundamental function on Arabidopsis development. We have performed phenotypic assays of homozygous mutants. We found statistically significant differences to wild plants on primary root length and rosette leaves number of mutant plant. These results strongly suggest that these Arabidopsis genes play a role in plant development especially on root and rosette structure. To study if Arabidopsis dfg10 genes are involved on the function of the endomembrane system we have analyzed the endocytosis route on the homozygous mutants. Results showed that mutants on both genes have similar internalization of the endocytic marker patterns compared to wild type plants. In conclusion, the dfg10 orthologs genes would be involved in Arabidopsis development. Further studies will be required to study the role of these genes on endomembrane system.

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19.

EFFECTS OF SORTIN2 ON ENDOCYTOSIS IN MODEL ORGANISMS SUCH AS *Saccharomyces cerevisiae* AND *Arabidopsis thaliana*.

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Sortin2 is a bioactive chemical compound that causes changes in cellular trafficking of carboxypeptidase-Y (CPY) in *Saccharomyces cerevisiae* and *Arabidopsis thaliana* (AtCPY), normally located in the vacuole, triggering its secretion to the extracellular. A search on an indexed mutant library of *S. cerevisiae* identified genes whose deletion conferred partial or total resistance to the effect of Sortin2. The Gene Ontology shows that most of them are directly or indirectly involved in the process of endocytosis. The resistant genes include *CLC1* and *SLA1*, whose gene products are directly involved in the formation and transport of clathrin vesicles, one of the main mechanisms of the cell endocytic route and also the mechanism how CPY is transported to the vacuole.
We have analyzed the effect of Sortin2 in the endocytosis of a fluorescent marker in wild type and mutant strains, *clc1* and *sla1*. The endocytosis marker FM4-64 binds to the plasma membrane and follows the endocytic route of clathrin-coated vesicles, toward to the vacuole through the endosomes and the pre-vacuolar compartment. Since the endocytosis mechanisms are highly conserved, a search of orthologous genes of *CLC1* and *SLA1* in *A. thaliana* was performed. Three orthologous genes were identified for *CLC1* however for *SLA1* none was found. In order to analyze the role of *AtCLC1* genes, insertional mutants were identify and homozygous T-DNA insertions lines were isolated. The transcript level of these genes was performed by means of semi-quantitative RT-PCR in the insertional mutants. Finally to visualize the effects of Sortin2 we analyzed the endocytosis of the FM4-64, where plants with homozygous insertion in *AtCLC1* genes show a distinct phenotype compared to wild type plants suggesting that the *AtClC1* is important to Sortin2 action. This work contributes to determine a possible common molecular target that may trigger the effects of Sortin2 in yeast and plants.

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20.

IDENTIFICATION OF A NOVEL NUCLEOTIDE SUGAR TRANSPORTER, AtUTr8, INVOLVED IN THE SYNTHESIS OF MUCILAGE *IN Arabidopsis thaliana*.

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Nucleotide sugar transporters (NSTs) are present in every eukaryotic organism. In plants has been proposed that these proteins are required biosynthesis of the polysaccharides present in the cell wall; however, to date only few NSTs have been characterized and their importance in this biological process has not been proved. To identify others NSTs, we performed an *in silico* search of NSTs that coexpress with genes involved in cell wall biosynthesis. From this analysis we identify a new NST and called it *AtUTr8*. Also we confirmed the subcellular localization of AtUTr8 fusing the protein to GFP. Additionally we analyze the phenotype of atutr8 mutant plants by ruthenium red staining and inmunohistochemical assays using antibodies against cell wall components. Our prior bioinformatics analysis shows that *AtUTr8* has a high degree of coexpression with genes involved in mucilage synthesis such as *GATL5* and *MUM4*. The AtUTr8 protein fused to GFP reveals Golgi apparatus localization, confirmed by colocalization with other fluorescent organelle markers. Subsequent analysis of two insertional lines on AtUTr8 has shown a reduction in the accumulation of pectinous mucilage. This phenotype seems to be related to the amounts of RG-I present in the mucilage of mutants compared to wild type seeds as determined by inmunohistochemical assays. These results suggest that AtUTr8 is essential for the biosynthesis of polysaccharides present in the mucilage. The overall results of this work evidence the important role of NSTs in the biosynthesis of cell wall in plants.

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21.

ROLE OF A PUTATIVE NUCLEOTIDE SUGAR TRANSPORTER IN POLLEN TUBE GROWTH

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Introduction: Nucleotide sugar transporters (NST) are membrane protein that are localized to the ER and Golgi apparatus, where they function to transport nucleotide sugars from the cytosol to the lumen of these organelles, to be use in glycosylation reactions by specific glycosyltransferases. In plants, most of these reactions in the Golgi apparatus are involved in the synthesis of wall material, such as pectins and hemicelluloses, but until now no evidence of NST involved in pectin synthesis has been obtained. We have identified a putative NST (At1g06890) that is probably involved in pectin synthesis during male gametophyte development, and characterized its expression pattern as well as the phenotype of two insertional mutants of this gene.

Methods: Pattern of expression of At1g06890 was analyzed by RT-PCR and qPCR. Insertional mutants for At1g06890 where identified in the SALK collection of the Salk Institute. Phenotype of mutants and wild type lines were studied at vegetative and reproductive levels.

Results: The putative NST At1g06890 shows a preferential expression in reproductive tissues when compared to vegetative tissues. The vegetative development of the insertional mutants of At1g06890 does not show considerable differences when compared to wild type plants, but the reproductive development of these mutants is affected in the male line since the pollen grains obtained from these plants are incapable of extend a normal pollen tube when germinated in vitro, although their viability and metabolic activity at dehiscence are similar to wild type.

Conclusions: These results suggest that the putative NST At1g06890 is important for pollen tube extension, and considering that pollen tube is made up only of cellulose and pectins, we conclude the this putative NST may be important for pectic wall material synthesis during pollen germination in *Arabidopsis thaliana.*

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22.

STRUCTURAL CHARACTERIZATION OF A NON SPECIFIC LIPID TRANSFER PROTEIN *(nsLTPs) in Lotus japonicus*

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Cuticle is an extra-cell, thin and continuous membrane that covers the leaves of the plants,one of its principal functions is to act like barrier against the loss of water, in response to the stress for drought. In *Lotus japonicus,* like the majority of the plants, it has been described that the fatty acids of long chain are important components of cuticular wax. It is thought that the lipid transfer proteins (LTPs) are involucrate in the process of formation of the cuticle. The LTPs are small, basic, soluble proteins, with extra-cell location and defined by their ability to facilitate transfer of lipids between membranes *in vitro*. This work focused on LjSGA.018057.1 sequence (a putative nsLTPs present in leaves of L japonicus), by phylogenetic analysis it was determined that belongs to the LTP I subfamily. Together, Using comparative modeling methodology was built the structure of protein LjSGA.018057.1, which was validated and refined with molecular dynamics simulation. Additionally, we explored the possibilities of interaction of a set of putative substrates with the LjSGA.018057.1 protein using molecular docking simulation. Our results suggest than the more stables conformation is the complex formed by protein and Palmitoil-CoA as substrate, which correlates with the high presence of palmitoyl-CoAs in the cuticle as aprecursor to the formation of this.

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23.

IMPLICATIONS OF LIPID TRANSFER PROTEINS (LTPs) IN CUTICLE WAX COMPOSITION DURING DROUGHT STRESS IN *Lotus japonicus*.

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Main ways of water lost in aerial sections of plants are stomatal and cuticle. During drought stress, the earliest response is stomatal closure. Second step of plant response is related with activation of gene expression and develops of sustainable maintenance. Identification of genes with relevant roles in drought tolerance is subject of study for several researchers. Cuticle is formed by hydrophobic layers covering aerial plant organs from primary stages of development. Cuticle is composed of cutin and wax which are synthesized by epidermal cells. Major classes of aliphatic cuticular wax component include alkanes, ketones, fatty alcohols, secondary alcohols, aldehydes, fatty acids and esters.

Transport of lipids from epidermal cells to cuticle is unknown. Mutants for ABC proteins have showed reduction in lipids loaded in cuticle and unusual lipidic cytoplasmic inclusions. Lipid transfer protein have been suggested mediate movement of lipids from dermal cells to cuticle, however the evidences are limited.

During this research have been evaluated composition of cuticular lipids in plants under drought stress and compared with optimal conditions using GC-MS. We selected from *Lotus japonicus* databases LTPs with expression patterns in shoots and stems and determined expression levels mediating qRT-PCR in leaves when plants were stressed at relative water content (RWC) of 60% during eight hours. We identified three genes coding for LTP type I which were induced during drought stress. Also, we analyzed sequence and identity structure for family I of LTP, including docking studies. We selected any candidate ligands which showed best binding affinity. Using the sequences for induced LTPs we designed two constructs in pKannibal vector for silencing overexpressed genes in *L . japonicus*. Results of mutants analysis will allow describe function of LTPs in plant drought stress.

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24.

REGULATION OF THE BORON TRANSPORTER EgBor1 IN *Eucalyptus globulus*: A PLAUSIBLE MODEL

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Boron is a micronutrient that plays an important role in plant cell wall biosynthesis. Nevertheless, an excess of boron in the soil causes severe damage to the respiratory tissue of the plant. In *Arabidopsis thaliana*, the *Atbor1* gene encodes a boron transporter that distributes this element throughout the plant.

A cDNA sequence encoding a *bor1* transporter was isolated from a *Eucalyptus globulus* cDNA library. This sequence contains several stop codons within the coding region. Initial bioinformatic analyses suggest that this interruption corresponds to an intron that may generate a truncated protein.

Egbor1 was overexpressed in *Saccharomyces cereviseae* to assess whether it was capable of restoring the phenotype of a mutant strain that lacks the boron transporter. It was also overexpressed in a wild type strain as a control. In both cases a significant increase in boron tolerance was observed, suggesting that the encoded transporter is functional. Subsequently, a western blot analysis showed that the expressed transporter corresponds to the product of the full-length protein, rather than the truncated protein.

Additional bioinformatic analyses showed that the intron presents several regulatory elements, therefore it may function as a promoter for a small protein encoded in the 3' region of *Egbor1*. Transient expression of GUS under the control of the intron sequence proved its capability to activate gene expression. Thus, we have identified three ORFs in the *Egbor1* sequence: a full length protein encoded by the spliced mRNA named fragment C, and two smaller proteins encoded by the 5' and the 3' regions of the non spliced mRNA named fragments A and B.

We are currently challenging the yeast mutant strain with all the putative encoded proteins to understand their specific role in boron transport.

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25.

FUNCTIONAL CHARACTERIZATION OF RNBPs UNDER BORON TOXICITY FROM *Arabidopsis thaliana*

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Boron (B) is an essential element for all organism development. In plant, the boron has been determined as essential for cell wall structure and function. B was found to be located in association with Rhamno-galacturonan II (RGII), a pectin polysaccharide. High concentration of boron is often associated to soil in semi arid and arid climates, B-toxicity symptoms are reduction of growing tissues and plant productivity. The molecular mechanism involved in coordination and integration of boron sensing remain to be elucidated. To investigate how the plant survive under conditions of B toxicity, we used T-DNA insertion mutants lines of *Arabidopsis thaliana* in two genes that encode RNA Binding Proteins (Salk Insertion Data base: AtRBPA, AtRBPB). These proteins are evolutionary conserved between monocotyledons and dicotyledonous plants. Under different B concentrations (0, 3, 6 and 10 mM boric acid), AtRbpa and AtRbpb displayed increased root hair length, but double mutant (AtRbpa-AtRbpb) showed decreasing root hair length in comparison to wild type. Furthermore, AtRbpa, AtRbpb and AtRbpa-AtRbpb showed impaired seed germination in 20mM boric acid. The AtRbpa-AtRbpb decrease tolerance to 20mM boric acid compared to AtRbpa, AtRbpb and wild type, suggesting that RNBPs are modulators of germination program under boron conditions. We hypothesize that post-transcriptional regulation mechanism have basic biological roles in regulation of boron toxicity and stress tolerance. Additionally, we have designed new strategies for understanding the molecular network that controls B toxicity in plants.

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26.

GENE EXPRESSION PROFILES FOR CELL WALL-MODIFYING PROTEINS INVOLVED IN BORON TOLERANCE.

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ATEXLA3 (*Arabidopsis thaliana* expansin-like 3), *PL* (*Arabidopsis thaliana* pectate lyase) and *PME* (*Arabidopsis thaliana* pectinesterase) are three genes whose protein products are possibly implicated in cell wall modification. The expression profiles indicated that they are mainly expressed in roots and are induced by boron excess (up to 10 mM H_3BO_3 added). In order to determine the role of these genes in boron tolerance and a possible relationship between them, we performed phenotype description and crossed analysis of gene expression in three insertion mutant lines subjected to high boron levels: SALK_146621 (*ATEXLA3*), SALK_017335 (*PL)*, and SALK_096342 *(PME*). *ATEXLA3* mutant showed overexpression of *ATEXLA3* gene, under normal and high boron conditions, while the other two mutants, *PL* and *PME*, were knock-out and knock-down for their respective genes. Interestingly, all three mutants displayed boron tolerance, and showed higher growth of primary root than wild type plants. The following results were obtained from crossed analysis of gene expression in boron tolerant mutants: In *ATEXLA3* mutant, *PL* and *PME* genes were suppressed under normal and high boron conditions compared with wild type plant. In *PL* mutant, *ATEXLA3* gene was highly overexpressed while *PME* gene reached the same expression level as in wild type plant under both conditions. Finally, *PME* mutant showed an increment of *ATEXLA3* gene expression and a strong suppression of *PL* gene compared with wild type plant under normal and high boron conditions.

The analysis suggests that ATEXLA3 plays a relevant and antagonic role with PME and PL in boron tolerance acquisition. Suppression of *PME* and/or *PL* genes leads to increases *ATEXLA3* gene expression and confers boron tolerance. On the other hand, suppression of *PL* doesn´t affect *PME* gene expression, suggesting that PME is upstream from PL in the cascade response.

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27.

INVOLVEMENT OF SIR2 IN RESPONSE TO GENOTOXIC STRESS *IN Arabidopsis thaliana*

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Plants are constantly exposed to biotic and abiotic stresses. Under certain conditions, stress can cause DNA damage, which activate the molecular repair machinery and leading to apoptosis in extreme damage, which reduces growth and productivity of plants.
Sir2 (Silent information regulator) belongs to the family of enzymes with histone deacetylase activity and to a lesser extent, ADP-ribosyl transferase. These enzymes use NAD as substrate, and are conserved in all organisms. In yeasts, this family is involved in transcription regulation via chromatin remodelling, silencing critical genes that contribute to the activation of cell survival mechanisms under stressful conditions, emphasizing the importance of NAD metabolism in the regulation of this response.
In this work we analyzed the importance of Sir2 homologues of *Arabidopsis thaliana* (At5g09320 and At5g55760) in genotoxic stress responses.
To characterize the role of Sir2, homozygous insertional mutants were selected for these genes. Wild type plants and selected mutants were exposed to stress by bleomycin or methyl methanesulfonate (MMS) in liquid medium, and UV radiation in soil. Tolerance to these stresses was determined by phenotypic analysis in each line.
We found an increased susceptibility to genotoxic stress in Sir2 mutants, reflecting a lower survival rate in treatments with MMS and UV, relative to control plants. Our results shows that Sir2 is actually participating in the regulation of genes involved in survival contributing to the development of resistance mechanisms against genotoxic stress.
This research could provide important information of Sir2 as a target for developing new strategies to improve plant resistance to stress.

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28.

MOLECULAR CLONING OF SIR2.1, A NAD-DEPENDENT HISTONE DEACETYLASE OF *Arabidopsis thaliana*

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Histone deacetylases (HDACs) are enzymes that catalyze the removal of acetyl groups from histones N-tails, process related to chromatin compaction and repression of gene expression. These enzymes are regulated differentially by abiotic stresses and hormones such as ABA. In *Arabidopsis*, there are 18 HDACs including 12 members of RPD3/HDA1 superfamily, four members of HD2 family and two members of SIR2 family (SIR2.1 and SIR2.2).
SIR2 (Silent Information Regulator protein2) is a NAD-dependent histone deacetylase family, which is phylogenetically conserved from prokaryotes to mammals. In addition to gene silencing, there is growing interest by the number of cellular processes that are being linked, like cellular regulation, fatty acids metabolism, apoptosis, and longevity by caloric restriction. Our work is focussed on the characterization of SIR2 genes from Arabidopsis and their involvement in stress responses. In order to achieve this goal, we have cloned SIR2.1 (At5g09230) into the entry vector pCR8 (Invitrogen). This construct was used to generate the expression vectors pGWB5-SIR2.1-GFP and pGWB8-SIR2.1-His by LR-recombination. Through a transient expression on tobacco leaves, followed by an RT-PCR, these vectors were found transcriptionally functional.
The vector pGWB5-SIR2.1-GFP will be used to determine the subcelular localization of SIR2.1, which is believed to be localized in mitochondrions, by in-silico analysis, while the other homolog of the SIR family (SIR2.2, At5g55760) has shown a typical nuclear localization. The other vector, pGWB8-SIR2.1-His, allows the expression of a fusion protein with a C-terminal 6xHis tag, necessary for immunoblotting using an α-his antibody. With this vector we will raise the expression levels of SIR2.1 in Arabidopsis plants stably transformed. Current progress in our research will be presented.

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29.

STUDY OF GERMINATION DELAY BY NICOTINAMIDE AND SIR2 INHIBITORS IN *Arabidopsis thaliana*

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Germination is a process in which seed undergoes several morphological modifications due changes in gene expression, regulated by environmental conditions (light, cold, water). These variations in gene expression leads to the production of a variety o hormones (ABA, GA, ethylene), determining the progress through different developmental stages. The identification of genes involved in plant development and their regulation, remains an area of interest in plant biology.

One of the mechanisms of gene regulation is accomplished by histone modification. Sirtuins are a gene family of NAD-dependent histone deacetylases (group III) and its product, nicotinamide, acts as an inhibitor of its activity. Sir2 was the first member described in yeast, and is involved in gene silencing of rDNA, telomere and mating loci (HM). So far, the role of Sir2 in *Arabidopsis* germination has not been described. In this work, we analyze the effect of different inhibitors of the germination process over Sir2 expression. This information will contribute to the understanding of the involvement of this gene and its product nicotinamide in germination.

To determine the mechanism and action site of Sir2 and nicotinamide in the germination process, we analyzed germination in wild type lines treated with nicotinamide (0-40mM), other inhibitors of Sir2 (sirtinol, trichostatin A) and with different germination inhibitors (glucose, ABA). The percentage and germination rate was determined. Statistical analysis was performed to choose and utilize mathematical models that describe the germination kinetics.

In this study we determined that nicotinamide produces a germination delay in *Arabidopsis thaliana.* Other sirtiun inhibitors such as trichostatin A and sirtinol induced a delay in germination similar to that generated by nicotinamide. Therefore Sir2 itself is a key element in plant development, specifically in the germination process.

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30.

STUDY OF THE PROMOTER *AtPht1:4* RESPONSE IN WHEAT AGAINST THE DEFICIT OF PHOSPHATE AND THE COMBINATION WITH DIFFERENT ABIOTIC STRESSES

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The choice of a promoter, according to the type specificity and spatio-temporal regulation, is of great importance in the regulation of transgenes in plants. The promoter *AtPht1;4* has a pattern of expression type-specific and root inducible by phosphorus (Pi) deficiency, which makes it extremely attractive for expression of transgenes in monocotyledonous plants of commercial interest, such as wheat. *In silico* analysis of *cis* regulatory elements present in *AtPht1;4* and other high-affinity phosphate transporters has suggested similarities between dicotyledonous and monocotyledonous. In addition, *AtPht1;4* presents *cis* regulatory elements in response to different environmental signals, in addition to Pi deficiency, suggesting a greater versatility in its response.

This study emphasized the response of the promoter *AtPht1;4* in wheat, compared to environmental signals often experienced by a crop under field conditions, such as heat, drought, cold and hypoxia. Through a system of type promoter-reporter gene, *AtPht1;4::GUS*, we evaluated the performance of three transgenic lines. Initially, a significant degree of silencing was observed in all of them. However, it was possible to establish GUS expression type-specific in roots and the induction of the transgene in deficit of Pi. Furthermore, the cellular localization of GUS expression was localized in the vascular tissue of differentiated roots, indicating a putative role of *AtPht1;4* in the transport of Pi into the xylem.

AtPht1;4::GUS expression quantificated by qPCR showed a significant induction when combine Pi deficiency with drought. In addition, a three hundred fold induction was reported in the cold condition as well as in the combination of cold and hypoxia treatment. It is important to mention that even with such transcriptional burst this induction could not be translated into higher levels of enzyme activity. This could be results from a low rate of translation and/or inhibition of GUS activity. Further analyses are necessary to clarify the disparity between GUS transcription levels and activity levels in transgenic wheat plants.

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31.

EFFECT OF OXIDATIVE STRESS ON THE VERNALIZATION RESPONSE USING SEVERAL ARABIDOPSIS ECOTYPES THAT EXPRESS THE *FRI/FLC* MODULE.

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Flowering in plants is under a tight genetic and environmental control. The most important pathways regulating this process are the photoperiodic and the vernalization pathway. Both converge in the master regulatory gene *FT* (*FLOWERING LOCUS T*), which encodes a small protein produced in the leaf vasculature that travels to the shoot apical meristem, activating the expression of the floral meristem identity genes. *FRIGIDA* is a dominant allele that repress flowering through the transcriptional activation of *FLOWERING LOCUS C* (*FLC*), which encodes a MADS-Box protein that repress *FT* expression. During vernalization, *FLC* promoter is remodeled and the gene becomes transcriptionally silenced, allowing *FT* expression. Most laboratory strains of Arabidopsis, as Col-0 and Ler, don't have a functional *FRI*/*FLC* module. In this work we have used several northern Arabidopsis strains that express high levels of *FLC* and thus have cold requirements to flowering. We have used these plants to determine the amount of cold that is necessary to flowering and found that mild oxidative stress could compensate for sub-optimal cold treatments. Transgenic plants expressing GUS under the control of *FLC* promoter show less GUS histochemical activity when growth on salicylic acid, which indicate that this response is controlled at the transcriptional level. Taken together our results suggest that northern accessions of Arabidopsis are an interesting model to study cold requirements and that mild oxidative stress could promote flowering through inactivation of *FLC*.

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32.

PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF *aca7*, A MUTANT DEFICIENT IN POLLEN DEVELOPMENT IN *Arabidopsis thaliana*.

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In our laboratory, we have developed a method based in expression data that allowed us to identify fast and efficiently, genes involved in pollen function and development. Using this method, we have identified At2g22950 as a candidate gene essential for pollen development, whose expression is limited to flowers and floral organs, especially anther and pollen grains, and it shows an accumulation kinetics of transcripts that reaches its peak at tricellular pollen stage and drastically drops at mature pollen stage. Bioinformatic analyses indicate that At2g22950 encodes a putative plasma membrane Ca^{2+} ATPase (ACA7) involved in calcium ion efflux from the cytoplasm to the apoplast. Two allelic mutants shows a high percentage of dead pollen grains in the mature flowers. Anther histological analysis shows strong problems at the PMI (pollen mitosis I) stage of development, which is consistent with the expression data determined by microarrays. *ACA7* is expressed exclusively in immature flowers, as could be observed in RT-PCR analysis in wild type plants, and the fusion protein ACA7:GFP localizes at the plasma membrane as shown by transient expression in tobacco leaves which confirms the results obtained through bioinformatic analyses. These results indicate that ACA7 might have an important role in pollen development suggesting an important role for Ca^{2+} in this process.

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33.

CHARACTERIZATION OF TWO DIVERGENT cDNAs ENCODING XYLOGLUCAN ENDOTRANSGLYCOSYLASE/HYDROLASE (XTH) EXPRESSED IN *Fragaria chiloensis* FRUIT.

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Chilean strawberry (*Fragaria chiloensis*) has emerged as a new berry fruit with excellent organoleptic characteristics. The fast softening of strawberries is a limiting step for their commercialization. Fruit softening has been shown to be related to cell wall degradation. Several enzymatic activities related to this process have been studied in strawberry fruit, however xyloglucan endotransglycosylase/hydrolase (XTH) enzymes have not been identified or characterized so far. Two XTH genes were identified in *Fragaria chiloensis*: Fc-XTH1 and Fc-XTH2. Their full-length cDNAs were isolated. Phylogenetic analysis suggests that both *F. chiloensis* XTH genes belong to distant phylogenetic groups of XTHs. By means of Real Time qPCR analysis, the expression level of each gene was analyzed at different developmental and ripening stages. The level of Fc-XTH1 transcripts increased during fruit softening congruent with a probable role during ripening, while transcripts level of Fc-XTH2 are high in developing fruit and low in soft fruit. On the other hand, by an ELISA assay XTH-related proteins were detected at all fruit stages, and by enzymatic assays high xyloglucan transglycosylating (XETA) and degrading (XDA) activities were recorded at the turning stage. The data presented confirms the existence of two divergent XTH genes in *F. chiloensis* fruit, with Fc-XTH1 probably involved in fruit softening.

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34.

IDENTIFICATION OF METABOLIC PATHWAY OF NONADIENOL, A CHARACTERISTIC VOLATILE COMPOUND IN *Fragaria chiloensis*

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Several volatiles compounds were identified in *Fragaria chiloensis* including a series of C9-aldehydes and alcohols. The green and fresh flavour of white strawberries detected by GC-MS and GC-O was attributed to E-2-Z-6-nonadien-1-ol. The identification of these compound, combined with enzymatic evidence, suggest that nonadienol are biosynthesized via unsaturated aldehydes from linolenic acid. A search for genes involved in the biosynthesis of nonadienol was carried out in a population of ESTs from *F. chiloensis* (MIFAB) and other Rosaceae databases. We were able to identified putative sequences of LOX and HPL, key enzymes in this pathway.

Expression of these genes was studied by qPCR at different tissues and development stages of F. *chiloensis fruits*. The expression profiles along fruit receptacle development and ripening showed that *FcLOX2* and *FcHPL* reaches their maximal level of expression in the stage S3 and decrease in the full ripening stage (S4).

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35.

FUNCTIONAL GENOMICS OF TREE BIOMASS: SEARCH FOR FRUIT DEVELOPMENT GENES THAT RESPOND TO CYTOKININS

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Cytokinins are hormones that control cellular proliferation and a broad spectrum of morphologic and metabolic processes that are related with tree biomass production. These processes are key research targets to improve biomass production.

Recent work has revealed that the cytokinin signal transduction pathway is a two-component system which includes the activation of target genes. The main goal of our work is to identify genes that are associated with fruit development and are potential cytokinin responsegenes. Using a Comparative Functional Genomics strategy, we are comparing cytokinin-responsive gene expression poplar wood formation and peach fruit development.

Bioinformatics analyses have shown that at least 112 genes respond to cytokinins in *Arabidopsis thaliana*. We have identified orthologs genes in peach fruit *(Prunus persica)* in an EST database and in the public peach genome sequence. Some of these genes encode transcriptional regulators, signaling proteins, developmental and hormonal regulators and cell wall proteins. To study the effects of cytokinins in peach fruit and quantify the relative transcript level of these putative genes, we have performed cytokinin treatments on peach fruits at five different developmental stages (25, 40, 65, 85 and 120 days post anthesis) and are performing gene expression analyses to identifygenes that cytokinin-responsive genes during fruit development.

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36.

DEVELOPMENT OF A SYSTEM TO INDUCE TETRAPLOID GRAPEVINE PLANTS (*Vitis vinifera L.*)

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The importance of grapevine culture in Chile makes necessary the development of own varieties that respond to the productive characteristics of the country, since all varieties commercially grown at this time, have been developed abroad. Genetic Improvement Programs based on sexual hybridization and selection of segregants, biotechnological techniques like genetic manipulation and strategies of generation of variability using chemical agents and radiation are being developed in grapevine. Considering this background, the increase of ploidy level is proposed as a source of genetic variability in this work. Colchicine treatments were aplied on embryogenic suspensions and also on meristematic tissues growing *in vitro*, in four varieties of grapevines. The treatment was aplied for short times, and then the tissues were cultured on regeneration media to recover complete plants. The evaluation and methodologies used to assess the treated plants were: i) Stomatal size and frequency by means of scanning electron micrograph; II) Number of chloroplasts in occlusives cells of stomata using confocal microscope; III) Evaluation of karyotype through chromosome count; IV) Nuclear DNA quantification by flow cytometry; v) Phenotype evaluation of the generated plants under greenhouse conditions. Until now it has been possible the evaluation of the plants derived from the treatments on meristematic tissues, being verified the existence of plants of the variety Red Globe 4X. The projections of this work are to evaluate the resistance to pathogens and abiotic stress and organoleptic quantification of biomass and others characteristics of interest. Additionally, with the obtained tetraploids plants, triploids plants could be obtained by crossovers with diploids varieties.

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37.

ENZYME ACTIVITY FOR H_20_2 REDUCTION IN *Polypogon australis* PLANTS TREATED WITH LIQUID WASTES FROM MINING ACTIVITIES (LWMA)

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Liquid wastes from mining activities (LWMA) have high heavy metal concentrations that can contaminate surface and ground waters. Constructed wetland systems are used for LWMA treatment using plants that can accumulate metals in roots, stems and/or leaves. *Polypogon australis* is a native grass that grows in copper polluted sites and accumulates the metal in their tissues, being able to tolerate up to 626 µM Cu^{+2} *in vitro*. The accumulation of heavy metals in tissues can give rise to excess concentrations of reactive oxygen species such as H_2O_2, resulting in oxidative damage at the cellular level. The removal of H_2O_2 is due to the activity of antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPx) and peroxiredoxins (Prdx). Plants of *P. australis* treated during one month with the LWMA Adit 71 from 'El Teniente' accumulate up to 18 ppm of Cu in their tissues without showing an altered phenotype. Under the LWMA treatment at different pHs (5,1 and 6,7); there was an increase in the activity of APX, CAT and GPx during the first 2 h. However, Prdx activity increased just after 48 h of treatment. The basal activity of the four enzymes was 2-10 times higher in roots than in leaves. These results correlates with the maintenance of H_2O_2 levels in both tissues, and different H_2O_2 reducing-enzymes are responsible for this under the stress caused by heavy metals.

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38.

MICROPROPAGATION AND ENCAPSULATION OF ALOE EXPLANTS AS AN ALTERNATIVE OF SYNTHETIC SEED PRODUCTION.

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The Aloe plants are spread mainly in asexual way. This is because the phenomena of self-incompatibility and protandric dichogamy present in various species of the genus and which prevents self-pollination of plants and affecting the fruit formation and seed production, thus reducing the chances of sexual propagation. The *in vitro* culture techniques for plants such as organogenesis and somatic embryogenesis are the basis for carrying out the synthetic seed technology; allow obtaining explants in short-time, ideal for encapsulating somatic embryos or other types of vegetative propagules in a polymer matrix. The object of the present work was to develop a protocol aimed to encapsulation of explants of Aloe for synthetic seed production. The direct and indirect organogenesis technique was implemented in *Aloe variegata* and *Aloe vera* to obtain shoots, those which were encapsulated in sodium alginate in presence of calcium chloride. For encapsulation trials were evaluated viability *in vitro* of the alginate capsules containing the vegetative propagules, using the following parameters: size and type of explants (shoot apical meristem), concentration of sodium alginate (1.0, 2.0 and 3.0%), concentration of calcium chloride (50, 75 and 100 mM) and times of exposure of sodium alginate and calcium chloride (10, 15 and 20 minutes), and viability *in vitro* of the calcium alginate gel capsules containing the vegetative propagules. We obtain a better result with sodium alginate concentration of 3% in combination with calcium chloride to 75 mM for an exposure time of 15 minutes. The best explants evaluated were buds, which show a good viability testing *in vitro* and also show an average growth of 5 mm in height per week since its sprouting from the alginate capsules.

39.

STUDY OF THE ROLE OF L-IDONATE DEHYDROGENASE IN TARTARIC ACID BIOSYNTHESIS DURING TABLE GRAPE (*Vitis vinifera* L.) DEVELOPMENT

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Flavor is the most important quality attribute determining consumer preference in fruit, including table grapes. This organoleptic property is directly influenced by pre- and post-harvest conditions and the genetic background of each cultivar, feature that in grapes is especially associated with the sugars and organic acids ratio. At ripening, tartaric acid is the most important organic acid in grapes, being also one of the major contributors to flavor. It has been observed that an important step in the synthesis of tartaric acid in grapes would be related to the L-Idonate dehydrogenase (L-IdnDH) enzyme; however, the molecular changes underlying this process are unknown for table grapes. Therefore, in order to understand the role of L-IdnDH on tartaric acid biosynthesis, we cloned and characterized the expression pattern of a *VvL-IdnDH* gene in two commercial varieties of table grapes, i.e. Thompson Seedless and Red Globe. The study was located at Los Andes and the sampling was performed weekly starting two weeks before *véraison* until commercial harvest. In general, as berry fruit development progressed, we observed differences in *VvL-IdnDH* gene expression levels, which would be affecting the changes and differences measured in tartaric acid content for both varieties. However, as already shown in the literature, this enzyme would have a major role in earlier stages of berry development. Therefore, further studies will be performed in order to better understand this and other key steps on tartaric acid biosynthesis.

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40.

DEVELOP OF NEW PROMISING TABLE GRAPE THROUGH CONVENTIONAL BREEDING.

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The Chilean Institute for Agricultural Research (INIA), has undertaken table grape breeding since year 1986, because the development of Chilean varieties represents an opportunity to increase competitiveness of the country in this matter. The INIA table grape breeding program goals include berry aspects (seedlessness, size, firmness, color, flavor, appearance), cluster structure (low labor and cost input), storage and shipping ability and harvest time. The breeding program is based mainly in the cross of seedless varieties, including embryo rescue and tissue culture, followed by field trials of obtained segregants and selections.

Two advanced selections named 5 and 5.56 were chosen and evaluated at pre-commercial level in two locations for three years. The evaluations included response to different cultural practices including bunch handling and application of growth regulator. Evaluations were carried out at growing season, harvest and post-harvest. The clusters were stored in cold room (0°C for 60 to 120 days) and shelf-life (20°C for 3 to 5 days) simulating usual commercial conditions.

Selection 5–INIA showed a 'Thompson Seedless' like characteristics, mead season harvest and fertile in basal buds. Berries were seedless with a natural size of 15.5-17 mm, good response to GA_3, obtaining a berry growth increasing up to 18.5-21 mm. 5-INIA produced cluster that did not require thinning, but little trimming. On the other hand, selection 5.56-INIA displayed characteristics that resembled to 'Midnight Beauty' variety, showing fertile basal buds. Berries were mainly seeded with full color and good size (16.0-17.5 mm). They showed responsiveness to GA_3 inducing berry growth and thinning, reaching berry diameter of 18.5-22.0 mm. 5.56-INIA ripening occurred together with 'Midnight Beauty' or slightly earlier and the clusters required low trimming.

These data show the potential of these selections and their possibility to be grown in a near future in Chile.

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41.

PLANTATIONS OF GUINDILIA TRINERVIS AT THREE ALTITUDES AND RESPONSE TO FERTILIZATION

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Guindilia trinervis is a native Chilean shrub that bears seeds with a high content of oil suitable for biodiesel production. It naturally grows at elevations of 1400 to 2200 m.a.s.l. in the central region of Chile. In this study, adaptation of artificial plantations to lower altitudes and plant response to fertilization were assessed. Plants grown in captivity, either from seeds or from cuttings, presented difficulties re-adapting when planted in their original ecosystem. Ten percent of juvenile plants established at 1750 m.a.s.l., survived after one year. At 1000 m.a.s.l. plant death reached 50 % and in controlled greenhouse conditions at 400 m.s.n.m. losses reached 15 % after one year. Plants cultured in the greenhouse grew vigorously and emitted flowers the third year. In the wild, adult plants were fertilized with doses of 0, 75N, 75 N + 30P, and 75N + 30 P + 30 K k/ha. The combined NPK treatment reduced growth and development of shoots, yet seed output was significantly increased. Considering 27% oil per seeds, NPK yielded 224 L/ha and was significantly higher than the other treatments. One genotype that was not included in the control group, yielded 445 L/ha of oil without fertilization. Plantations in captivity at 1000
m.a.s.l. supplemented with nitrogen and phosphorous did not increase shoot growth. In the greenhouse, nitrogenous fertilization increased leaf and stem biomass at 1 g/plant, but not root biomass. Increasing amounts of nitrogen decreased biomass and plants showed signs of toxicity at 16 g/plant. Currently the curve of nitrogen demand is being revised. The results suggest that plantations of Guindilla in its natural habitat or at lower altitudes are difficult and require further studies. Fertilization with NPK increases seed biomass but nitrogen is toxic at high doses. Seed production is more affected by genotype than by fertilization.

42.

STUDY OF PHYSIOLOGICAL CHANGES IN RACHIS OF RED GLOBE TABLE GRAPE (*Vitis vinifera L.*) ON POSTHARVEST STORAGE

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Although there are cultural differences concerning the appropriate quality of table grapes, the overall characteristics of the cluster (berries plus rachis) are important conditions for consumers. In the literature, several papers describe table grape berry changes during development and postharvest life, but rachis physiology has not been studied with similar attention. Red Globe is one of the most important table grape varieties for export in Chile. However, during long-term storage or extended shipping conditions of Red Globe clusters, rachis undergoes a loss of quality resulting in a poor condition and lack of fresh appearance. This reduces the value and selling potential at the receiver or retail market, even though the quality of berries is normal. Therefore, the present work was focused to study rachis quality as response on prolonged cold storage. Stored rachis under usual storage conditions (air, 0°C) showed anatomical changes as epidermis disruption (SEM). In addition, alternative strategies were studied such as application of cytokinin (6-benzyladenine) at harvest and controlled atmosphere storage of clusters. Cytokinin and controlled atmosphere treatments helped to reduce the rate of quality lost based on image scanning analyses, suggesting the hypothesis that senescence-modulated effects are associated with rachis quality loss.

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* Balic, I. and Moreno A. are supported by CONICYT-Doctoral fellowship.

43.

THE EXPRESSION OF XTHS IN THE CHILEAN STRAWBERRY FRUIT IS MODULATED BY HORMONAL TREATMENTS

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The expression of XTHs genes is regulated in plant tissues by different factors including hormones. Treatments of tomato with synthetic auxin (2,4-D) showed a differential expression of LeEXT and LeXET2 genes. It has been shown in rice that expression of XET increases by increasing levels of auxin and decreases with increasing levels of gibberellin (GA3). In cherimoya (*Annona cherimola* Mill), a climacteric fruit that softens very quickly after harvest and resembles strawberry fruit, the application of 1-MCP, an inhibitor of ethylene perception, promotes a delay in the expression of AcXETs compared to control fruit. In the Chilean strawberry (*Fragaria chiloensis*) two XTH genes were identified: Fc-XTH1 and Fc-XTH2. It is known that auxin has a repressive effect on the expression of genes associated with strawberry softening such as pectate lyase, endoglucanase and expansins. Therefore, treatments with different hormones such as auxin, gibberellins, abscisic acid (ABA), ethylene and 1-MCP were performed in order to establish their effect on the expression of *F. chiloensis* XTHs isoforms. The analysis performed by Real Time qPCR indicates that there is a decrease in the expression of both isoforms with GA3 and ABA treatments. Auxin and ethylene treatments show an inhibitory role in XTH expression, whereas 1-MCP treatment promotes an increase in Fc-XTHs expression level. The results show that the expression of both Fc-XTH1 and Fc-XTH2 isoforms are regulated by hormonal treatments in the Chilean strawberry fruit.

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44.

AROMA FORMATION DURING RIPENING OF GOLDENBERRY FRUIT AND SUBSTRATE SUPPLY ASSAY.

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Goldenberry fruit (*Physalis peruviana*) is a climacteric fruit with a pleasant aroma 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 goldenberry fruit the aroma is provided mainly by esters, which are synthesized by the enzyme alcohol acyl transferase (AAT). To understand the biosynthesis of esters in goldenberry fruit, the ester production profile and AAT activity were determine during ripening development. In addition, yellow stage fruit was supplied with four different alcohols: ethyl-, butyl-, hexyl- and octyl-alcohol. After treatment, the fruit was maintained at 20 °C for up to 6 days and the volatile production rate determined. An increase in ester production was found during ripening progress, which is coincident with an increase of total AAT activity. AAT activity was higher against hexyl alcohol as substrate than to butyl alcohol. In addition, goldenberry fruit treated with hexyl and octyl alcohol displayed intense changes in ester production profile. These results suggest that ester formation in goldenberry fruit depend on AAT activity and substrate availability.

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45.

HYPOXIA INDUCED BUD-BREAK IN ENDODORMANT GRAPEVINE BUDS, BUT UP-REGULATES DIFFERENTLY HYPOXIC RESPONSIVE GENES *ADH2*, PDC AND *Susy2* AND AOX THAN DORMANCY BREAKING COMPOUNDS HYDROGEN CYANAMIDE AND SODIUM AZIDE

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It has been reported that dormancy breaking compounds hydrogen cyanamide (H_2CN_2) and sodium azide (NaN_3) increase the production of ethanol and acetaldehyde in grapevine-buds, indicating that both compounds produce a metabolic shift towards the fermentative pathway. Here we demonstrated that this metabolic shift is due to the inhibition of COX, the final enzyme of the mETC suggesting that resp ct of hypoxia treatments on the sprouting of endodormant grapevine buds and the effect of dormancy breaking compounds and hypoxia on the expression of hypoxic responsive genes. ADH2, AOX and PDC are upregulated by H_2CN_2 and NaN_3, but not by hypoxia, while Susy2 was lately increased by the three treatments.

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46.

EVALUATION OF THE Q-10 GENE EXPRESSION OF *Deschampsia antarctica* UNDER CONDITIONS OF COLD AND SALINITY STRESS

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Several environmental factors can affect growth and development of plants. Throughout the evolution of these species have evolved mechanisms allowing them to tolerate and adapt to adverse environmental conditions.

Deschampsia antarctica (Family poaceae) is the only grass that grows naturally in the area of Antarctica. This species has attracted the attention of many scholars because of its ability to survive in the harsh Antarctic environment. Is regularly exposed to cold conditions and high salinity, some studies suggest that this ability is due to the coordinated expression of genes associated with abiotic stress. Our group has identified a group of genes differentially expressed under conditions of Antarctica, including Q-10 Quinone, a coenzyme of lipid nature. Q-10 Coenzyme is an essential substance for the production of energy from carbohydrates, it acts as a carrier of electrons necessary for the oxidative phosphorylation process that occurs in cellular mitochondria,also plays an important role as an antioxidant in many organisms, protecting against free radical damage.

To analyze the expression of this genewas carried out relative quantification of transcription Q-10 in *Deschampsia antarctica* plants in different salinity conditions in combination with cold.

The results confirmed differences in the expression of this gene in the conditions tested.

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47.

MOLECULAR AND HISTOLOGICAL COMPARISON BETWEEN SEED AND VEGETATIVE PROPAGATED *Deschampsia antarctica* PLANTS.

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Deschampsia antartica and *Colobanthus quitensis,* are the only vascular plants that inhabit the Maritime Antarctic. Both have awakened scientific interest due to their biochemical, physiological and molecular mechanisms that allow them to survive in such extreme conditions. It has been reported that seed viability in *D. antarctica* is close to 1% in natural conditions, and needs a long period of dormancy•break to germinate in laboratory conditions. For that reason, routinely, in the laboratory *D. antarctica* is vegetatively propagated. Seeds from a spike collected on December (Summer) 2009 from Rey Jorge Island, in Maritime Antarctic, germinated in laboratory without previous treatment. A non•esterille soil was used, and the percentage of germinated plants greatly surpasses previous reports. Therefore, it was necessary to verify if the germinated individuals are *D. antarctica* or if they correspond to soils contaminant•plant species. ISSR was used to compare germinated and vegetatively•propagated individuals of *D. antarctica*. Nine ISSR primers were used for molecular level comparisons. Due to the polymorphic similarity with the vegetative•propagated plants, the most informative primer ($(CT)_nRG$) was used and no differences were detected between both types of individuals. Additionally, preliminary histological comparison did not show any relevant differences between individuals. This is the first report of high germination percentage of non•treated seeds in *D. antarctica* and the first study to use ISSR for the species. We propose that *D. antarctica* seeds need the extreme conditions of Antarctic autumn – winter for dormancy•break, potentially achieving higher germination percentage than previously assumed. More studies are requiered to unravel the physiological processes behind seed germination in antartic population *of D. antarctica.*

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48.

SUCROSE PHOSPHATE SYNTHASE (SPS) EXPRESSION AND ENZYMATIC ACTIVITY IN *Colobanthus quitensis* FROM CONTINENTAL AND ANTARTIC POPULATIONS AND ITS RELATIONSHIP WITH SUCROSE ACCUMULATION.

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Colobanthus quitensis ranges from Mexico to southern Antarctic Peninsula, where is one of the two vascular plants reported. It grows from 0 to 4200 m a.s.l. showing considerable morphological variability along its range. In Chile, *Colobanthus quitensis* can be found at high altitudes in La Parva (the Andes) and close to sea level in King George Island (the Antarctic) and Punta Arenas. Antarctic and La Parva populations are considered cold•resistant ecotypes. Plants from Punta Arenas, however, have been less studied. Sucrose plays an essential role in osmoregulation and cryoprotection in many plant tissues during cold conditions. *C. quitensis* has been shown to accumulate sucrose in response to cold. In plants, sucrose synthesis is catalyzed by sucrose phosphate synthase (SPS) and sucrose phosphate phosphatase (SPP). SPS is strictly regulated, and different SPS isoforms are expressed according to the tissue, developmental stage, and environmental conditions. We evaluated SPS accumulation and SPS activity in three *C. quitensis* populations (Antarctic•Ant•, Punta Arenas•PA•and La Parva•Par•). Plants from all populations were growed alternatively at 4°C and 15°C and at two photoperiods (21/3 and 8/16). Data from plants collected in the field is also reported. Additionally, total soluble sugar (TSS) and sucrose accumulations were determined. In order to identify different SPS isoforms, we used primers designed from SPS conserved regions of different plants species. Continental populations (PA y Par) showed higher SPS protein expression and SPS activity than Ant. PA plants showed higher TSS accumulation in field and laboratory conditions. The highest sucrose accumulations was observed in the 4°C, 21/3 treatment, and also in roots tissues at laboratory conditions. At least, two SPS isoforms were amplifyed from *C. quitensis* leaves. This is the first report including PA individuals of C. quitensis. Understanding SPS patterns in a widely distributed plant such as *C. quitensis* can contribute to elucidate adaptative mechanisms of cold resistance.

49.

ISOLATION AND CHARACTERIZATION OF A SODIUM/PROTON EXCHANGER (*EgNhx1*) FROM *Eucalyptus globulus*.

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Salinity is one of the major factors effecting the growth and yield of plants. Saline environments inhibit cell division and expansion, and results in slower cell growth and smaller plants. In plants, vacuolar Na^+/H^+ antiporters can pump Na^+ into vacuolar to reduce Na^+ toxicity and maintain a high K^+/Na^+ ratio in cytosol to alleviate salt stress. Overexpressing *AtNHX1* in *Arabidopsis* resulted in transgenic plants capable of growing in the presence of 200 mM NaCl, with an increased activity of vacuolar Na^+/H^+ antiporter. We had previously isolated from an *E. globulus* cDNA library a clone that contained the EgNhx1 coding region. To prove its functionality, we complemented the *Saccharomyces cerevisiae* Δ*nhx1* mutant strain with the full length EgNhx1. While the yeast mutant strain was sensitive to high concentrations of salt, the mutant strain complemented with the full length cDNA grew normally. Likewise, the overexpression of the full length EgNHX1 on wild type yeast resulted in an improved growth rate at high salt concentration when compared to untransformed wild type yeast.

To determine if EgNHX1 had the same effect in plants, we transformed *Arabidopsis thaliana* Columbia with the full-length cDNA under the control of the CaMV35S constitutive promoter. We already have 35 T1 lines that are currently being grown in control conditions to perform functional analyses.

To investigate the expression pattern of EgNHX1, we subjected *Eucalyptus globulus* seedlings to different abiotic stress treatment: salt, drought or heat stress. Our results revealed that within the first four hours EgNhx1 transcripts exhibit their highest peak when subjected to drought stress.

50.

Vitis vinifera GENOME ANNOTATION IMPROVEMENT USING NEXT-GENERATION SEQUENCING TECHNOLOGIES AND NCBI PUBLIC DATA

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Grapevine is the most widely cultivated and economically important fruit crop in Chile. Besides its economic importance and huge diversity, grapevine constitutes *per se* a model for woody plant species. Our aim is to develop a national biotechnological platform to support new genome sequencing technologies capabilities to Chilean grapes breeding programs, which will be used in germplasm selection for interest traits as well as SNPs, splicing variants and new genes identification. We analyzed data from commercial varieties of *Vitis vinifera* and wild species of *Vitis* genus, obtained using Illumina sequencing technology, from two RNA-seq experiments performed by Zenoni *et al.* (2010) and Myles *et al.* (2010), both available at NCBI. The sequence reads were aligned onto the 12X draft sequence based on Pinot Noir 40024 genome whereas the reported data was aligned onto 8X genome. We aligned 116,665,608 reads selecting hits with at most two mismatches. Over 55% of the reads showed only one alignment to the reference genome, 19% had multiple matches and 26% of them were unmatched. 172,284 unique putative SNPs were detected using a Q-score of 20, coverage over 8x and variability over 25%. 72% of those were exclusive to *Vitis vinifera* varieties, 21.4% appeared only in wild *Vitis* species and 5.9% were common to wild and cultivated samples. Variants of putative alternative splicing (PAS) were identified using *ad hoc* Perl scripts and SAM tools. 80,175 PAS variants were filtered by a preliminary Blast search against Swissprot Database, and results showed that just 1,676 (2%) of PAS coded for a complete protein sequence with a maximum E-value of e^{-10}. According with the current results, we have improved the *Vitis vinifera* genome annotation in 4.8% and also our knowledge about *Vitis* evolutive phylogenetic relationships. Our future perspectives involve the integration of our developing experiments from transcriptomics and proteomics approaches.

Financed by Genoma-Chile, Millenium Nucleus in Plant Biotechnology, grant FONDEF G07I-1002, Basal PFB-16, Basal-CMM grant, and Mecesup Program.

51.

CHERRY TRANSCRIPTOME: A TOOL TO DEVELOP NEW VARIETIES

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Chile is the main cherry exporter from the south hemisphere, despite producing only 1.6% of the world production. Until now, our country has been able to compete with the rest of the south hemisphere producers, but this situation, without technological investments is not sustainable over time, due to a market which is increasingly more competitive as well as increased restrictive access to new varieties. Within this context, our Cherry Breeding Program is establishing a technological platform where agronomic and molecular tools can converge to potentiate the competitiveness of our chilean cherry industry by establishing a Cherry Marker Assisted Breeding Program. One of the objectives of this project is to develop new molecular tools based on the cherry genome/transcriptome. Using 454 FLX technology, we have obtained 478,403 reads from leaf and fruit total mRNA from cherry varieties Bing, Lapins and Rainier (6 cDNAs libraries). Using the CLC Genomic Workbench suit, we have assembled 5,124 contigs from all fruit reads and 4,310 contigs from all leaf reads. Afterwards, and through automatic functional annotation (Blast2GO), followed by manual annotation, we have annotated 65% of both contigs sets. We have also made a differential expression analysis based on Digital Northern technique. Our preliminary results suggest that 712 genes are differentially expressed between the 3 varieties in fruit, and 540 in leaf. In addition to the identification of these variety-specific differentially expressed genes, these sequences are being analyzed for molecular markers that may be used this Chilean Cherry Marker Assisted Breeding program.

This research was supported by Innova-Corfo 07CN13PBT-167 and Millennium Nucleus in Plant Cell Biotechnology (PCB) ICM P06-065-F.

52.

IDENTIFICATION AND CHARACTERIZATION OF BOR TRANSPORT FAMILY IN GRAPEVINE.

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Boron (B) is an essential microelement for plants. The primordial function of B is undoubtedly its structural role in the cell wall. Despite the great importance of B for plants, the range of concentrations between deficiency and toxicity is narrow. For these reason plants have evolved an efficient system of B uptake and transport using a mix of efflux carrier named BOR proteins. These genes encoded the BOR proteins have been described as a family in arabidopsis and rice genome. In this work, we have identified and characterized the BOR family in the fruit tree *Vitis vinifera*. We carried out a search for homologous proteins into Grape Genome Browser Platform (Genoscope), using as template AtBOR1 protein. We identified six gene models that encode putative proteins with sizes ranging from 720-650 amino acids. These genes were named *VvBOR1* to *VvBOR6*. Using Real Time-PCR we demonstrated that the six genes show different patterns of expression, suggesting that may have complementary roles. All of them are expressed in flowers and *VvBOR2* is the only gene that is expressed in all tissues examined (root, leaf, flower, fruit and seed). We evaluated whether the expression of these genes is dependent on the presence of B. After 24 hours of treatment, either in deficit or excess, no significant changes in gene expression of VvBORs was detected. To evaluate the role of these putative B transporters, we cloned the coding region of *VvBOR2* and were overexpressed in arabidopsis plants. Transgenic plants compared with arabidopsis wild type plants have the same normal and sensitive phenotype under control and excess of B respectively, but were more tolerant to B deficiency. The characterization of B transport will provide more information to understand the homeostasis of this element in plants.

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53.

IDENTIFICATION OF CANDIDATE TRANSCRIPTION FACTORS FOR ENDOMEMBRANE SYSTEM GENES REGULATION THROUGH CONSTRUCTION AND ANALYSIS OF AN INTRACELLULAR PROTEIN TRANSPORT REGULATORY NETWORK.

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The Endomembrane System (ES) is a fundamental component of every eukaryotic cell and corresponds to a set of compartments physically and functionally interconnected by cargo vesicles trafficking through the secretory and endocytic pathway. The ES regulation allows a preferential vesicles routing to a particular compartment during different biological processes. This regulation is carried out mainly post-translationally. However recently has been published evidence showing transcriptional regulation of ES genes coordinating protein trafficking.

Using online-available *Arabidopsis thaliana* databases we generated a regulatory network to find candidate transcription factors (TF) that could regulate ES genes. We selected 98 ES genes of Intracellular Protein Transport GO and searched for TF whose assigned binding-sites are over-represented in their promoters generating a Transcriptional Network. A Co-expression Network was constructed between TF and ES genes using microarray data. Integrating both networks the Intracellular Protein Transport Regulatory Network was obtained. This corresponds a non-random network with 125 edges, 122 nodes (91 TF, 31 ES gene neighbors). The candidates TF were ranked using two criteria: higher degree to ES genes than all Arabidopsis genes in the Transcriptional Network and high degree in the Co-expression Network. This allowed to select seven candidates TF for the regulation of ES genes. One of them has been previously characterized as ES genes regulator supporting that the Network and the selection criteria are robust.

To analyze if the ES-putative-target gene of the candidates TF are being transcriptional regulated we have looked their transcript level under known-drugs treatments that alter the ES into microarray data. The ES genes transcript levels changes in response to the analyzed treatments. We are analyzing the effect of different ES-impairing drugs over ES genes subset by means of qRT-PCR.

The bioinformatic approach we have used allowed us to select seven TF as strong candidates for regulation of ES gene neighbors.

54.

NOVEL *Arabidopsis thaliana* IONIC HOMEOSTASIS-INVOLVED GENES CONFER DIFFERENT HEAVY METAL TOLERANCE IN *Saccharomyces cereviseae.*

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To develop organisms that resist ionic stresses, we are studying mechanisms that regulate ionic homeostasis. We are looking for genes that might be involved in ion transport. Since plants are able to adapt to different environmental changes, we decided to look into the *Arabidopsis thaliana* genome in order to find novel genes involved in this process. The searching and selection of them were performed analyzing co-expression networks, based on large-scale microarray data analysis in the Arabidopsis public database. Using a neighborhood analysis of the coexpression network, a list of genes that are likely involved in ion transport in Arabidopsis has been obtained. Out of those genes, we are focusing on genes that have highly probability of being involved in ionic homeostasis. We want to investigate their capability to confer tolerance against salts and heavy metals due to the high concentration are toxic to many organisms. In order to test the novel genes function we are using yeast and plants as an experimental model. We have cloned these genes to express them in both models and we have checked their correct molecular design and subsequent expression of interesting proteins. Using episomal vectors the plant genes were transformed in yeast to evaluate the tolerance given by these genes to resist high concentrations of metals. We have seen that the expression of several genes changes the tolerance of the transformed yeast to different concentrations of copper, cobalt and cadmium. We are currently testing the transformed yeast on different conditions such as mixture of the mentioned cations and also consider other metals like sodium, zinc and nickel. In addition we have begun the plant stable transformation and subsequent evaluation of them respect to their tolerance to heavy metals. We expect that this approach allows identifying novel plant genes involved in ionic homeostasis.

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55.

"DETERMINATION OF STRUCTURAL ELEMENTS INVOLVED IN pH SENSITIVITY IN KAT1 CHANNEL OF *A. THALIANA*"

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Potassium ions (K^+) are required for plant growth and development. K^+ transport generates cell turgor necessary for stomatal opening. This last event require an apoplasm acidification (around 22.5 pH units), that is perceived by voltage gated inward potassium channels (K_{in}) allowing its opening with lower energy cost. It is proposed that the extracellular pH sensors in stomata K_{in} channel are histidines exposed to the apoplasm. However, in KAT1, a K_{in} channel of *Arabidopsis thaliana*, mutations in the unique histidine (H267) exposed to solvent, do not alter its pH dependence. H267 is in the pore of KAT1, as part of the sequence GYGDXH. KAT1 has a phenylalanine (F) at position X of this motif, while most of the other K_{in} channels have leucine at that position. The KAT1 homology model shows that F266 and H267 side chains are very close (< 4.5 Å). These data suggest that in KAT1:

1. H267 cannot sense pH changes, because F266 displace its pKa to undetectable values.

2. The pH sensing mechanism involves acidic residues exposed to the apoplasma, with a pKa around 4.1.

Both hypotheses were corroborated experimentally. It was determined that E240 is involved in sensitivity to pH in KAT1. In addition, through quantum mechanics calculations can be concluded that if the X position is occupied by leucine, histidine sensor could be protonated and deprotonated. On the contrary, if the position is occupied by phenylalanine, these events cannot occur at physiological pH. Phenylalanine established a cationπ interaction with H267, displacing its pKa and preventing it functioning as pH sensor in KAT1.

Thanks: DAADConicyt project NiaPoc

56.

STRUCTURAL AND TRANSCRIPTIONAL CHARACTERIZATION OF *PIRUVATE DESCARBOXYLASE (PDC), ALCOHOL DEHYDROGENASE (ADH) AND LACTATE DEHYDROGENASE (LDH)* GENES EXPRESSED IN ROOTS OF STONE-FRUIT ROOTSTOCKS (*PRUNUS SPP.*) EXPOSED TO HYPOXIA STRESS

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In several of the Chilean places in which stone fruits are currently cultivated, the excessively clay soils with poor drainage is one of the most limiting edaphic factors for the stone-fruit orchards development. In such soils, heavy rain or excessive irrigation causes them to become waterlogged. Under this situation, air pockets in soil become filled with water during saturation and a hypoxic (low oxygen levels) condition followed by anoxia is created. From the physiologically point of view, when plant roots are exposed to hypoxic conditions the aerobic respiration is inhibited, yielding low energy (hypoxia blocks oxidative phosphorylation and ATP is generated by the cytosolic glycolysis). Furthermore, under hypoxia the fermentation of pyruvate to the major end products, ethanol or lactate, is activated to yield NAD+ for sustaining the anaerobic metabolism. In stone fruit trees, the tolerance to many environmental stresses is mediated in part by the characteristics of the rootstock. Most of the *Prunus* rootstocks are classified as hypoxia sensitive, although differences among genotypes regarding their ability to tolerate this abiotic stress have been reported. However, the molecular bases of such responses are scarcely understood. In order to characterize the *Prunus* rootstock molecular responses to hypoxia stress, *Piruvate Descarboxylase (PDC), Alcohol Dehydrogenase (ADH) and Lactate Dehydrogenase (LDH) ESTs* from roots of three *Prunus* rootstock (peach, plum, cherry) exposed to waterlogging were isolated and transcriptionally analyzed. An increment in the *ADH, PDC* and *LDH* expression was observed in the rootstocks studied suggesting a role of fermentative pathways in the adaptative responses of these species to hypoxia stress.

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57.

IN SILICO ANALYSIS OF THE EXPRESSION OF GENES INVOLVED IN NAD SALVAGE PATHWAY IN *Arabidopsis thaliana* IN DEVELOPMENTAL STAGES AND STRESS CONDITIONS

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Plants are frequently faced to several stresses under natural conditions. They show tolerance, given mainly by molecular adjustments, that determines the survival and colonization of different species. In this sense, the study of genes involved in NAD Salvage Pathway has become a fundamental aspect for the improvement of plants against stress, because through this pathway, NAD regeneration takes place, wich is a substrate for enzymes that improve the tolerance of plants under these conditions (ei SIR2 and PARP).
In this work, we used the Genevestigator Software to perform an *in Silico* Analysis of genes involved in NAD Salvage Pathway in *Arabidopsis thaliana*, at different stages of development and at different stress conditions. In addition, we identified mutants that activate and repress this pathway in basal conditions.
We found that during different Arabidopsis developmental stages, there is an increase in the expression of several genes involved in this pathway, especially on flowering time. However, it was noted that the expression levels of genes such as Sir2 and PARP, have different expression pattern depending on the stage of plant development.
On the other hand, the study of these genes compared to stress conditions revealed that PARP1 (At4g02390) and PARP2 (At2g31320) are activated by genotoxic stress, while PARP3 (At5g22470) is activated sharply against drought, osmotic stress and ABA, indicating the possible implication of this gene family in DNA repair; Sir2.1 (At5g09230) was activated by light, UV and drought (to a lesser degree) and Sir2.2 (At5g55760) was slightly activated by nitrate and glucose. These data suggest that functions of many genes of NAD Salvage Pathway in plants are closely related and they are required to maintain the integrity of the plant during its development and against different stress types.

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58.

IDENTIFICATION OF PUTATIVE PEACH TRANSCRIPTIONAL REGULATORY NETWORKS ASSOCIATED WITH COLD AND/OR RIPENING RESPONSE GENES

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Previously, we have reported the identification of clusters of genes that are co-expressed under different postharvest conditions using digital expression analyses (Vizoso et al., 2009). *In silico* analyses of the upstream regulatory regions of these co-expressed genes in the recently completed peach genome has enabled us to identify conserved cis-regulatory elements in these co-expressed genes. Candidate transcription factors responsible for this co-regulation are being identified using comparative analyses with Arabidopsis. Additionally, we have begun functionally characterizing these candidate transcription factors using transient over-expression analyses in peach fruits.

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59.

A COLORFUL SIDE OF THE U TRIANGLE: PHYTOENE SYNTHASE GENE FAMILY IN THREE BRASSICA SPECIES.

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The three diploid (*B. nigra, B. oleracea, B. rapa*) and three allotetraploid (*B. carinata, B. juncea, B. napus)* species of Brassica, known as the "triangle of U" are one of the best model systems for the study of polyploidy. In this study, our goal was to determine whether phytoene synthase (PSY) gene families exist in *B. rapa* (AA), *B. oleracea* (CC) and the interspecific hybrid *B. napus* (AACC). Based on synteny information between the closely related genomes of *Arabidopsis thaliana*, *B. rapa* and *B. oleracea*, we were able to identify 3 genomic regions where PSY genes may reside in both *B. rapa* and *B. oleracea*, and 6 genomic regions in *B. napus*. Using an overlapping-PCR strategy, we have cloned and identified 2 BraA.PSY, 3 BolC.PSY and 5 BnaX.PSY genes. The existence of PSY gene families was further confirmed using DNA-SSCP and Southern-blot analyses. Expression profiling of PSY genes in these three Brassica species was performed by RT-PCR using primers targeted to conserved regions. Expression of at least one paralog, albeit at different levels, was observed for every tissue studied. To determine the existence of subfunctionalization of the different PSY paralogs in each species, we conducted cDNA-SSCP analysis. This analysis revealed that in *B. napus* one PSY paralog is preferentially expressed in petals and another preferentially expressed in photosynthetic tissues. This evidence is suggestive of the existence of a chromoplast-specific carotenoid biosynthetic pathway in Brassicas. Knowledge from this study will aid in future development of transgenic and conventional cultivars with carotenoid-enriched oil and further confirm Brassicas as a suitable model for the study of subfunctionalization of paralogous genes in polyploids.

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60.

FUNCTIONAL CHARACTERIZATION OF *AGAMOUS-like 11* GENE OF *Vitis vinifera (VvAGL11)* IN HETEROLOGOUS SYSTEMS, A KEY STEP TOWARDS THE MOLECULAR CHARACTERIZATION OF GRAPEVINE SEEDLESSNESS

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Seedlessness in table grape is one of the most desired traits for consumers and breeding programs. The integration of genomic resources with classic genetic experiments allowed us to identify and propose *VvAGL11* as the major gene responsible for the genetic control of seedlessness in grapevine. Genetic, transcriptional and molecular evidence was generated to support this hypothesis.

In *Arabidopsis*, *AGL11,* also known as *SEEDSTICK* (*STK*), is a D functional MADS-box gene that acts redundantly with other members of the *AGAMOUS* family to specify ovule and carpel identity, funiculus growth and seed abscission. It is also necessary and sufficient to promote ovule development.

In grapevine, the seedless allele characterization reveals several polymorphisms in the regulatory region that might be causatives of the detected misexpression of *VvAGL11* in seedless genotypes. Polymorphisms identified in the coding region are not exclusive to seedless genotypes and no causative effect could be attributed to them.

To provide further evidence that *VvAGL11* is the cognate homolog of *STK*, and to further investigate the role of *VvAGL11* in grapevine ovule and seed development, the *VvAGL11* cDNA was transcriptionally fused to the CaMV35S promoter. To define the functional elements in the minimal promoter and to provide evidence that seedlessness is due to a misexpression of *VvAGL11* caused by polymorphisms in these elements, several seedless and seeded alleles are being characterized and studied at transcriptional level. Furthermore, the Green Fluorescent Protein was transcriptionally fused to the seedless and seeded *VvAGL11* regulatory regions and allele-specific expression is being analyzed.

These constructions are being used for genetic transformation of *Arabidopsis* (wt and endogenous mutant *stk*) and tomato. Preliminary results reveal that overexpression of *VvAGL11* in *Arabidopsis* results in an accelerated transition to flowering and differentiation of apical inflorescence meristems into ectopic terminal flowers.

The work is supported by grant 08CT11PUD-07 from INNOVA-CORFO.

61.

TARGETED IDENTIFICATION OF GENES REQUIRED FOR POLLEN DEVELOPMENT IN *Arabidopsis thaliana*.

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Male gametophyte development in plants is a complex process that ends with the production of mature pollen grains. In the past years, a few genes encoding proteins required for pollen development and function has been identified in Arabidopsis and other species, however, our knowledge of the molecular actors involved in these essential processes are still very limited. Our laboratory has developed a methodology that, based of expression data, has allowed us to identify candidate genes involved in pollen development and function. Our hypothesis proposes that in the group of genes that are expressed preferentially in the final stages of pollen development, there are an over-representation of genes involved pollen development and function, specifically in pollen germination and pollen tube growth. Our methodology consists on first select candidate genes for functional analysis based on publicly available microarray data, identify mutant plants in candidate genes searching *in silico* using the data bases available for Arabidopsis and finally characterize the phenotype of mutant plants. Different stains are used to evaluate viability, morphology and metabolism in the developmental stage. Pollen functionality is assayed by *in vitro* germination assays and pollen tube growth and structure are analyzed by bright field microscopy. From 157 candidate genes found we have characterized 114 of them. Since now, we have identified plants that show alterations in pollen development (dead pollen production) and others that show alterations in germination and/or pollen tube growth. Genes identified encodes proteins involved in cell signaling, cell wall metabolism and calcium homeostasis, among others.

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62.

GENERATION AND ANALYSIS OF SUPRESSIVE SUBTRACTIVE HYBRIDIZATION LIBRARIES OF GRAVITROPIC RESPONSE IN RADIATA PINE SEEDLINGS.

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Coniferous trees develop compression wood in response to gravitropic stimuli. In nature this response can be generated by growth in slope, exposure to snow or high winds. However, genes and the molecular mechanism involved in this phenomenon are still unknown. We studied the expression of genes in response to gravitropic stimulation induced at 45° inclination of *Pinus radiata* D. Don seedlings. Total RNA was extracted at different inclination time and considering two half of the stem (inferior and superior half). This tissue was obtained through longitudinal section of the zone of curvature. From these RNAs 8 ESTs suppressive subtractive hybridization libraries (SSH) were generated containing 2683 clones with fragment sizes between 100 and 1500bp. The sequences were assembled, analyzed and categorized by FunCat, highlighting differences in expression between superior and inferior half and across the different times of inclination in groups of genes related to metabolism, signal transduction and cellular transport among the others. In the group of genes involved in metabolism are include some related to cell wall remodeling and sugar metabolism. Another important group was transcription factors. Candidate genes were selected and analyzed by qPCR. Genes involved in hormonal signal transduction and metabolic pathways, especially phenylpropanoids, are indeed differentially expressed at both side of stem during the gravitropic response.

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63.

MOLECULAR MODELLING AND SITE-DIRECTED MUTAGENESIS 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. This attribute is a complex character determined by a set of low molecular weight volatile compounds. In mountain papaya *(Vasconcellea pubescens)* the aroma is formed mainly by esters. The esterification step is catalyzed by alcohol acyl-transferase enzymes (VpAAT1) that transfer an acyl-CoA to an alcohol. To gain insight about the mechanism of action of VpAAT1 at the molecular level, the comparative modeling methodology was used to build the enzyme's structure, which was refined with molecular dynamics simulation. The obtained model showed the active site HTMSD motif located in a loop between the sheet 7 and the helix 5. The analysis of this motif showed to the residue H166 exposed to the solvent channel. To understand the importance of this residue, the H166A mutation was evaluated *in silico*. Significant changes on interaction energy and ligand's orientation in the solvent channel was found, after molecular docking simulation of VpAAT1 H166A, showing unfavorable interaction energies with different alcohols and acyl-CoA substrates. Finally, *in vitro* site-directed mutagenesis analysis showed a loss in the enzymatic activity of VpAAT1 mutant enzyme, confirming the functional role of residue H166 during the VpAAT1 catalysis. The obtained results are discussed in term of the differences in the interaction of residue H166 with different substrates and the enzymatic activity experimentally observed.

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64.

IDENTIFICATION AND EXPRESSION ANALYSIS OF *VvSDH1*, A PUTATIVE SORBITOL DEHYDROGENASE OF GRAPEVINE (*Vitis vinifera*)

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Sorbitol is the main product of photosynthesis and the form in which carbon is translocated via the phloem in species belonging to the Rosaceae family, which includes peaches (*Prunus persica*), pears (*Pyrus* spp) and apples (*Malus* x *domestica*). Once in the carbon sink organ, a proportion of this sugar alcohol is metabolised to fructose via NAD-dependent sorbitol dehydrogenase (SDH). Nevertheless, SDH has also been found in non-Rosaceae species, such as tomato (*Solanum lycopersicum*), soya (*Glycine max*) and maize (*Zea mays*), in which the main phloem-translocated carbon is sucrose.

Sorbitol presence in non-Rosaceae species has been linked to stress conditions, such as drought or stress due to high or low temperatures. As a first approach to determine the physiological role of sorbitol and SDH in these species, we have identified a putative SDH from grapevine (*Vitis vinifera*), using a reverse genetics approach which we have named VvSDH1. VvSDH1 possesses all the molecular characteristics and the conserved domains present in the SDHs of other species, and is expressed in various organs of the plant, as determined by RT-PCR. However, bioinformatic studies using the microarray database Plexdb show that *VvSDH1* expression is momentarily increased under drought conditions, but that it is not influenced by cold stress. In order to determine the enzymatic activity of the enzyme, *VvSDH1* cDNA has been cloned into plant binary vectors under the control of two different promoters: cauliflower mosaic virus 35S and the fruit-specific polygalacturonase promoter from tomato. In order to test the functionality of these constructs, tobacco leaves and tomato fruits will be transiently transformed using *Agrobacterium tumefaciens*. Advances in the progress of these experiments will be discussed.

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EXPRESSION AND BIOCHEMICAL ANALYSIS OF *AtSDL*, A PUTATIVE SORBITOL DEHYDROGENASE IN *Arabidopsis thaliana*.

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Sorbitol is the main product of photosynthesis in *Rosaceae* species (apples, peaches, plums, among others). This sugar alcohol is produced in source organs (mature leaves) and transported through the phloem to sink organs (roots, fruits, immature leaves), where, by the action of sorbitol dehydrogenase (SDH) it is oxidized to fructose, which can be either stored or metabolised. On the other hand, in almost all other plant families, including *Solanaceae* and *Brassicaceae*, sucrose is the principle sugar transported. Nevertheless, sorbitol and/or SDH activity have been detected in some of these species, yet their physiological role has yet to be understood. As an approach to determine the roles of SDH in non-sorbitol translocating species, we have identified a protein in *Arabidopsis thaliana* with >75% aminoacid identity with previously-characterised plant SDHs, named AtSDL. RT-PCR expression analysis showed that *AtSDL* is expressed in multiple organs. In addition, analysis of *Arabidopsis* plants which have been stably-transformed with the *AtSDL* promoter::GUS fusion construct, revealed systemic expression except in roots and petals where only the vasculature was stained. An analysis of the promoter region of *AtSDL* revealed the presence of multiple *cis*-regulatory elements that respond to different stimuli, which will allow the quantification of expression under these conditions. Additionally, a monoclonal antibody has been raised against AtSDL for use in western blot assays and subsequent applications in the determination of the subcellular localization of this enzyme. Finally, using *in vitro* translation, bacterial, yeast and plant expression systems, recombinant AtSDL is being expressed in order to determine the substrate specificity of the enzyme.

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