VI Reunión biología vegetal

30 de noviembre • 02 de diciembre de 2011
Pucón, Chile

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Scientific Program

Wednesday – November 30th, 2011

Hotel Check-in starts at 12 PM – Gran Hotel Pucón Reception

12:00 – 17:00 Registration – Gran Hotel Pucón Pre Foyer
12:00 – 17:00 Posters & Exhibits Set up –Lonquimay Room

15:00 – 15:30 Opening Welcome –Araucanía Room
Haroldo Salvo-Garrido & María L. Federico, CGNA.

15:30 – 16:30 Plenary Lecture I –Araucanía Room

Dr. Andrew Sharpe, "Sequencing and assembly of Brassica crop genomes". NRC Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, Saskatchewan, S7N 0W9, Canada - International Collaborator FONDECYT 1100732
Chair: Federico Iñiguez-Luy

16:30-17:15 Coffee Break – Gran Hotel Pucón Foyer

17:15-18:45 Oral Session I –Araucanía Room
Chairs: Federico Iñiguez-Luy & Andrés Zurita Silva

17:15 **Identification of polymorphism between different sweet cherry varieties using next generation sequencing, GeneScan analyses of SSRs and RosBREED iscan bead chip analyses. Carolina Klagges**, Nicolás Briceño, Ingrid Araya, Fernanda Rodríguez, Lee Ann Meisel

17:35 **Marker assisted selection for the introduction of double resistance to powdery mildew in grapevine. Camila Almendra-Claëys**, Carolina Serrano, Sarolta Hoffman, Pal Kozma, Patricio Arce-Johnson

17:55 **High throughput SNP genotyping in *Brassica napus* L.: SNP detection in genomic areas associated to traits of agronomical and nutritional importance**. Cristell Navarro, **Wayne E. Clarke**, Daniel Gerhardt, Humberto Gajardo, Andrew Sharpe, Isobel P. Parkin, María L. Federico, Federico L. Iñiguez-Luy

18:15 **Evidence of microsynteny between *Lupinus luteus* and *Medicago truncatula* genomes. Lorena Parra**, Cristell Navarro, Marcos Olivos, Joshua Udall, Jeff Maughan, Haroldo Salvo-Garrido, Iván Maureira-Butler

19:00 – 21:00 Welcome Cocktail - Poster Session I –Lonquimay Room
(ODD number posters)

21:15 Dinner – Gran Hotel Pucón Calafquén Restaurant

Thursday – December 1st, 2011

Open access to Posters & Exhibits – Lonquimay Room 9:00 – 21:00

7:00-8:45 Breakfast – Gran Hotel Pucón Calafquén Restaurant

9:00 – 10:00 Plenary Lecture II –Araucanía Room

Dr. Manuel Rodríguez Concepción, "Regulation of plant carotenoid biosynthesis: lessons from Arabidopsis". Centre for Research in Agricultural Genomics, Campus UAB, Bellaterra, CRAG/CSIC, Barcelona, España. International Collaborator CSIC España/Universidad de Chile 10/11-2
Chair: Claudia Stange

10:00- 11:00 Oral Session II –Araucanía Room
Chairs: Claudia Stange & María L. Federico

10:00 **Molecular strategies to study the function of *Icyb2*, a putative lycopene β-cyclase from *Daucus carota* (carrot). Carolina Rosas,** Claudia Stange

10:20 **Retention of triplicated phytoene synthase (*PSY*) genes in brassica napus l. And its diploid progenitors during the evolution of the *Brassiceae*. Pablo Cárdenas**, Humberto Gajardo, Isobel Parkin, Federico Iñiguez-Luy, María L. Federico

10:40 **Use of plants as bioreactors: expression of the RNA of three different epitopes of Hepatits C virus (HCV) in tomato plants. Susan Hitschfeld**, Nicolas Daneri, Francisca Jauregui, Patricio Arce-Johnson

11:00-11:30 Coffee Break – Gran Hotel Pucón Foyer

11:30- 13:00 Oral Session III –Araucanía Room
Chairs: Rodrigo Gutiérrez & Ma. Alejandra Moya

11:30 **Expression of an optimized *Argopecten purpuratus* antimicrobial peptide in *E. Coli* and evaluation of the purified recombinant protein by *in vitro* challenges against important plant fungi. Christian Montes**, Eduardo Tapia, Patricia Rebufel, Alberto Paradela, Gloria Arenas, Humberto Prieto

11:50 **Cytokinin signaling and nitrate induced root growth in *Arabidopsis thaliana*. Pamela A. Naulin**, Karem Tamayo, Rodrigo A. Gutiérrez

12:10 **TGA1 and TGA4 transcription factors control nitrogen responses in *Arabidopsis thaliana* roots. José M. Alvarez**, Eleodoro Riveras, Diana E. Gras, Elena A. Vidal, Orlando Contreras-López, Felipe F. Aceituno, Karem P. Tamayo, Rodrigo A. Gutiérrez

12:30 **ATPRP3 polar localization within the root hair cell wall is regulated by both secretory and endocytic mechanism(s). Cecilia Rodríguez-Furlán**, Mary Tierney, Ariel Orellana

13:00-14:30 Lunch – Gran Hotel Pucón Calafquén Restaurant

15:00- 16:00 Plenary Lecture III –Araucanía Room

Apoyo a la Formación de Redes Internacionales entre Centros de Investigación 2011 Bilateral Seminar Series:

Dr. Steve Robinson, "Resisting the Freeze". Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, Saskatchewan S7N 0X2, Canada.
Chair: Haroldo Salvo, PhD.

16:00-17:20 Oral Session IV –Araucanía Room
Chairs: Loreto Holuigue & Gabriel León

16:00 **Functional characterization of novel genes associated to ionic stress in *Arabidopsis thaliana* and *Saccharomyces cerevisiae*. Daniela Urbina**, Matías Freire, Aliosha Figueroa, Alexander Vergara, Lorena Norambuena

16:20 **Physiological, biochemical and molecular responses in three eucalyptus species that differs in their tolerance to drought stress. Pino M.T.**, Selles G., Balboa M., Milla. E., Romero P., Rojas P., Ortiz O., Molina M.P., Gutiérrez B

16:40 ***GRXS13* plays a key role in protection against photooxidative stress and cold acclimatization in Arabidopsis. Ema Olate**, Daniel Laporte, Marcela Salazar, Julio Salinas, Loreto Holuigue

17:00 **Heterologous expression of an artificial microRNA derived from grapevines. Alejandra Ramírez**, Álvaro Castro, Humberto Prieto

17:20-17:50 Coffee Break – Gran Hotel Pucón Foyer

18:00 Business Meeting – VII Plant Biology Meeting Organization –Araucanía Room

19:00 – 21:00 Poster Session II –Lonquimay Room

(EVEN number posters)

21:15 Dinner – Gran Hotel Pucón Calafquén Restaurant

22:30 After Dinner Get Together –Casino Enjoy, Amura Lounge

Friday – December 2nd, 2011

Hotel Check-out before 12 PM – Gran Hotel Pucón Reception

Open access to Posters & Exhibits – Gran Hotel Pucón Multicancha (10:00-17:00)

7:00-9:15 Breakfast – Gran Hotel Pucón Calafquén Restaurant

9:15-10:00 **Application Seminar: "Illumina MySeq™ System: the next revolution in personal sequencing"**. Dr. Veridiana Cano, Illumina, Sao Paulo, Brazil.

10:00-11:00 Plenary Lecture IV –Araucanía Room

Apoyo a la Formación de Redes Internacionales entre Centros de Investigación 2011 Bilateral Seminar Series:

Dra. Isobel Parkin, "An Integrated Systems Approach to Studying Seed Quality Traits in *Brassica napus*". Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, Saskatchewan S7N 0X2, Canada.
Chair: María Laura Federico

11:00-13:00 Oral Session V –Coñaripe Room
Chairs: Michael Handford & Verónique Amiard

11:00 **Do potatoes have a single evolutionary history? What proportion of the genome supports this history? Flor Rodríguez,** David Spooner

11:30-12:00 Coffee Break – Multicancha

12:00 **Genetic characterization of the cluster structure of grapevine. José Correa**, Denisse Laborie, Maribel Mamani, Carlos Muñoz, Manuel Pinto, Patricio Hinrichsen

12:20 **Identification and functional analysis of molecular factors involved in the cytokinin response pathway in peach fruits. Camilo Avendaño Miralles**, Fernanda Rodríguez Rojas, Héctor Duchens Silva, Soledad Cabrera, Juha Immanen, Herman Silva, Ykä Helariutta, Lee Ann Meisel

12:40 **Identification of novel nucleotide sugar transporters involved in pectin biosynthesis. Henry Temple**, Ignacio Moreno, Francisca Blanco, Macarena Greve, Omar Sandoval, Ariel Orellana

13:00-14:30 Lunch – Gran Hotel Pucón Calafquén Restaurant

15:00- 16:00 Oral Session VI –Araucanía Room
Chairs: Patricio Hinrichsen & León Bravo

15:00 **Relationship between the size of berries and the sugar content in contrasting phenotypes of grapevine (*Vitis vinifera L.*).** Cristian Valdés, Daniela Olivares, José Correa, Denisse Laborie, Herman Silva, Patricio Hinrichsen, **Manuel Pinto**

15:20 **Photosynthetic light responses may explain vertical distribution of Hymenophyllaceae species in a temperate rainforest of southern Chile. María José Parra**, Karina I. Acuña, Angela Sierra-Almeida, Camila Sanfuentes, Luis J. Corcuera1, León A. Bravo

15:40 **Molecular and physiological study of postharvest rachis browning of table grape cv Red Globe. Iván Balic**, Adrián Moreno, Claudia Huerta, Ariel Orellana, Bruno Defilippi, Reinaldo Campos-Vargas

16:00 Closing Ceremony – Coñaripe Room

Posters should be removed by 5 PM.

1

SEQUENCING AND ASSEMBLY OF BRASSICA CROP GENOMES

Andrew Sharpe[1], Matthew Links[2], Chushin Koh[3], Carling Tallon[1], Rob Wood[2], Carrie Haimanot[2], Brittany Polley[1], Jacek Nowak[1], Faouzi Bekkaoui[1], Isobel Parkin[2]
andrew.sharpe@nrc-cnrc.gc.ca

[1]NRC-Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9, Canada
[2]Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, S7N 0X2, Canada

The Canadian Canola Sequencing Initiative (CanSeq) is spearheaded by the National Research Council of Canada Plant Biotechnology Institute and Agriculture & Agri-Food Canada, and brings together Genome Alberta and nine private partners from all over the world. The goal of this initiative is to produce draft public genome sequences for each of the ancestral *Brassica* species (*B. rapa*, *B. oleracea*, and *B. nigra*) that have led to the development of major commodity oilseed crops - *Brassica napus* (canola), *Brassica juncea* (mustard), and *Brassica carinata* (Ethiopian mustard, now being explored as a platform for the production of industrial bioproducts). By developing these foundational genomic resources, each partner organization will be able to use the data to help understand key genes in the plant's development, identify traits of interest, and ultimately to develop new varieties of the crops that have desired properties, such as drought tolerance, disease resistance, and increased yield.

The 3-year project is based in Saskatoon with the bulk of the sequencing being done on high-throughput, next-generation DNA sequencers. One component of the CanSeq project has been a contribution to the Multinational *B. rapa* Genome Sequencing Project that generated a publically available genome sequence for this species. CanSeq has also been engaged with other international partners to develop an assembly of the *B. oleracea* genome. This approach used data from multiple sequencing platforms together and has resulted in the production of a new genome assembly which is currently being validated. This effort together with the current status of our efforts in *B. nigra* and the amphidiploid Brassica crops will be described.

2

REGULATION OF PLANT CAROTENOID BIOSYNTHESIS: LESSONS FROM ARABIDOPSIS

Manuel Rodríguez-Concepción[1]
manuel.rodriguez@cragenomica.es

[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB, Campus UAB Bellaterra, 08193 Barcelona, Spain.

Carotenoids are one of the most abundant groups of natural pigments found in nature. Plant carotenoids provide color to roots, flowers and fruits, but also play central roles in photosynthesis and photoprotection. Additionally, their oxidative cleavage generates apocarotenoids such as the hormones abscisic acid (ABA) and strigolactones that regulate plant development and responses to external stimuli. Carotenoids are also important components of the human diet, as precursors of essential retinoids (including vitamin A) and protective antioxidants. The molecular mechanisms controlling plant carotenogenesis are not well understood yet, but recent work in the model plant *Arabidopsis thaliana* is providing new insights on the regulation of carotenoid biosynthesis at both transcriptional and post/transcriptional levels.

The metabolic precursors for plant carotenoid biosynthesis derive from the methylerythritol 4-phosphate (MEP) pathway and are shared by other plastidial pathways leading to the production of different isoprenoid-end products such as gibberellins and the side chain of tocopherols and chlorophylls. The identification and characterization of *Arabidopsis* mutants resistant to the inhibition of the pathway has provided evidence of several mechanisms controlling the levels and activities of key rate-determining enzymes at the post-transcriptional level. In particular, specific plastidial protease and chaperone systems appear to act together to ensure proper levels of active enzymes of the MEP pathway under normal growth and also in response to environmental challenges. MEP-derived precursors are specifically channelled to the carotenoid pathway by the enzyme phytoene synthase (PSY). Environmental factors such as light and salt stress are important regulators of PSY accumulation at the gene expression level. Our recent work has shown that the only gene encoding PSY in *Arabidopsis* is repressed in dark-grown seedlings by direct binding of the phytochrome-interacting transcription factor PIF1 to specific motifs in the PSY promoter. During deetiolation, PIF1 is degraded upon interaction with photoactivated phytochromes, resulting in a rapid derepression of PSY gene expression and a burst in the production of carotenoids in coordination with chlorophyll biosynthesis and chloroplast development for an optimal transition to photosynthetic metabolism. PIF1 and other PIFs also regulate PSY gene expression and carotenoid biosynthesis in response to changing light conditions in shoot tissues of deetiolated plants. In roots, however, PSY expression is feedback-regulated by ABA and strigolactones by a mechanism that does not involve PIFs but a different family of transcription factors.

3

RESISTING THE FREEZE

Steve Robinson[1]
steve.robinson@agr.gc.ca

[1]Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, S7N 0X2, Canada.

Agricultural productivity of the Canadian Prairies is restricted due to a short growing season that is delimited by the presence of damaging frosts. To protect themselves from such stresses, temperate plants have the ability to enhance their freezing tolerance upon exposure to low but above freezing temperatures, a process know as cold acclimation. The physiological adjustments and changes in gene expression that occur during this process are being elucidated. Most notably, a family of transcription factors (*CBF*) have been identified that control the cold regulated (*COR*) genes. It has been demonstrated that these gene themselves are induced by low temperature but their potential to manipulate freezing tolerance is hampered by numerous pleiotropic effects. It is therefore important to identify additional genes acting independently or in concert with *CBF* that will enable our ability to manipulate freezing tolerance. The use of these genes, where their expression is targeted to particular developmental stages will enable the length of the growing season to be extended, resulting in both an increase in yield potential and stability.

We report on the use of complimentary genomics strategies that have been utilised to investigate cold acclimation and the development of freezing tolerance among species in the Brassicaceae. The use of forward and reverse genetic screens in Arabidopsis has revealed additional genes that are important to this trait. In addition, a comparative genomics approach assessing the extent of the variation present among highly adaptive species such as *Pringlea antiscorbutica* (Kerguelen cabbage) has been undertaken. This has resulted in the generation of extensive transcriptome data from these species that will reveal additional strategies to improve this valuable trait.

4

UTILISING AN INTEGRATED SYSTEMS APPROACH TO ELUCIDATE SEED QUALITY TRAITS OF *BRASSICA NAPUS*

Wentao Zhang[1], Erin Higgins[1], Steve Robinson[1], Pierre Fobert[2], **Isobel Parkin**[1]
isobel.parkin@agr.gc.ca

[1]Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, S7N 0X2, Canada
[2]NRC-Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9, Canada.

Oilseed rape, *Brassica napus*, is an economically important crop with seed products that are invaluable for human nutrition and as renewable sources for various industrial applications. The overall value of *B. napus* seed is determined by quality traits including oil and protein content and composition, along with some anti-nutritive components such as glucosinolates, fibre, erucic acid, and phytate. These quality traits exhibit quantitative inheritance controlled by complex interacting gene networks. To dissect this genetic architecture, we have utilised an integrated systems analyses of the developing seeds from a large double-haploid population of spring type *B. napus*. This population was generated from a diverse cross between a cultivated *B. napus* line and a newly re-synthesized line and was extensively genotyped using a combination of RFLP, SNP and SSR markers. Firstly, phenotypic quantitative trait loci (QTL) controlling major seed quality traits including fibre, oil, total glucosinolates etc., were mapped by traditional methods; secondly, gene expression quantitative loci (eQTL) were identified and mapped using transcriptome data from Agilent customized *B. napus* 44K arrays. Using these two sets of data, a weighted gene co-regulatory network was constructed to correlate phenotypic and gene expression data. By integrating the results from the QTL mapping, eQTL mapping, and identified gene co-regulatory networks, key gene networks comprising the quantitative genetic architecture controlling *B. napus* seed quality traits were uncovered. This study provides insights into dissecting complex traits with a systems biology approach in a polyploid species.

OS 1

IDENTIFICATION OF POLYMORPHISM BETWEEN DIFFERENT SWEET CHERRY VARIETIES USING NEXT GENERATION SEQUENCING, GENESCAN ANALYSES OF SSRS AND ROSBREED ISCAN BEAD CHIP ANALYSES

Carolina Klagges [1], Nicolás Briceño[1], Ingrid Araya[1], Fernanda Rodríguez[1], Lee Ann Meisel[1]
lmeisel@gmail.com

[1]Millennium Nucleus in Plant Cell Biology and Biotechnology; Plant Molecular Genetics Laboratory, Center of Plant Biotechnology, Andres Bello University, Av. República 217, 837-0146 Santiago

Recent technical advances such as Next Generation Sequencing as well as Genescan and Goldengate analyses have the potential to dramatically increase the number of molecular markers that may be used to genotype fruit varieties, segregating populations and potentially interesting new varieties. An international initiative in the Rosaceaes community has led to the development of the RosBREED Cherry iSCAN Bead Chip which contains 5,733 SNPs as well as over 227 RosCOS markers that are conserved among different Rosaceaes species. Using this bead chip, as well as Genescan analyses of peach SSRs and HRM analyses of RosCOS SNPs and putative SNPs identified from 454 sequences, we have identified over one thousand molecular markers polymorphic between four sweet cherry varieties.

Funded by INNOVA 07CN13PBT-167, ICM P06-065-F, USDA-NIFA-SCRI grant #2009-51181-05808(RosBREED) and PBCT R-11a

OS 2

MARKER ASSISTED SELECTION FOR THE INTRODUCTION OF DOUBLE RESISTANCE TO POWDERY MILDEW IN GRAPEVINE

Camila Almendra-Claëys[1], Carolina Serrano[1], Sarolta Hoffman[2], Pal Kozma[2], Patricio Arce-Johnson[1]
caalmend@uc.cl

[1]Departament of Molecular Genetics and Microbiology. Faculty of Biological Sciences. Pontificia Universidad Católica de Chile.

[2]University of Pécs, Research Institute of Viticulture and Enology, Pécs, Hungary

Erisiphe necator is one of the most severe fungal pathogens in grapes, being able to infect both berries and leafs, causing the Powdery Mildew disease. The infection implies important productive losses in different places. Therefore, several applications of commercial fungicides during the grape productive cycle are required. Almost all *Vitis vinifera* varieties are sensitive to this pathogen; however different resistance loci have been described in grapes and related species. *Run1* (Resistance to *Uncinula necator 1*, according to the former name of the fungus) and *Ren1* (Resistance to *Erisiphe necator 1*) are the most studied loci. Nevertheless, the fungus has the chance to break down the resistance mechanism in monogenic resistant plants. Because of this, an effort to introduce two of these resistance loci in table grapes varieties is being made through genetic improvement. Using crossing and backcrossing strategies, we obtained a progeny named "P09-105" in which two resistance loci, *Run1* from '*Muscadinia rotundifolia'* and *Ren1* from the *Vitis vinífera* '*Dzhandzhal kara'* have been incorporated. We are analyzing P09-105 population using marker assisted selection. Susceptible plants (*ren1/run1*), mono-resistant plants (*REN1/run1* or *ren1/RUN1*) and double resistant plants (*REN1/RUN1*) were identified using microsatellites analysis and capillary electrophoresis. Finally with Trypan blue staining we can observe a correlation between the molecular analysis and the fungal presence in selected plants. The propagation of the double resistant plants and their crossing with table grape varieties will provide an advantage, due to the low probability of a fungal mutation capable of breaking down the double resistance. In addition, the use of fungicide treatments will decrease with the consequent economic and health benefits for the country.

Acknowledgements: Consorcio Tecnológico de la Industria Hortofrutícola, Fundación para la Innovación Agraria-FIA, Millennium Nucleus for Plant Functional Genomics

OS 3

HIGH THROUGHPUT SNP GENOTYPING IN *BRASSICA NAPUS* L.: SNP DETECTION IN GENOMIC AREAS ASSOCIATED TO TRAITS OF AGRONOMICAL AND NUTRITIONAL IMPORTANCE

Cristell Navarro[1], **Wayne E. Clarke**[2], Daniel Gerhardt[3], Humberto Gajardo[1], Andrew Sharpe[4], Isobel P. Parkin[2], María L. Federico[1], Federico L. Iñiguez-Luy[1]
federico.iniguez@cgna.cl

[1]Centro de Genómica Nutricional Agroacuícola (CGNA), CONICYT-REGIONAL, GORE LA ARAUCANIA, R10C1001, Unidad de Genómica y Bioinformática, Temuco, Chile.

[2]Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 0X2, Canada.

[3]Nimblegen-Roche NimbleGen, 500 South Rosa Road, Madison, WI 53719.

[4]National Research Council of Canada, Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, SK, Canada, S7N 0W9.

Targeted enrichment of specific genomic regions allows for large-scale resequencing in species with large and complex genomes. This approach coupled with the advent of next generation sequencing technologies (NGS) provides an attractive alternative to assess and characterize the levels of natural genomic variation in crops species of economical importance. In this study, we combined Roche NimbleGen sequence capture microarray technologies with NGS Roche 454 Life Science chemistry (454FLX-T) to discover single nucleotide polymorphisms (SNPs) in 50 specific genomic areas previously associated to yield, yield component traits, seedling vigor, seed quality and a disease resistance trait in five allopolyploid *Brassica napus* L. (AACC, 2n=38) genotypes. Sequence information was compiled into 890 FASTA files annotated from scaffold bins and raw genomic data, totaling approximately 51 Mb (36Mb and 15 Mb corresponding to the A and C genome, respectively). A 2.1 million feature sequence capture arrays representing 93.4-98.3% target coverage was used to hybridize the five *B. napus* genotypes. Captured DNA sequenced with 454FLX chemistry yielded an average number of 917,702 reads with an average total of 345,199,848 bases and an average length of 370 bp. On average 80% of the NGS reads mapped back to the reference genome providing great coverage of the examined genomic locations. SNP markers were detected using the CLC bio's Genomic Workbench software and a combination of *in house* build Perl scripts protocols. A total of 60,000 putative haploSNP markers were identified based on their unique flanking sequence and polymorphic frequencies within genotypes and between the reference genome. Putative haploSNP were classified by their genomic context (distribution of interrogated region, coding vs. non-coding and transition vs. transversion SNPs). This classification was used to select a subset of putative SNP markers to be validated in segregating populations.

Authors 1 and 2 contributed equally. This work has been funded by FONDECYT 1100732.and Agriaquaculture Nutritional Genomic Center (CGNA), CONICYT-REGIONAL, GORE LA ARAUCANIA, R10C1001. We acknowledge INIA for its support providing experimental fields and infrastructure

OS 4

EVIDENCE OF MICROSYNTENY BETWEEN LUPINUS LUTEUS AND MEDICAGO TRUNCATULA GENOMES

Lorena Parra [1,2], Cristell Navarro [1], Marcos Olivos [1], Joshua Udall[3], Jeff Maughan[3], Haroldo Salvo-Garrido[1], Iván Maureira-Butler[1]
ivan.maureira@cgna.cl

[1]Centro de Genómica Nutricional Agroacuícola (CGNA), CONICYT-REGIONAL, GORE LA ARAUCANIA, R10C1001, Unidad de Genómica y Bioinformática, Temuco, Chile.

[2]Instituto de Investigaciones Agropecuarias, Carillanca, Temuco, Chile
[3]Plant and Wildlife Science Dept., Brigham Young University, Provo, UT, USA

Next generation sequencing has allowed the building up of massive amount data, which have opened new opportunities for gene discovery, genome evolution, and macro and minor scale synteny studies. Probably, the greatest impact of this sequencing era has been achieved on orphan crops where combination of new EST data bases with already developed model species genomic platforms has facilitated the understanding of these minor crop genomes. *Lupinus luteus* is a legume orphan crop with great potential in human and animal nutrition due to its high protein and sulfur amino acids content and relatively low amount of antinutriotionals. We have started a genomic initiative to aid the genetic improvement of this crop, including massive sequencing of EST libraries, development of molecular markers, association studies, and comparative genomics with model species such as *Medicago truncatula*. We constructed *Lupinus luteus* EST libraries from seed, root, leaf and flower tissues and carried out massive 454 sequencing, which yielded, after full assembly, a total of 64,973 non redundant expressed sequences. Sequences were mapped in silico on the *M. truncatula* genome using Blastn with an E value < 1 e -20 and alignment information displayed as featured tracks in a GBrowse platform. Blast matches were homogeneously distributed on the eight *Medicago* chromosomes. The occurrence of microsynteny between *L. luteus* and *M. truncatula* was evaluated by PCR amplification and sequencing of genomic DNA blocks of *L. luteus* using primers designed to amplify coding sequences and intergenic regions of contiguous genes based on the physical map of *M. truncatula*. Around 40% of targeted *L. luteus* regions yielded positive Medicago equivalent DNA blocks. We are currently using this approach to fine map and/or identify genes or genomic regions previously associated to nutritional and agronomic traits.

This research was funded by project FONDECYT 1090759 and Agriaquaculture Nutritional Genomic Center (CGNA), CONICYT-REGIONAL, GORE LA ARAUCANIA, R10C1001. We acknowledge INIA for its support providing experimental fields and infrastructure

OS 5

MOLECULAR STRATEGIES TO STUDY THE FUNCTION OF LCYB2, A PUTATIVE LYCOPENE B-CYCLASE FROM DAUCUS CAROTA (CARROT)

Carolina Rosas[1], Claudia Stange[1]
cstange@uchile.cl

[1]Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile

In plants, carotenoids are isoprenoid pigments synthesized in plastids and are involved in photosynthesis, photoprotection and abscisic acid synthesis. In addition, b-carotene, the main carotenoid of carrots, is precursor for vitamin A and possesses high antioxidant properties. Lycopene b-cyclase (LCYB), which catalyzes the conversion of lycopene into b-carotene is one of the most important enzymes involved in carotenoid biosynthesis. In *Daucus carota*, two lcyb genes have been described (lcyb1 and lcyb2). During development, Dclcyb2 is expressed in leaves and roots but preferably in the mature storage root. Phylogenetic and aminoacidic analysis showed that Dclcyb2 gene is linked with the lycopene cyclases that are expressed in chromoplasts enriched organs. Here, we demonstrated that LCYB2 was targeted to plastids by using transient expression of LCYB2-GFP fusion protein in tobacco. We also determined that carrot Dclcyb2 presents LCYB function by means of heterologous complementation in BL-21/ΔCrtY *E.coli* strains. The expression of Dclcyb2 in tobacco (*Nicotiana tabacum*) showed an increase of around 2 fold of b-carotene in transgenic lines related to wild-type plants. In order to understand the molecular basis of a higher carotenoid content in transgenic lines, expression levels of Dclcyb2 and endogenous Ntpsy1 and Ntpsy2 were evaluated by quantitative PCR. We observed that the expression of Dclcyb2 induces the modification of the carotenogenic pathway in tobacco, by means of the induction of Ntpsy1 and Ntpsy2 key genes. Taken together, these results indicate that carrot lcyb2 codifies for a functional plastid-targeted LCYB.

Acknowledgement to FONDECYT 11080066

OS 6

RETENTION OF TRIPLICATED PHYTOENE SYNTHASE (*PSY*) GENES IN *BRASSICA NAPUS* L. AND ITS DIPLOID PROGENITORS DURING THE EVOLUTION OF THE BRASSICEAE

Pablo Cárdenas[1], Humberto Gajardo[1], Isobel Parkin[2], Federico Iniguez-Luy[1], María L. Federico[1]
maria.federico@cgna.cl

[1]Centro de Genómica Nutricional Agroacuícola (CGNA), CONICYT-REGIONAL, GORE LA ARAUCANIA, R10C1001, Unidad de Genómica y Bioinformática, Temuco, Chile

[2]Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 0X2, Canada

The extent of genome redundancy exhibited by Brassica species provides a model to study the evolutionary fate of multi-copy genes and the effects of polyploidy in economically important crops. Phytoene synthase (*PSY*) catalyzes the first committed reaction of the carotenoid biosynthetic pathway, which has been shown to be rate-limiting in *Brassica napus* seeds. In *Arabidopsis thaliana*, a single *PSY* gene (*AtPSY*) regulates phytoene synthesis in all tissues. Considering that diploid Brassica genomes contain three *Arabidopsis-like* subgenomes, the objectives of the present work were to determine whether *PSY* gene families exist in *B. napus* (AACC) and its diploid progenitor species, *Brassica rapa* (AA) and *Brassica oleracea* (CC); to establish the level of retention of Brassica *PSY* genes; to map *PSY* gene family members in the A and C genomes and to compare Brassica *PSY* gene expression patterns. A total of 12 PSY homologues were identified, 6 in *B. napus (BnaX.PSY.a-f)* and 3 in *B. rapa (BraA.PSYa-c)* and *B. oleracea (BolC.PSY.a-c)*. Indeed, with six members, *B. napus PSY* gene family is the largest described to date. Sequence comparison between *AtPSY* and Brassica *PSY* genes revealed a highly conserved gene structure and identity percentages above 85% at the coding sequence (CDS) level. Altogether, our data indicates that *PSY* gene family expansion preceded the speciation of *B. rapa* and *B. oleracea*, dating back to the paralogous subgenome triplication event. In these three Brassica species, all *PSY* homologues are expressed, exhibiting overlapping redundancy and signs of subfunctionalization among photosynthetic and non photosynthetic tissues. This evidence supports the hypothesis that functional divergence of *PSY* gene expression facilitates the accumulation of high levels of carotenoids in chromoplast-rich tissues. Thus, functional retention of triplicated Brassica *PSY* genes could be at least partially explained by the selective advantage provided by increased levels of gene product in floral organs.

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OS 7

USE OF PLANTS AS BIOREACTORS: EXPRESSION OF THE RNA OF THREE DIFFERENT EPITOPES OF HEPATITS C VIRUS (HCV) IN TOMATO PLANTS

Susan Hitschfeld[1], Nicolás Daneri[1], Francisca Jauregui[1], Patricio Arce-Johnson[1]
sahitsch@uc.cl

[1]Departament of Molecular Genetics and Microbiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile.

The use of plants to produce antigens in order to treat human diseases is a promising system because it has many advantages; there are not expenses associated with purification, transport, maintenance of a cold chain and sterile delivery. In the present work we describe the generation of transgenic tomato plants expressing genes of the HCV (Hepatitis C Virus), an important pathogen which affects more than 170 million of people around the world. The treatment for the illness has serious adverse effects and a low success rate. We used E1 and E2 genes that code for glycoproteins of the virus and core gene whose expression is essential for the viral nucleocapside. The tomato plant was chosen as a model for transformation because its short life cycle and its fruit is eaten raw, avoiding the antigen degradation. Each one of the three genes were cloned in binary vectors under the control of the 35S promoter. These vectors were inserted in *Agrobacterium tumefaciens* and they were used to transform tomato explants. Several shoots were regenerated and the transgene integration was confirmed by PCR and the presence of the transcript by RT-PCR. Finally we obtained 5 transgenic lines of plants with the E1 gene, 3 with E2 and 3 with core. We observed that all the lines with the E1 gene expressed the transcript but only 2 of the three lines of E2 and core were expressing it. New constructions using a gene fusion of HCV core to an endoplasmic reticulum signaling peptide KDEL are being transformed in tomato plants to increase recombinant protein accumulation in the plant cells. As a projection, we expect the detection of transgenic proteins by Western blot and also we hope to observe immune response triggered in rats fed with genetically modified plants.

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OS 8

EXPRESSION OF AN OPTIMIZED ARGOPECTEN PURPURATUS ANTIMICROBIAL PEPTIDE IN E. COLI AND EVALUATION OF THE PURIFIED RECOMBINANT PROTEIN BY IN VITRO CHALLENGES AGAINST IMPORTANT PLANT FUNGI

Christian Montes[1], Eduardo Tapia[2], Patricia Rebufel[1], Alberto Paradela[3], Gloria Arenas[2], Humberto Prieto[1]
hprieto@inia.cl

[1]Instituto de Investigaciones Agropecuarias, La Platina Research Center, Santa Rosa 11610 La Pintana, Santiago, Chile

[2]Biotechnology Doctoral Program, Universidad Técnica Federico Santa María-Pontificia Universidad Católica de Valparaíso, Av. Brasil 2950, Valparaíso, Chile

[3]Laboratorio de Proteómica, Centro Nacional de Biotecnología, CSIC, c/Darwin 3 28049, Madrid, España

Antimicrobial peptides (AMP) have been widely described in several organisms from different kingdoms. We recently designed and evaluated a synthetic version of an AMP isolated and characterized from *Argopecten purpuratus* hemocytes. This study describes the generation of a chimaeric gene encoding for Ap-S, the use of this construct to transform *E. coli* strain BL21, and the evaluation of the purified recombinant Ap-S (rApS) as an antifungal agent. The proposed gene coding for rAp-S consists of 93 nucleotides arranged downstream from the IPTG-inducible T7 promoter. The best synthesis conditions were obtained after *E. coli* cultivation at 26°C for 3 h, which allowed for the production of an rApS-enriched fraction containing the peptide at 249 µM. Mass spectrometry analysis of the purified rApS (3085.80 Da) showed the addition of a glycine residue on its N-terminal end derived from vector design and peptide purification. The purified rApS fraction was assayed for antifungal activity by direct addition of purified rApS elution to potato dextrose agar media at a final concentration of 81 nM. These assays showed important growth inhibitions of both biotrophic (*Fusarium oxysporum, Trichoderma harzianum*) and necrotrophic (*Botrytis cinerea, Alternaria* spp.) plant fungi in that the hyphae structures and spore count were affected in all cases. The strategy of cloning and expressing rAp-S in *E. coli*, the high yield obtained and its successful use for controlling plant pathogenic fungi suggest that this molecule could be applied to agricultural crops using various management strategies.

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OS 9

CYTOKININ SIGNALING AND NITRATE INDUCED ROOT GROWTH IN *ARABIDOPSIS THALIANA*

Pamela A. Naulin[1], Karem Tamayo[1], Rodrigo A. Gutiérrez[1]
pamela.naulin@gmail.com

[1]Departamento Genética Molecular y Microbiología. Pontificia Universidad Católica de Chile

Nitrate is an essential macronutrient for plant growth and development. Nitrate and other nitrogen (N) nutrient/metabolites can act as potent signals to control gene expression in plants. Transcriptomics analyses have now provided thousands of nitrate-reponsive genes in Arabidopsis. These studies are a valuable basis for the identification of mechanisms involved in regulating plant responses to changes in N availibility. Research from our group and others suggest that plant hormones, and especially cytokinin, play a key role in the nitrate response. In order to evaluate the importance of cytokinin for the nitrate response, we performed phenotypic analysis under different conditions of nitrogen in cytokinin perception and biosynthesis mutants. Both, perception and biosynthetic mutants exhibited shorter roots when grown with nitrate as the only nitrogen source. This result indicates that cytokinin is necessary for the nitrate stimulation of Arabidopsis root growth. To explain the observed phenotypes, we performed histological analysis of the root tip in cytokinin perception mutants. We found that the root tip of mutant plants have decreased cell division and elongation as compared to wild type plants when grown under nitrate conditions. In addition, mutant plants did not show the characteristic cellular patterns observed in the root tip of wild-type plants under these experimental conditions. These results suggest that cytokinin signaling is important for the maintenance of the cellular characteristics that allow active cell division and elongation in the root tip in response to nitrate availability.

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OS 10

TGA1 AND TGA4 TRANSCRIPTION FACTORS CONTROL NITROGEN RESPONSES IN ARABIDOPSIS THALIANA ROOTS

José M. Álvarez[1], Eleodoro Riveras[1], Diana E. Gras[1], Elena A. Vidal[1], Orlando Contreras-López[1], Felipe F. Aceituno[1], Karem P. Tamayo[1], Rodrigo A. Gutiérrez[1]
jmalvarh@gmail.com

[1]Departamento Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile

Nitrogen (N) nutrient and metabolites regulate plant growth and development and act as potent signals to control gene expression in Arabidopsis. Using an integrative bioinformatics approach we identified TGA1 and TGA4 as putative regulatory factors that mediate N responses in Arabidopsis thaliana roots. We showed that both TGA1 and TGA4 mRNAs accumulate strongly and quickly after nitrate and nitrite treatments in root organs. Phenotypic analysis of tga1 and tga4 double mutant plants indicated that TGA1 and TGA4 are necessary for both primary and lateral root growth in a nitrate dependent manner. Global gene expression analyses revealed that 97% of the genes with altered expression in the tga1/tga4 double mutants are regulated by nitrate treatments indicating these transcription factors have a specific role in nitrate responses in Arabidopsis roots. Among the nitrate-responsive genes that depend on TGA1/TGA4 for normal regulation of gene expression, we found the nitrate transporters NRT2.1, NRT2.2 and the nitrite reductase (NIR) genes. Specific binding of TGA1 to its cognate DNA sequence on the target gene promoters was confirmed by chromatin immunoprecipitation assays. These results identify TGA1 and TGA4 as important regulatory factors of the nitrate and nitrite response in Arabidopsis roots.

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OS 11

ATPRP3 POLAR LOCALIZATION WITHIN THE ROOT HAIR CELL WALL IS REGULATED BY BOTH SECRETORY AND ENDOCYTIC MECHANISM(S)

Cecilia Rodríguez-Furlán[1], Mary Tierney[2], Ariel Orellana[1]
cec.rodriguez.f@uandresbello.edu

[1]FONDAP Center for Genome Regulation; Núcleo Milenio en Biotecnología Celular Vegetal; Centro de Biotecnología Vegetal, Universidad Andrés Bello
[2]Plant Biology Department, University of Vermont

Root hairs are extensions of epidermal cells that elongate by tip growth using secretory and endocytic mechanisms. Current models of cell wall assembly suggest that endocytosis is important in establishing ECM structure during growth. ATPRP3 and ATPRP1, two structural Proline-Rich Proteins, are secreted into the ECM at the tip of growing hairs. Here we further characterized the role of polarized secretion and possible endocytosis in trafficking ATPRP3 and ATPRP1 to the root hair cell wall. Root hairs of 3-day-old seedlings expressing AtPRP1::ATPRP1-GFP and AtPRP3::ATPRP3-GFP were analyzed using confocal microscopy. Treatments were performed with 15uM FM4-64 (5-30 min) and 100uM BFA (30 min). Vesicles containing ATPRP1-GFP and ATPRP3-GFP moved in an anterograde to retrograde direction and were concentrated at the root hair tip. Incubation with the endocytic tracer FM4-64 showed labeling at the plasma membrane as well as punctate structures (the putative early endosomes) within root hairs. These punctate structures showed co-labeling with AtPRP3-GFP but not with AtPRP1-GFP. Brefeldin A treatment, which inhibits secretion but not endocytosis, resulted in the co-accumulation of ATPRP3-GFP and FM4-64 in BFA compartments in root hairs. This contrasted with ATPRP1-GFP labeling that was found outside of the BFA bodies. Thus we conclude that both ATPRP1 and ATPRP3 present in trafficking vesicles of the endomembrane system are both secreted to the root hair tip. However, the localization of ATPRP3 within the growing cell wall is controlled by secretory and endocityc mechanism(s).

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OS 12

FUNCTIONAL CHARACTERIZATION OF NOVEL GENES ASSOCIATED TO IONIC STRESS IN *ARABIDOPSIS THALIANA* AND *SACCHAROMYCES CEREVISIAE*

Daniela Urbina[1], Matías Freire[1], Aliosha Figueroa[1], Alexander Vergara[1], Lorena Norambuena[1]
daniurbina@gmail.com

[1]Laboratorio de Biología Molecular Vegetal. Facultad de Ciencias, Universidad de Chile

Ionic stress is a strong problem in plant growth and development. Depending of latitude, weather or geographic and geologic characteristics, soil land field is changing in metals and salt content. For this reason, many efforts have been driven to understand and characterize the molecular machinery that plants use to adapt and tolerate this kind of stress. In this direction, using co-expression analysis of public global expression profiles of *Arabidopsis thaliana*, we have found 55 new genes as candidates to be associated to the biological process of ionic transport. We have called them ITRG for Ion Transport Related Genes , which were ranked according with highest correlation coefficient in order to prioritize their study. Out of the 55 genes we have selected 12 ITRG with the best probability to be involved in ionic stress. To evaluate the role of these ITRG we have chosen two strategies: reverse genetic approach and heterologous expression in *S. cerevisiae*. The loss of function of the selected genes confers sensitivity to high salt condition in Arabidopsis, suggesting they are necessary to plant ionic stress. On the other hand, over expression of these genes confers differential sensitivity of *S. cerevisiae* growth in high concentrations of salt and heavy metals. Finally, our results suggest that ITRG are associated to ionic stress response in plants. Furthermore they are able to disturbed the salt and heavy metal sensitivity in yeast. Over all co-expression analysis has been a robust tool to find novel ionic stress-related genes. The molecular function of ITRG will give new hints of ionic tolerance mechanisms in plants.

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OS 13

PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR RESPONSES IN THREE EUCALYPTUS SPECIES THAT DIFFERS IN THEIR TOLERANCE TO DROUGHT STRESS

Pino M.T.[1], Selles G.[1], Balboa M.[1], Milla. E.[1], Romero P.[1], Rojas P.[2], Ortiz O.[2], Molina M.P.[2], Gutiérrez B[2]
mtpino@inia.cl

[1]Instituto de Investigaciones Agropecuarias (INIA)
[2]Instituto de Forestal (INFOR)

Early studies in some Eucalyptus species showed different growth rate in response to water stress. In areas with low precipitations (⩽150mm per year*), Eucalyptus camaldulensis* Dehnh and *Eucalyptus cladocalyx* F. Muell showed higher plant survival and higher growth rate than *Eucalyptus globulus Labill*; suggesting that these species should have different adaptation mechanisms to cope drought stress. In the present study, the main goal was to determinate physiological, biochemical and molecular responses in three Eucalyptus species that differs in their tolerance to drought stress. The Eucalyptus species studied were *E. globulus*, *E. camaldulensis* and *E. cladocalyx*. Two water availability treatments were evaluated in five replications by species; T1 well-watered plants (-1MPa) and T2 non-watered plants (-2.8MPa). Eucalyptus plants were grown in pots under greenhouse conditions (25ºC, 16/8h day/light photoperiod). Soil water availability (%) was measured with FDR Decagon Devices (ECHO20), and recorded every 30min. by data logger (EM50). Differences were observed in stomatal conductance (gs), net photosynthetic rate (A), intercellular CO2 concentration (Ci), stem water potential (ψmd), osmoprotectors and gene expression. *E. camaldulensis* and *E. cladocalyx* showed higher osmotic adjustment than *E. globulus*; proline content was higher in those species in particular after 7 days under water restrictions. Similar results were observed for total soluble sugars. In addition and under drought stress treatments, quantitative Real-Time PCR analysis showed that gene expression of pyrroline-5-carboxylate synthetase (Pc5s), dehidryn (Dhn10) and CBF-like genes were best observed in *E. camaldulensis* and *E. cladocalyx* under drought stress condition. Suggesting, various mechanisms are involved in Eucalyptus drought tolerance. Acknowledgements, this research was carried out with financial support from CORFO-INNOVA (06CN12PFT-70).

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OS 14

GRXS13 PLAYS A KEY ROLE IN PROTECTION AGAINST PHOTOOXIDATIVE STRESS AND COLD ACCLIMATIZATION IN ARABIDOPSIS

Ema Olate[1], Daniel Laporte[1], Marcela Salazar[1], Julio Salinas[2], Loreto Holuigue[1]
eyolate@uc.cl

[1]Laboratorio Biología Molecular Vegetal. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile

[2]Departamento de Biología Medioambiental, Centro de Investigaciones Biológicas-Consejo Superior de Investigaciones Científicas, Ramiro de Maeztu 9, 28040 Madrid, Spain

Glutaredoxins (GRXs) belong to the antioxidant and signaling network involved in the cellular response to oxidative stress. In spite of the high number of GRX genes in plant genomes, the biological functions and physiological roles of most of them remain unknown. Here we report the functional characterization of the Arabidopsis GRXS13 gene, that codes for two CC-type GRX isoforms. The transcript variant coding for GRXS13.2 isoform is the predominantly expressed under basal conditions and the one that is induced by photooxidative stress produced by high light (HL) and methyl viologen (MeV), and also by cold stress. To determine the role of GRXS13.2 in the plant response to photooxidative and cold stress we have analyzed tolerance and oxidative damage after HL and MeV tretaments and cold acclimatization in different transgenic lines that silence (sil) and over-express (OE) GRXS13 gene. Transgenic lines where the GRXS13 gene has been knocked down show increased basal levels of superoxide radicals and reduced plant growth. These lines also display reduced tolerance to MeV and HL treatments, and reduced cold acclimatization capacity. These stress conditions are characterized by increased production of reactive oxygen species. Consistently, lines over-expressing the GRXS13.2 variant show reduced MeV- and HL-induced damage. Furthermore, alterations in GRXS13 expression also affect superoxide levels and the ascorbate/dehydroascorbate ratio after HL-induced stress. These results indicate that GRXS13 gene expression is critical for limiting basal and oxidative stress-induced reactive oxygen species (ROS) production. Together, these results place GRXS13.2 as a member of the ROS-scavenging/antioxidant network that shows a particularly low functional redundancy in the Arabidopsis GRX family.

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OS 15

HETEROLOGOUS EXPRESSION OF AN ARTIFICIAL MICRORNA DERIVED FROM GRAPEVINES

Alejandra Ramírez[1], Álvaro Castro[2], Humberto Prieto[3]
hprieto@inia.cl

[1]Biochemistry Undergraduate Program, Universidad de Santiago de Chile.
[2]Biotechnology Doctoral Program, Universidad de Santiago de Chile.
[3]Biotechnology Lab., La Platina Research Center, Instituto de Investigaciones Agropecuarias, Santiago, Chile.

Micro-RNAs (miRNAs) regulate a wide range of processes in plants, including development, abiotic stress tolerance and antiviral defenses. In recent years, some 16,772 miRNAs have been detected, 163 of which are related to *Vitis vinifera*. Vvi-MIR319e is a grapevine miRNA from the MIR319 miRNA family. It is expressed in several tissues including tendrils, leaves, stems, roots, berries, calli and inflorescences. In the present work, artificial versions of vvi-MIR319e were designed to target the Green Fluorescent Protein (GFP) reporter gene. The mature miRNA in vvi-MIR319e was modified and two artificial vvi-MIR319 versions were generated; the first synthetic version was meant to target the central region of GFP mRNA, and the second was designed to focus on the 3' end of the same reporter gene. Transient transformation assays were implemented on *Nicotiana benthamiana* leaves by co-agroinfiltration in order to express both the synthetic miRNAs and the 35S::GFP vectors. The results show that both synthetic miRNA constructs successfully silenced GFP, though the silencing patterns were different. The use of artificial miRNAs for candidate gene evaluation in Vitis spp. is discussed and proposed in this article based on those results.

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OS 16

DO POTATOES HAVE A SINGLE EVOLUTIONARY HISTORY? WHAT PROPORTION OF THE GENOME SUPPORTS THIS HISTORY?

Flor Rodríguez[1,2] and David Spooner[1]
flor.rodriguez@inia.cl

[1]University of Wisconsin-Madison, Wisconsin, USA.

[2]*INIA CRI* Remehue, Osorno, Chile.

Solanum section *Petota* is taxonomically difficult, partly because of interspecific hybridization at both the diploid and polyploidy levels. There is much disagreement regarding species boundaries and affiliation of species to series. Phylogenies reconstructed with only one or a few independently inherited loci may be unresolved or incongruent due to taxon and gene sampling, horizontal gene transfer, or differential selection and lineage sorting at individual loci. In an effort to remedy this situation, we examined the utility of conserved orthologous set (COSII) nuclear loci to elucidate the phylogenetic relationships among diploid and polyploid *Solanum* species.

When total evidence is invoked, one single predominant history is highlighted within and among the three main clades. It also supports the hypothesis of the North and Central American B-genome origin of the tuber-bearing members of *Solanum* sect. *Petota* and shows a clear division between A genomes in clades 3 and 4, and B genomes in clade 1+2. On the other hand, when a prior agreement approach is invoked other potato evolutionary histories are revealed but with less support. Concordance analyses revealed and summarized the extensive discordance among COSII markers. This study confirms and quantifies the utility of using DNA sequences from different parts of the genome in phylogenetic studies to avoid possible bias in the sampling.

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OS 17

GENETIC CHARACTERIZATION OF THE CLUSTER STRUCTURE OF GRAPEVINE

José Correa[2], Denisse Laborie[1], Maribel Mamani[1], Carlos Muñoz[2], Manuel Pinto[1], Patricio Hinrichsen[1]
phinrichsen@inia.cl

[1]INIA, La Platina, Santa Rosa 11.610, Santiago
[2]Universidad de Chile, FCA, Santa Rosa 11.315, Santiago

The rachis is the part of the grapevine cluster where berries are attached and the size, number and angle of insertion of its ramifications have important management implications for table grape production. The study of the genetic parameters controlling cluster structure is critical. Therefore, a total of 23 traits, including rachis length (*rl*), rachis fresh weight (*rw*), first shoulder length (*sl*), number of ramifications (*ri*) and total number of berries (*tb*), were measured in a 140 progeny coming from a 'Ruby Seedless'×'Sultanina' cross, during 2 consecutive seasons. A mixed model was used to determine the genotypic variance, calculated by the Restricted Maximum Likelihood. Results indicate that the genotypic variance was significant for all evaluated traits. The *rl*, *rw* and *sl* exhibit the largest genetic variance. On average, broad sense heritability of these traits was ~65%, with *rl* showing the largest value (75%). Also, the Best Linear Unbiased Predictors (BLUPs) of the genotypic effects were calculated. Multivariate factorial analyses of BLUPs showed highly significant correlations (r~68.2%) among the BLUPs of *rl*, *rw*, *ri* and *sl*, and were responsible for 27.8% of the total variance (first factor). A quantitative trait loci (QTL) analysis showed that the linkage group 18 (LG18) and LG5 harbored significant QTLs for the first factor traits in both seasons, and that these QTLs were supported mainly by a paternal additive effect. The correlation structure together with QTLs analyses revealed possible pleiotropic effects. The QTLs detected indicate that these traits have a clear genetic basis and, due to their contribution to the total variance, they are probably good determinants of the genetic diversity of the cluster architecture.

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OS 18

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF MOLECULAR FACTORS INVOLVED IN THE CYTOKININ RESPONSE PATHWAY IN PEACH FRUITS

Camilo Avendaño Miralles[1], Fernanda Rodríguez Rojas[1], Héctor Duchens Silva[2], Soledad Cabrera[2], Juha Immanen[3], Herman Silva[2], Ykä Helariutta[3], Lee Ann Meisel[1]
lmeisel@gmail.com

[1]1Plant Molecular Genetics Laboratory, Plant Biotechnology Center, Andrés Bello University, Av. República 217, Santiago, Chile.

[2]Laboratorio de Genómica Funcional y Bioinformática, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile.

[3]Plant Molecular Biology, Institute of Biotechnology, P.O.B 56, University of Helsinki, FIN-00014 Helsinki, Finland.

Cytokinins are plant hormones involve in several physiological processes, such as, cell division, seed development, vascular differentiation, etc. The cytokinin response pathway plays a role in biomass accumulation in plant species such as Poplar and Arabidopsis. Fruit development is a process in which there is a dramatic increase in the accumulation of biomass in a short period of time. Peach (*Prunus persica*) is a commercially relevant fruit-tree species, whose genome was recently sequenced. During peach fruit development, cytokinin levels change suggesting a regulatory role of this hormone during fruit formation. In order to identify molecular factors associated with fruit development, first we performed bidirectional Blasts to search for putative orthologs of the Arabidopsis cytokinin response pathway in six gene families. The first three gene families correspond to cytokinin homeostasis genes and the last three families correspond to cytokinin response pathway. We analyzed the expression patterns of several putative cytokinin pathway genes during peach fruit development and the effects of exogenous cytokinin application on their expression levels. We observed that the expression of many of these genes was related to fruit development and that their expression was modified by exogenous cytokinin treatment. Finally, we functionally analyzed several of these genes by transient transformation on peach fruits overexpressing the response regulators *PpRR1* and *PpRR4* in order to analyze the expression of downstream genes. We observed that overexpression of *PpRR1* and *PpRR4* are each individually sufficient to induce the expression of cytokinin response pathway genes in peach fruit. Our results indicate that the cytokinin response pathway is conserved between peaches and Arabidopsis. This is the first report that demonstrates functionality of cytokinin response transcription factors in peach fruits.

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OS 19

IDENTIFICATION OF NOVEL NUCLEOTIDE SUGAR TRANSPORTERS INVOLVED IN PECTIN BIOSYNTHESIS

Henry Temple[1], Ignacio Moreno[1], Francisca Blanco[1], Macarena Greve[1], Omar Sandoval[1], Ariel Orellana[1]
aorellana@unab.cl

[1]FONDAP Center for Genome Regulation, Núcleo Milenio en Biotecnología Celular Vegetal, Centro de Biotecnología Vegetal, Universidad Andrés Bello

Nucleotide sugar transporters (NSTs) have been proposed to be required for biosynthesis of non-cellulosic plant cell wall polysaccharides of diverse and complex structure; however, to date only few NSTs have been characterized and their role in this biological process has not been directly evidenced. To identify others NSTs we performed an *in silico* search of putative NSTs that co-express with genes involved in cell wall biosynthesis. From this analysis we identified two putative NSTs called AtUTr8, AtUTr9 which co-express with pectins biosynthesis genes. We confirmed the subcellular localization of these NSTs fusing the protein to GFP. Additionally, we analyzed the phenotype of the mutants of these genes by ruthenium red staining and inmunohistochemical assays using antibodies against cell wall components. Our results indicate a Golgi apparatus localization of AtUTr8 and AtUTr9 confirmed by co-localization with other fluorescent organelle markers. Also analysis of insertional lines on these genes has shown a reduction in the accumulation of pectinous components in different Arabidopsis tissues. *atutr8* and *atutr9* mutants show a reduction in the accumulation of pectinous seed coat mucilage. This phenotype seems to be related to the amounts of RG-I present in the seed mucilage of mutants compared to wild type seeds as determined by inmunohistochemical assays. Furthermore, qRT-PCR analysis shows high co-expression degree of these NSTs with genes directly involved seed mucilage synthesis. These results suggest that these NSTs are essential for the biosynthesis of pectinous polysaccharides present in seed coat mucilage; efforts to measure the activity of these putative NSTs are underway. The overall results of this work show the important role of NSTs in the biosynthesis of the cell wall in plants.

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OS 20

RELATIONSHIP BETWEEN THE SIZE OF BERRIES AND THE SUGAR CONTENT IN CONTRASTING PHENOTYPES OF GRAPEVINE (VITIS VINIFERA L.)

Cristian Valdés[1], Daniela Olivares[2], José Correa[2], Denisse Laborie[1], Herman Silva[2], Patricio Hinrichsen[1], **Manuel Pinto**[1]
mpinto@inia.cl

[1]Instituto de Investigaciones Agropecuarias, CRI La Platina
[2]Universidad de Chile, Facultad de Ciencias Agronómicas

The aim of this study was to determine the sugar content during the development of the berries and the relationship with the size in six contrasting genotypes of grapevine grouped in large (LB) and small (SB) berry phenotypes. Fresh and dry weight, volume, soluble solids, and sugar content based on HPLC detection (glucose and fructose) were measured at pre-veraison (36 days after anthesis, (DAA), veraison (76 DAA), post-veraison (85 DAA) and maturity (105 DAA) when the berries reached 18°Brix. Berry growth was measured weekly since pre-veraison. Differences in berry size between the two groups were observed from pre-veraison. At maturity, LB group had a larger berry diameter and volume than SB group. From veraison, the water accumulation was higher in LB group. Although the action of soluble solids accumulation on the increase of the osmotic potential was similar (~0.165 MPa/ °Brix), in both phenotypic groups, the effect of this potential on the volume was clearly higher in the LB group. In addition, before veraison, in both groups the glucose/fructose ratio (glu/fru) was >1. At veraison this proportion is slightly higher in LB than in SB. After veraison, glu/fru decreased in both groups. These results indicate that in SB group, the increase in osmotic potential has a lower capacity to incorporate water into the berries and to increase their volume. On the contrary in LB phenotypes an increase in the osmotic potential has a high capacity to increase the berry volume. Furthermore, the accumulation of glucose and fructose is independent from the phenotype and the type of sugar accumulated is not related to the volume increase of the berries.

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OS 21

PHOTOSYNTHETIC LIGHT RESPONSES MAY EXPLAIN VERTICAL DISTRIBUTION OF HYMENOPHYLLACEAE SPECIES IN A TEMPERATE RAINFOREST OF SOUTHERN CHILE

María José Parra[1], Karina I. Acuña[1], Ángela Sierra-Almeida[2], Camila Sanfuentes[2], Luis J. Corcuera[1], León A. Bravo[3]
mariapar@udec.cl

[1]Laboratorio de Fisiología Vegetal, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas. Universidad de Concepción, Casilla 160-C, Concepción, Chile

[2]ECOBIOSIS, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile

[3]Departamento de Ciencias Agronómicas y Recursos Naturales. Facultad de Ciencias Agropecuarias y Forestales; Center of Plant, Soil Interaction and Natural Resources Biotechnology BIOREN, Universidad de la Frontera, Casilla 54-D, Temuco, Chile

The diversity and abundance of epiphytic Hymenophyllaceae species decrease with the tree height in a secondary forest of Southern Chile. While some species are restricted to lower parts of the host (<60 cm), with light availability around 10-100µmol m-2 s-1, other species occupy the whole host height (>10 m), where light availability exceeds 1000 µmol m-2 s-1. This vertical distribution is explained mainly by the relative humidity and by canopy openness of the forest. Although these species are considered as shade tolerant plants, little is known about their light tolerance. Thus, our aim was to assess the photosynthetic light responses of two filmy species with contrasting vertical distribution in a temperate rainforest of Southern Chile. We compared light tolerance of *Hymenoglossum cruentum* (Hcru) and *Hymenophyllum dentatum* (Hden), by measuring gas exchange, light energy partitioning at PSI and PSII, NPQ components, and chlorophyll contents. Hden showed lower maximum net photosynthesis (Amax) than Hcru, but the former species keeps its Amax across a wider light range. Amax of Hcru declined abruptly at PPFDs >75 umol photons m-2 s-1. Consistently, Hcru, the most shady plant, showed higher chlorophyll contents. Differences in light energy partitioning at PSI and PSII were consistent with gas exchange results. Hence, both species allocate absorbed energy mainly toward photochemistry instead of heat dissipation at their respective light saturation points. Above saturation, Hcru have higher heat dissipation than Hden, consistently with the depoxidation state of VAZ. Regarding PSI, differences between species were only found at moderate light intensities where PSI heat dissipation in Hcru was caused by donor side limitation, while in Hden by acceptor side limitation. Differences in photosynthetic responses to light between Hcru and Hden suggest different levels of light tolerance, which could explain their contrasting vertical distribution in the forest of Southern Chile.

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OS 22

MOLECULAR AND PHYSIOLOGICAL STUDY OF POSTHARVEST RACHIS BROWNING OF TABLE GRAPE CV RED GLOBE

Iván Balic[1], Adrián Moreno[2], Claudia Huerta[1], Ariel Orellana[2], Bruno Defilippi[3], Reinaldo Campos-Vargas[1]
reinaldocampos@unab.cl

[1]Universidad Andrés Bello, Fac. Ciencias Biológicas, Centro de Biotecnología Vegetal
[2]Universidad Andrés Bello, Fac. Ciencias Biológicas, Centro de Biotecnología Vegetal; FONDAP CRG 15090007
[3]Instituto de Investigaciones Agropecuarias, INIA La Platina

Red Globe is one of the most important varieties of table grapes for export in our country. Rachis browning is one of the main problems affecting the quality and marketing of table grape clusters. Usually this process has been associated with water loss. Actually, we propose that browning could be associated in some degree to a senescence process. In this regard, we carried out a transcriptional analysis by qRT-PCR of 31 senescence-associated genes (SAG). The expression of these genes was evaluated at harvest and after 90 days storage at 0°C with air or controlled atmosphere (CA) according to the parameters that normally are followed by industry. In addition, we evaluated the effect of the application of cytokinin (Ck) as retardant of senescence process, which was applied one day before harvest. Our results showed a significant effect in delaying the development of browning with CA and Ck just after cold storage. However, following the refrigerated period plus 2 days at 20°C (shelf-life), only the Ck treatment had a significantly lower percentage of browning compared to control. The analysis of the relative levels of mRNA of candidate genes showed differences between treatments, where a down regulation of transcription of several genes was observed in samples under controlled atmosphere. In the Ck treatment only few differences were observed as compared with the control. In addition, all SAG were evaluated at 0°C and 20°C for 48 h. In this case half or 3/4 of studied genes showed statistically differences at 0°C or 20°C respectively. Taken together, our results suggest that rachis browning is a complex process involving at least several genes associated to senescence in plants.

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P 1

DATA MINING AS A TOOL TO IDENTIFY CANDIDATE GENES IN CROP PLANTS

Jonathan Maldonado[1], Andrea Morales[1], Loreto Prat[1], Lee Meisel[2], Herman Silva[1]
jomaldon@gmail.com

[1]Laboratorio de Genómica Funcional y Bioinformática, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile

[2]Laboratorio de Genética Molecular Vegetal, Centro de Biotecnología Vegetal, Universidad Andrés Bello

Since the first protein sequence was obtained on 1951 by Frederick Sanger, the amount of biological digital data has been growing exponentially. Today, with the next generation sequencing technologies, we need specialized tools and trained professionals to mine this data and obtain useful results. One of the focuses of our group is to act as a filter of public and private digital data. This will allow to explore large amount of data generated mainly by high-throughput technologies, and then, obtain limited subsets of useful data according to our projects objectives. This new subset can be used by others PIs to make better decisions on their experiments. We work with 4 plant species in different projects but our bioinformatic approach is based on a standard pipeline that runs from raw data pre-process to unigene annotation and digital northern analysis. Our current studied species are peach (*Prunus persica*), white strawberry (*Fragaria chiloensis*), sweet cherry (*Prunus avium*) and quinoa (*Chenopodium quinoa*). The transcriptome data has been obtained from Sanger, 454 FLX, and Illumina sequencing. In peach, our transcriptome data has allowed us to propose candidate genes related to chilling injury and also, we have contribute with the International Peach Genome Initiative on gene validation. In white strawberry we were able to propose a subset of genes related to aroma biosynthesis. We also had collaboration with the Strawberry Genome Sequencing Consortium in gene validation. Sweet cherry data is being used mainly to search for genetic markers that could be used on breeding programs and we are using digital northern analysis to search for candidate genes involved in cracking of the fruit. Finally, with Quinoa data we are searching for novel genes related to drought stress tolerance that could be used on susceptible crops.

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P 2

MOLECULAR MODELLING AND DOCKING SIMULATIONS OF TWO POLYGALACTURONASE PROTEINS FROM FRAGARIA GENUS.

Rodrigo Díaz[1], **Carlos Gaete-Eastman**[1], Raúl Herrera[1], María Alejandra Moya-León[1]
cgaete@utalca.cl

[1]Laboratorio de Fisiología Vegetal, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca

Chilean strawberry (*Fragaria chiloensis*) fruit shows a faster softening rate compared to *Fragaria ananassa* cv Chandler during ripening. Recently, in our lab a higher polygalacturonase enzymatic activity between these two strawberry species was found, correlating with the fruit softening. To gain insight about the mechanism of action of the FcPG1 and FaPG1 at molecular level, the comparative modeling methodology was used to build the structure of both enzymes, which were validated and refined with molecular dynamics simulation. The resulting models showed that both protein folds into a right-handed parallel β-helix with 7 complete turns. The polygalacturonase's active site is composed by three Asp, one Arg and one Lys residues, which were located in several β-sheets in the floor of a cleft between two loops. Additionally, the possibilities of interaction with model substrates (octagalacturonic acid) with the polygalacturonase protein using molecular docking simulation was explore. For the interaction with octagalacturonic acid, the results suggested that the most stable conformation of the PG-Pectin complex correspond to the FcPG1-OctGal complex, compared to FaPG1-OctGal complex, supporting the experimental data. Finally, the analysis of structural differences between these two PG proteins suggests that just few amino-acid changes are enough to alter the protein structure and the subsequent interaction with the substrate.

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P 3

BZ2-TFES IS INVOLVED IN REGULATION OF ENDOMEMBRANE SYSTEM MORPHOLOGY, TRAFFICKING-GENE EXPRESSION AND PRIMARY ROOT GROWTH IN *ARABIDOPSIS THALIANA*.

Lorena A. Pizarro[1], Arantzazú Bidegain[1], Marcela Rojas-Pierce[2], Lorena Norambuena[1]
lorepizarro@gmail.com

[1]Plant Molecular Biology Laboratory. Faculty of Science. University of Chile
[2]Department of Plant Biology. North Carolina State University. Raleigh, NC

The endomembrane system has a central and essential role ensuring the correct protein destination being fundamental in every cellular and physiological function. The regulation of protein trafficking through endomembrane system is performed mostly at post-translational level. However, there is raising evidence that trafficking is regulated at transcriptional level by means of changes in gene expression of endomembrane system genes involved in trafficking, called trafficking-genes. We are characterizing transcription factors that are putative trafficking-genes regulators, called TFES. The characterization of TFES is being performed by means of loss of function analysis using T-DNA insertional mutants analyzing endomembrane system morphology and trafficking using confocal microscopy. We found that bZ2-TFES mutant line has defects in the endomembrane system compartment shape and in protein markers localization. Our results show that bZ2-TFES mutant line has endoplasmic reticulum morphology altered. Furthermore, It has impaired localization of Golgi apparatus and vacuole marker suggesting that bZ2-TFES is involved in ES functioning. By means of real-time PCR we have found this mutant has changes in the expression level of trafficking genes such as Sar1. Interestingly, at physiological level bZ2-TFES mutant has longer primary root, however, germination rate and general development seems to be normal. These results suggest that bZ2-TFES is involved in regulation of trafficking-genes with impact in ES morphology and functioning. Those regulations could be implied in primary root growth. Further analysis are needed for unravel the connection between endomembrane system transcriptional regulation and primary root growth.

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P 4

TRANSCRIPTOMIC NETWORK ANALYSIS OF *A. THALIANA* REVEALS SPECIFIC GENE EXPRESSION IN COLD, SALT AND UV-B CONDITIONS

Eduardo A. Sagredo Campos[1], Jaime A. Espinoza[2], Carolina Bizama[3], Gustavo Cabrera[3], Ana Gutiérrez Moraga[2]
sagredo1989@gmail.com

[1]Carrera de Biotecnología, Universidad de La Frontera, Temuco, Chile
[2]Programa de Doctorado en Ciencias Mención Biología Celular y Molecular, Universidad de La Frontera, Temuco, Chile
[3]VentureL@b, Universidad Adolfo Ibañez, Santiago, Chile

In this work, a transcriptional network analysis was performed in order to identify gene co-expression groups (clusters) which are specifically expressed in ultraviolet light B (UV-B), salt or cold stress in *Arabidopsis thaliana* plants. For this purpose, public datasets (GEO5626, GEO5623 and GEO5621, Affymetrix(r) Arabidopsis ATH1 platform) containing expression values of A. thaliana leaves under UV-B, salt and cold stress during 24 hours were used. This analysis was carried out using Biolayout Express3D software and the gene ontologies enrichment was performed using DAVID v6.7. We found twenty seven clusters which their expression profiles changed only under UV-B, salt or cold stress conditions. Ten clusters showed specifically expression changes under UV-B, related to phosphoylation kinases, proteolysis functions and pathogen related responses. Ten clusters were specifically under cold stress conditions, three clusters showed an up regulated expression profiles, related with ethylene signaling response. The remaining clusters present a down regulated expression profiles without enrichments. Finally seven clusters shows differential expression related with salt stress response. Five of them including an up regulated expression, involving the abscisic acid pathway, oxidative stress response and secondary metabolic processes. Additionally, two clusters showed a down regulated expression profiles enriching plant development structures. This analysis allows us to identify particular genes and functional process related to A. thaliana under different stress conditions in order to identify new markers to characterize differential abiotic stress response.

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P 5

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF ERF115 GENE CODING FOR A TRANSCRIPTION FACTOR INVOLVED IN TOLERANCE TO HIGH SALINITY STRESS IN *ARABIDOPSIS THALIANA*.

Luis León [1], **Mariola Tobar**[1], Eva Villarroel[1], Loreto Holuigue[1]
mptobar@uc.cl

[1]Laboratorio de Biología Molecular Vegetal, Núcleo Milenio en Genómica Funcional de Plantas, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile.

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 identifying 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 plant growth and production. With the purpose 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. Differentially expressed genes under high salinity stress were identified and clustered according to their expression profiles. We performed a gene network analysis of genes induced in roots by salinity and we selected the *ERF115* transcription factor that showed the greatest number of interactions in the network. Using RT-qPCR we proved that *ERF115* is selectively induced in roots by high salt treatments. Moreover, we identified and characterized a homozygous insertional mutant line *erf115/erf115*, which is null for ERF115 expression. Phenotypic analyses were accomplished and we detected that the mutant line had a dwarf phenotype compared to WT plants under salinity stress (300 mM NaCl). To identify putative targets, a network analyses was executed and we found putative targets of ERF115, which have the gene ontology term "*reponse stress and oxidative stress response*". These results suggest an important role for ERF115 in the tolerance to salinity stress by inducing genes involved in the response to this stress.

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P 6

FROST TOLERANCE IN *EUCALYPTUS GLOBULUS*, ROLE OF ELIPS, LTP AND DHN1

Hita Barraza[1], Claudia Flores[2], Daniela Salgado[1], Marta Fernández[3], Sofía Valenzuela[2]
hita.barraza@gmail.com

[1]Facultad Ciencias Forestales, Universidad de Concepción, Concepción, Chile
[2]Centro de Biotecnología, Universidad de Concepción, Concepción, Chile
[3]Genómica Forestal SA, Concepción, Chile

Eucalyptus globulus is the second most important tree species in Chile, after *Pinus radiata* and it is used to obtain wood and pulp due to its high growth rate, but it has low cold tolerance, especially in young plants, limiting the area in which this species can be planted. Despite this *E. globulus* has the capacity to be cold acclimated, acquiring tolerance to freezing after exposure to low temperatures. Two genotypes of *E. globulus* contrasting in frost tolerance, were exposed to an acclimation treatment to analyze the effect of low temperatures in the relative expression of three genes which are involved in different biological functions and have been related to response in cold stress. ELIPs (early light-inducible protein) are proteins, which are induced by light after a period of darkness and have been associated with light stress and with cold stress in other species. LTP (Lipid Transfer Protein) is related with cell growth plant adaptations to environmental changes, signaling for plant defense, defense against pathogens, cuticle formation among others. Dehydrin1 (DHN1) belongs to the second group of LEA proteins (late embryogenesis abundant). There is not a clear function for this protein, but are commonly induced by abiotic stress that involve cellular desiccation and it has been proposed that dehydrins may stabilize cellular membranes via conformational changes in the K-segment under stress conditions. All three genes showed significant differences in their expression levels between the contrasting genotypes after exposing them to low temperatures. As well, differences in the transcript level of these genes among non-acclimated plants and acclimated plants were clearly observed.

P 7

RELATIVE EXPRESSION OF ASCORBATE PEROXIDASE AND AGAMOUS TRANSCRIPTS IN RESPONSE TO COLD ACCLIMATION IN *EUCALYPTUS GLOBULUS* LABILL.

Darío Navarrete-Campos[1], Marta Fernández[3], Javier Latorre[1], Jorge Velásquez[2], Valeria Neira[3], Sofía Valenzuela[1]
darnacos@gmail.com

[1]Facultad de Ciencias Forestales, Universidad de Concepción, Concepción, Chile
[2]Centro de Biotecnología, Universidad de Concepción, Concepción, Chile
[3]Genómica Forestal S.A., Centro de Biotecnología, Universidad de Concepción, Concepción, Chile

Low temperatures affect the growth and development of plants, limiting their distribution and productivity. *Eucalyptus globulus* is an important forest specie used for cellulose pulp production, but is affected by freezing temperature. One way to increase cold tolerance in plants exposed to low non-freezing temperatures, is by a process called cold acclimation; in which, the plant acquires temporary tolerance to low temperatures, involving gene expression, physiological and biochemical changes, such as an active transcription of multiple genes, proteins and metabolites accumulation that lead to the protection of the cell structures integrity and functionality from freezing damages. Some genes involved in this protection are: ascorbate peroxidase (*apx*) and agamous (*agm*), *apx* encodes an enzyme involved in the metabolism of hydrogen peroxide in response to oxidative stress, which is an important component of cold stress and *agm* encodes a transcription factor MADS box-like protein that has a DNA binding site allowing the transcription of genes involved in flowering, photoperiod and vernalization in plants. The objective was to study the relative expression of *apx* and *agm* at low nonfreezing and freezing temperature under controlled photoperiod. Two genotypes of *E. globulus* contrasting in their cold tolerance were subjected to a cold-acclimation treatment. This study reports differences in the relative expression of *apx* and *agm* among both genotypes and the different temperatures employed during the profile. According to the results, the importance in the accumulation of *agm* transcripts and the decline in *apx* transcript levels in response to cold acclimation in *E. globulus* is discussed.

P 8

PRECONDITIONING TREATMENT OF *PRUNUS PERSICA* FRUITS ALLEVIATES CHILLING INJURY SYMPTOMS AND CORRELATES WITH CHANGES IN ANTIOXIDANT CAPACITY AND GENE EXPRESION INVOLVED IN REDOX METABOLISM

Leonardo Pavez[1], Felipe Olivares[1], González Mauricio[1]
leopavez@gmail.com

[1]Laboratorio de Bioinformática y Expresión Génica, INTA, Universidad de Chile, Santiago, Chile.

Peaches are sensitive to low temperature and develop chilling injury (CI) symptoms during refrigerated storage. Preconditioning is a thermal treatment that consists in maintaining stone fruits immediately after harvest and prior to cold storage at 20 °C for 24 h in special chambers aimed to extend fruit market life reducing chilling injury symptoms. In this work, cv. 'Elegant lady' fruits exposed to preaconditioning treatment alleviates symptoms of CI after 21 days to cold storage (4 °C), in mesocarp tissue we determinated that the antioxidant capacity quantify by trolox equivalents in fruits preaconditionated was lower than control fruits with chilling injury symptoms. In experiments of RT-qPCR, the relative mRNA abundance of *CuZn-SOD*, *catalase* and *glutathione reductase* was significantly greater in control CI fruits, suggesting that at the transcriptional level the cold storage in CI fruits increased the expression of genes associated with reactive oxygen species metabolism. Finally, to unravel the molecular changes to underlying this phenomenom a large-scale transcriptome analysis has been conducted using mPEACH1.0 microarrays, preliminary analysis indicates an increase in the expression of genes related to redox metabolism in control chilling injury fruits, whereas in preacondionated normal fruits, there is a trend toward the activation of genes related to cell wall metabolism. We will discuss the involvement of these genes in previously characterized metabolic pathways.

P 9

RELATIONSHIP BETWEEN COMPATIBLE SOLUTES AND LEA PROTEINS (DEHYDRINS) ACCUMULATED DURING HYDRIC STRESS ON FREEZING RESISTANCE

Rafael Rubilar[1], León Bravo[3], **Alexis Velásquez**[4], Stefanía Anselmi[4]
alexvelasquezsaez@gmail.com

[1]Forest Productivity Cooperative, North Carolina State University, Virginia Polytechnic University and State University and Universidad de Concepción.

[2]Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile.

[3]Departamento de Cs Agronómicas y Recursos Naturales, Facultad de Cs Agronómicas y Forestales, Universidad de La Frontera, Temuco, Chile.

[4]Facultad de Ciencias Forestales, Universidad de Concepción, Concepción, Chile.

Low temperatures limit *Eucalyptus* plantations expansion and productivity in Chile restricting more valuable species to coastal regions with oceanic influence. Low temperatures, particularly frosts, generate irreversible tissue damages limiting plant survival at early growth. However, plants under cold hardening or acclimation process may succeed at harsh environments. Cold acclimation imply a series of biochemical changes that allow better resistance to freezing temperatures. In parallel, there is evidence that plants under moderate hydric deficits present changes in protein synthesis and in the solutes accumulation that could be related with frost resistance suggesting cross-linkages with low temperature physiological changes. We evaluated the importance of the compatible solutes and dehydrins (DHNs) accumulated during hydric stress and frost resistance. Five genotypes (EgA1, EgA2, EgM2, EgB1, Egxn) corresponding to *E. globulus* varieties of high, middle and low productivity and one high yield *E.globulus x E. nitens* hybrid were selected and carried to pre-down xylem potentials of -0.5MPa, -2.0MPa and -3.0MPa. Once each genotype reached the expected potential plants were submitted to temperatures profiles of 20/12ºC, 8/4ºC and 8/4ºC with pulses of -2ºC. Finally, all the plants were exposed to a -6ºC frost to evaluate survival. Proline content, total soluble carbohydrates (CST) and DHNs extraction were evaluated from excised leaves. Our results suggest that water deficit seems to be more important in solutes accumulation and in frost resistance than low temperatures in cold acclimation, meanwhile DHNs accumulation didn´t exhibited differences among the treatments. Genotypes showed no differences in solutes accumulation but differences in DHNs were found with EgM2 with the highest accumulation. Genotype GN showed the faster response in DHNs accumulation showing the highest survival followed by EgM2 suggesting that speed of response could be critical.

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P 10

THE EFFECT OF LIGHT ENVIRONMENT ON THE DESICCATION TOLERANCE OF HYMENOPHYLLACEAE SPECIES FROM THE CHILEAN TEMPERATE RAIN FOREST

Alejandra Flores-Bavestrello[1], Luis J. Corcuera[1], **León A. Bravo**[2]
lbravo@ufro.cl

[1]Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile

[2]Departamento de Ciencias Agronómicas y Recursos Naturales, Facultad de Ciencias Agronómicas y Forestales; Center of Plant, Soil Interaction and Natural Resources Biotechnology, BIOREN, Universidad de La Frontera, Temuco, Chile.

Filmy ferns (Hymenophyllaceae) are epiphytes characterized by fronds with a single-cell thick lamina, lack of cuticle, differentiated epidermis and stomata. They are poikilohydric, because no barrier exists to prevent water loss. Although they are associated with humid and shade environments, their vertical distribution varies throughout the trunks. Filmy ferns undergo light and humidity variability in the vertical gradient, being abrupt in higher zones. Species from the top zone are exposed in a greater proportion to direct light, while those located in the basal zone, receive mainly diffuse light. This work aims to study desiccation tolerance in Hymenophyllaceae species under diffuse and direct light. It is hypothesized that photoinactivation caused by desiccation at the photosynthetic apparatus in fronds of Hymenophyllaceae species will be greater under direct light than diffuse light. As a result, the recovery capacity from the desiccated state will be greater under diffuse light. Consequently, species from top of the gradient will be less affected by direct light. In order to test these hypotheses, we characterized their natural environment and determined RWC, Fv/Fm and photosynthetic pigments during a desiccation/rehydration cycle. We observed that (a) There was no difference in photoinactivation under direct and diffuse light, in both desiccation and rehydration treatments. (b) There was a relation between photoinactivation and vertical distribution only during desiccation. Those species with a vertical distribution along the entire trunk, photoinactivated at higher RWC, compared to species that inhabit the basal zone of the trunk and (c) The xanthophylls cycle is active during desiccation and rehydration under direct and diffuse light treatments. Therefore, light direction does not significantly affect desiccation tolerance. Species able to reach the top of the vertical gradient are able to respond earlier to water loss, consistently with the abrupt daily humidity and light variability on the upper canopy.

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P 11

CHALCONE SYNTHASE GENES AND ANTHRACNOSE RESISTANCE IN *LUPINUS ALBUS*

Manuel A. Muñoz[1], Ricardo R. Riegel[1]
manuelmun@gmail.com

[1]Institute of Plant Production and Protection, Faculty of Agricultural Science, Universidad Austral de Chile

Chalcone synthase (CHS) is the first enzyme in the pathway branch for isoflavonoid biosynthesis. These secondary metabolites play important roles in adaptative mechanism such as plant defense against pest and pathogen, protection against UV light, symbiosis with *Rhizobium* and flower pigmentation. This work aims to investigate relationship between CHS and pathogen attack, focusing in potentially develops tools for *Lupinus albus* breeding process towards antracnose resistance. In a first step we described *CHS* gene copies, elaborated a phylogenetic reconstruction and analyzed expression profiles in infected and healthy tissues in order to gain insight about divergence and functionality of these genes. We found three putative *CHS* isoforms. The structure of these genes inferred by means of bioinformatic tools revealed presence of one intron flanked by two exon. Intron was highly variable among isogenes. Predicted amonoacidic sequence showed existence of conserved residues corresponding to functionally important domains in exon 2. Phylogenetic analysis showed that these isoforms are highly divergent, each one sharing a common ancestor with a previously described sequence of *L. luteus*, suggesting that they evolved by duplication before species divergence. One of these (*CHS1*) showed and altered expression during pathogen attack. Subsecuently, we analyzed the association between intervarietal diversity in *Lupinus albus* and phenotypic variability of resistance to infection by *C. lupini*. Resistance was measured in inoculated cotyledons. Through linkage disequilibrium analysis and Tree scanning method we discovered one SNPs associated to variation in resistance inside Exon 2. Haplotype network testing revealed an haplotype associated to extreme susceptibility. In conclusion, *CHS* gene is involved in defense reaction in conjunction with others genetic components still unknown. More genetic components must be discovered to explain all the variation in resistance to this disease. However, markers derived of this study can be used to discard susceptibility

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P 12

CHARACTERIZATION OF THE GENE ALS (ACETOLACTATE SYNTHASE) AND MUTATIONS THAT CONFER RESISTANCE TO HERBICIDES IN QUINOA

Camilo Alexander Mestanza Uquillas[1], Ricardo Roberto Riegel Schlegel[2]
lalomestanza@hotmail.com

[1]Escuela de Graduados, Fac. de Ciencias, UACh, Valdivia, Chile. Universidad Técnica Estatal de Quevedo, UTEQ, Quevedo, Ecuador.

[2]Fac. de Ciencias Agrarias, Inst. de Producción y Sanidad Vegetal, Lab. de Biotecnología Silvoagrícola, UACh, Valdivia, Chile.

Quinoa (Chenopodium quinoa Willd) is an allotetraploid spieces whose parental diploid are still unknown, is a pseudocereal with great food value and nutritional qualities have been recognized worldwide. The main limitation for mass cultivation is weed control. Acetolactate synthase (ALS or AHAS) is an enzyme that catalyzes the first step in the synthesis of branched chain amino acids, particularly valine, leucine and isoleucine, is considerably important because it is the target of several herbicides, including imidazolinone. Currently, there are species with herbicide resistance gene associated with ALS gene. The first objective of this work was to characterize the ALS genes in quinoa, hypothesizing that is an allotetraploid species, so more than one copy exists of the ALS gene and their sequences differ from each other. DNA was extracted from the variety Regalona Baer and mutant lines (M4-M5) that were treated with ethyl methane sulphonate (EMS) that presented different levels of resistance to Onduty, post-emergent herbicide imidazolinone. Described primers for other species were used and also primers were designed to analyze conserved regions between related species. The results show that the gene quinoa ALS is not interrupted by introns with 2001 bp, generating a protein of 667 aa. Appearance of two peaks in a single nucleotide position in the chromatogram shows polymorphisms between the sequences, which is a sign of the existence of two different copies of the gene. This study allowed to know the full gene sequence ALS, may continue with the analysis of the sequences in the mutant lines to identify mutations that confer resistance to herbicides, this will allow to generate breeding strategies and deliver to market new varieties resistant to herbicides, thus solving one of the limiting factors for production.

P 13

FUNCTIONAL CHARACTERIZATION OF VITIS VINIFERA AGAMOUS-LIKE 11, THE MAJOR CANDIDATE GENE RESPONSIBLE FOR SEEDLESSNESS IN GRAPEVINE.

Braulio Soto[1], Nallatt Ocarez[1], Marcos Guerrero[1], Nilo Mejía[1]
nmejia@inia.cl

[1]Laboratorio de Biotecnología, INIA La Platina. Av. Santa Rosa 11.610, La Pintana, Santiago, Chile.

Seedlessness is one of the most desired traits in the chilean table grape industry. The use of genomic resources, genetic and transcriptional experiments allowed us to identify and propose *VvAGL11* as the major gene responsible for seedlessness in grapevine. So far, *VvAGL11* function has not been functionally validated yet. Predicted peptides from model species, STK/AGL11 from Arabidopsis, TAGL11 from tomato and FBP7/11 from petunia are highly conserved and reveal high homology with VvAGL11. Furthermore, *AGL11*, *TAL11* and *FBP7/11* show a similar expression pattern during floral and seed developmental stages. Additionally, FBP7/11 and AGL11 were validated functionally as members of the D MADS-box family. In grapevine, *VvAGL11* expression was detected particularly in mature carpels and developing seeds and fruits. In seedless varieties *VvAGL11* remains unexpressed at these key developmental stages. We propose *VvAGL11* as the class D MADS-box responsible for ovule and seed development in grapevine. Expression of *VvAGL11* in heterologous system, wild type and stk mutant of Arabidopsis, under control of CaMV 35S strong promoter reverses the mutant phenotype and produces an overdeveloped stigma at early stages of flower development. Otherwise, the seedless allele characterization reveals several polymorphisms in the regulatory region, to define the functional elements in the promoter and provide evidence that seedlessness is due to a miss-expression of *VvAGL11* caused by polymorphisms in these elements, several seedless and seeded alleles are being functionally characterized and used to drive the expression of Beta Glucuronidase reporter gene. The allele-specific expression was analyzed by transient transformation of tobacco and tomato. Differential expression patterns were found between seedless and seeded alleles of *VvAGL11*.

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P 14

A FUNCTIONAL GENOMICS STRATEGY TO STUDY FRUIT RIPENING IN MOUNTAIN PAPAYA FRUIT (*VASCONCELLEA PUBESCENS*)

Tamara Méndez[1], Carlos Gaete-Eastman[1], María Alejandra Moya-León[1]
biotamas@gmail.com

[1]Laboratorio de Fisiología Vegetal, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca

Fruit ripening involves several changes, and in climacteric fruits ethylene triggers the ripening process. The molecular mechanism is still under study, but several genes have been identified and demonstrated to be involved in fruit softening, aroma production or color changes. Mountain papaya (*Vasconcellea pubescens*) is an exotic fruit native from the Andean regions of South America. The ripening of mountain papaya fruit is characterized by changes in fruit firmness, ethylene production, color development, soluble solids, titratable acidity and respiration, confirming the climacteric behavior of the fruit and the relevance of firmness on fruit quality. Recently, the involvement of ethylene in the expansin transcript accumulation during ripening has been reported. No additional molecular information is available considering the other molecular players involved in the ripening process of this climacteric fruit. The present work describes a strategy to identify new key genes related to ripening of mountain papaya fruit using a functional genomics approach. With this aim, mountain papaya fruit samples at different ripening stages were collected. After an exhaustive physiological characterization of them, six different ripening stages were selected with physiological differences among them. Then, RNA extractions were performed, mRNAs were separated, cDNA were synthesized and sequenced through 454 pyrosequencing. With the 454 ESTs reads, an EST processing pipeline will be applied, to obtain the number of singletons and tentative contigs, and the functional annotation report. To improve the performance in the annotation process the tropical papaya (*Carica papaya*) genome we will use as a reference genome. The sequences and Bioinformatics analyses will be used to build a database and the candidate genes identified will be functionally tested by qPCR.

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P 15

SEQUENCE COMPARISON OF CANDIDATE GENES ASSOCIATED WITH RESISTANCE TO FUSARIUM CIRCINATUM IN PINUS RADIATA.

Ángela A. Carrasco[1], Sofía Valenzuela[1], Sofía Valenzuela[2], Eugenio Sanfuentes[1], Eugenio Sanfuentes[2], Álvaro Durán[3]
angelacarrasco@udec.cl

[1]Facultad de Ciencias Forestales, Universidad de Concepción. Casilla 160-C. Concepción. Chile
[2]Centro de Biotecnología Universidad de Concepción. Casilla 160-C. Concepción. Chile
[3]Bioforest S.A. Camino a Coronel km 15. Concepción. Chile.

Fusarium circinatum causes the disease known as pitch canker in Pinus and Pseudotsuga species. This disease is characterized by abundant resin production in the plant stems, trunks or branches of infected trees, which in some cases, cause death of the host. The disease was first reported in the southeastern U.S. in 1946. It has now been reported in the U.S. West Coast, Mexico, Japan, South Africa, Spain and Uruguay. In Chile, the fungus was firt reported in 2001, in nurseries of the VIII region, being now present in nurseries from the VI to the X region. Pinus species have different degrees of susceptibility to the pathogen, where P. radiata is one of the most susceptible. Quantitative phenotypic variation and intermediate heritabilities in response to F circinatum have been observed by studies of controlled inoculations in different families of P. radiata, suggesting the existence of a genetic component related to resistance. The aim of this study was to determine whether the candidate genes AXR, SOD-chl, CESA3, GATAbp2 and COMT2.1, related to resistence to F. circinatum in Pinus taeda, are present in P. radiata and compare the inter and intra-specific nucleotide sequences. The results show that the 5 candidate genes were present in the genomic DNA of P. radiata, having a high sequence identity with the homologs in P. taeda and other Pinus species. The genes AXR, SOD-chl, COMT2.1 and CESA3 showed similarity with the putative conserved domains for the corresponding proteins. This is the first report for the nucleotide sequences of SOD and COMT2.1-chl in P. radiata.

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P 16

ADVANCES IN THE IDENTIFICATION OF PUTATIVE BIOMARKERS ASSOCIATED TO BERRY SIZE TRAIT IN TABLE GRAPES, USING A MASSIVE TRANSCRIPTOMIC APPROACH (RNA-SEQ)

Claudia Muñoz[1,4], Alex Di Genova[2,4], Alejandro Maass[2,4], Mauricio González Agüero[3], Ariel Orellana[1,4] and Patricio Hinrichsen[3]
camunoz2009@gmail.com

[1]Centro de Biotecnología Vegetal, Universidad Andrés Bello
[2]Laboratorio de Bioinformática y Matemática del Genoma, Centro de Modelamiento Matemático
[3]Centro Regional de Investigación INIA-La Platina
[4]Centro de Regulación del Genoma (CRG)

The development and maturation of grape berries has been intensely studied because of the uniqueness of this process in plant biology and molecular regulation. Our aim is to identify genetic factors associated with berry size and seedlessness, which can be used as biomarkers for the selection of new table grapes varieties. We have analyzed data from a high-throughput sequencing of cDNA (RNA-Seq) using Illumina sequencing technology. We used 47 table grapes samples obtained from a set of 12 segregants and the parents of a Ruby x Sultanina crossing, from anthesis, fruit-setting and 6-8 mm berry diameter stages, including in the latter stage an application of GA3. Segregants represent contrasting phenotypes for berry size, including seedless and seeded individuals. After quality trimming (Q-value ≤ 20), 477 millions of reads with an average length of 47 bp, were aligned onto the 12X draft grapevine reference sequence, with at most two mismatches. In order to identify differentially expressed (DE) genes related to berry size which expression is independent and dependent of seed presence, total reads between contrasting phenotypes i.e. large berry size-seedless and large berry size-seeded were compared with small berry size-seedless from the same phenological stages using Samtools, Bedtools and libraries edgeR and limma of R statistical software. Also, alignment reads were analyzed to detect SNPs. Our preliminary results detected 4,833 differentially expressed genes after eight comparisons: 2,245 of which were up-regulated while 2,588 were down-regulated. In addition, 196,253 putative SNPs were detected. Our next step will be to search for putative candidate genes associated to berry size, analyzing the current population of differentially expressed genes, determining the metabolic pathways involved as well as SNPs and others polymorphisms (INDELs, SSRs) in those genes in order to propose putative selectable markers for berry size to be applied in table grapes breeding.

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P 17

IMPROVEMENT OF TABLE GRAPE BREEDING PROGRAM: GENE ASSISTED SELECTION FOR SEEDLESSNESS.

Nilo Mejía[1], **Carolina Uquillas**[1], Ariel Pinolef[1], Patricio Hinrichsen[1]
nmejia@inia.cl

[1]Instituto de Investigaciones Agropecuarias, INIA, La Platina

The use of progenies issued from breeding programs, combined with molecular genetic tools and genomics allowed us to identify a major candidate gene (VvAGL11) for the seedlessness. VvAGL11 was identified within limits of a major QTL characterized at sequence, transcriptional and genetic level in the experimental progeny derived from the cross of Sultanina x Ruby Seedless. Developed intragenic marker explains up to 70% of phenotypic variation and has a perfect allele-phenotype association in the experimental progeny. The seedless allele reveals a partial dominance over the seeded allele. In the present work we performed validation experiments for the intragenic VvAGL11 marker. Association analysis was performed with a population issued from fourteen different progenies derived from ten common seedless parental genotypes. PCR genotyping and capillary electrophoresis were performed in these seedlings and parental genotypes to obtain genetic profiles. Seeds or stenospermocarpic seeds rudiments were phenotyped weighting seed fresh weight of a hundred and fifty grapes taken from three randomly selected grapes bunches. Seedless allele was in heterozygosis state in all tested parental genotypes. Six different alleles were identified within the analyzed progenies. Up to eleven different genotypes were identified. Association analysis reveals that the developed intragenic marker can be routinely used for assisted selection of seedless offspring and parental genotypes. The dominance behavior of the seedless allele over most of the seeded counterparts was also detected in these progenies. The developed marker was validated for breeding purposes; it has the potential to increase the efficiency and efficacy in the process of obtaining new seedless varieties. The dominance effect of the seedless allele and the use of molecular markers allow the production of large seedless progenies without the intercrossing of seedless grapes and the subsequent in vitro embryo rescue.

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P 18

EXPRESSION ANALYSIS OF THE GIBBERELLIN METABOLIC PATHWAY USING MASSIVE RNA-SEQ ON DISTINCT PHENOTYPES OF A SIBLING POPULATION OF SEEDLESS TABLE GRAPES

Gonzalo Ravest[1], Sebastián Silva[1], Alex DiGenova[2], Alex DiGenova[3], Claudia Muñoz[4], Alejandro Maass[2], Alejandro Maass[3], Manuel Pinto[1], Patricio Hinrichsen[1]
phinrichsen@inia.cl

[1]INIA La Platina
[2]Centro de Modelamiento Matemático, Universidad de Chile
[3]Centro FONDAP de Regulación del Genoma
[4]Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, UNAB

Berry size is an important trait for the commercial success of table grapes, with larger berries more desirable than smaller ones. The berry development and final size is controlled by several genetic and environmental factors, but the gibberellins pathway is thought to be key in these processes. Using a set of 12 individuals of a sibling population of 'Ruby Seedless'× 'Sultanina' clustered on four distinct phenotypes, an RNA-sequencing approach (Illumina) was performed to obtain transcription and sequence information for these individuals in three stages of berry development (50% flowering, fruit-setting and berries of 6-8 mm). The comparative analysis of the transcript data-set and the evaluation of key genes of the gibberellin pathway revealed a clear correlation between development stage, phenotype and the level of expression for the GA-oxidases that participate in the last steps of biosynthesis of the hormone and in their inactivation. For instance, gene expression of GA20-oxidase, a key enzyme of the gibberellins pathway, show considerable expression levels at 50% flowering stage, lowering during fruit-setting and with no noticeable expression on 6-8 mm berries. Consistently, in the case of GA3-oxidase (the enzyme catalyzing the activation of GAs), the peak of the expression was detected during fruit-setting. Finally, GA2-oxidase, the enzyme that initiates the inactivation of GAs, is expressed mainly on 6-8 mm berries. Also, GA receptors (GAI-like genes) and repressor genes (DELLA) were studied to obtain a wider picture of the differential expressions of GA-related genes during the berry development and in contrasting phenotypes regarding berry size. Further evaluations of the gene expression level by qPCR will provide a more detailed panorama of the expression level of each one of these genes, allowing the selection of candidate genes that could be tested as potentially useful markers to be applied in the assisted selection of new genotypes.

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P 19

ISOLATION AND CHARACTERIZATION OF NEW *PSEUDOMONAS FLUORESCENS* ISOLATES WITH ANTAGONISTIC ACTIVITY AGAINST THE VECTOR OF THE *GRAPEVINE FANLEAF VIRUS*

Hayron Canchignia[1], Michael Seeger[2], Myriam Gonzalez[2], Álvaro Castro[3], Eduardo Tapia[1], Luis Ortega[3], Humberto Prieto[4]
hprieto@inia.cl

[1]Biotechnology Doctoral Program, Universidad Técnica Federico Santa María-Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.

[2]Molecular Biology and Environmental Biotechnology Lab, Universidad Técnica Federico Santa María.

[3]Biotechnology Doctoral Program, Universidad de Santiago, Santiago, Chile.

[4]Biotechnology Lab., La Platina Research Center, Instituto de Investigaciones Agropecuarias, Santiago, Chile.

Grapevine Fanleaf Virus (GFLV) is one of the diseases that cause major losses in grape cultivars in Chile. The main vector described for this virus is the nematode *Xiphinema index*. *Pseudomonas* strains have been used as bio-control agents for fungi, bacteria and nematodes and the activity reported to be mediated by the production of antibiotics such as 2,4-diacetylphloroglucinol (Phl). In this research project, new local *P. fluorescens* isolates were obtained, characterized and assessed for their antagonistic activity against *X. index*. These new isolates were compared to the highly active *Pseudomonas* strain CHA0 during *in vitro* challenges in which whole bacterial extracts were produced by culturing in King B-agar dishes. The results showed that 3 h after interaction between extracts and *X. index*, the isolates RE4 and CHA0 had eliminated all of the nematodes. The application of bacterial supernatants showed that CHA0 and RE4 have the same level of efficacy, resulting in the elimination of all *X. index* individuals after three hours of treatment. No Phl antibiotic was detected in RE4 cultures or supernatants, which suggests that this antagonism is the result of a mechanism other than that which is deployed by CHA0. As a result, RE4 is proposed as a new *Pseudomonas fluorescens* isolate with nematicide activity that is not mediated by Phl production and is able to control *X. index* under *in vitro* assays.

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P 20

DEVELOPMENT OF A NEW STRATEGY FOR SILENCING OF GENE-ISOFORMS IN *VITIS VINIFERA* L.USING ARTIFICIAL MICRO-RNAS

Álvaro Castro[1], Alejandra Ramírez[2], Catalina Álvarez[3], Blanca Olmedo[3], Marisol Muñoz[3], Humberto Prieto[3]
alvarocastrowei@gmail.com

[1]Doctorado en Biotecnología, Facultad de Química y Biología, USACH.
[2]Bioquímica, Facultad de Química y Biología, USACH.
[3]Laboratorio de Biotecnología, CRI La Platina, INIA.

The amount of a given mRNA can be regulated at postranscriptional level by small 21-nucleotides RNAs called micro RNAs (miRNAs). The interaction in plants between a miRNA and its target mRNA is mediated by the enzymatic complex RISC, which has RNAse activity. miRNAs drive the cleavage in a sequence-specific fashion. The seed region, i.e. nucleotides 2 to 8 in the miRNA, is responsible for the initial recognition of the target mRNA, which is cut between nucleotides 10 and 11. In this work we have proposed that the presence of a non-complementary nucleotide at position 10 or 11 prevents the cleavage of the target mRNA. Two artificial miRNAs were designed from a *Vitis vinifera* endogenous pre-miRNA (*vvi*-MIR319e), both sequences possess a specific recognition region (21 bp) for the green fluorescent protein (GFP) gene isoform sGFP S65-T (GFP1). These recognition regions are not complementary at positions 10 or 11 with the GFP gene isoform mGFP5-ER (GFP2). Two stable transgenic grapevine lines expressing either the GFP1 or the GFP2 gene isoforms, were subjected to transient expression with our artificial microRNAs directed against GFP1. Results showed a selective silencing of leaves and calli from GFP1 grape lines and not in the same type of tissues obtained from GFP2 grape lines. Results are discussed focused on the relevance of these nucleotide arrangements in synthetic miRNA design.

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P 21

GENETIC DIVERSITY OF ARAUCARIA ARAUCANA BY ANALYSIS OF TWO CLUSTERING APPROACHES

Glenda Fuentes[1], **Javier Saavedra**[2], Eduardo Ruíz[1], Cristian Torres[3], Freddy Mora[2]
glfuente@gmail.com

[1]Laboratorio de Sistemática Molecular, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile

[2]Facultad de Ciencias Forestales, Departamento de Silvicultura, Universidad de Concepción, Concepción, Chile

[3]Laboratorio de Genómica y Biodiversidad, Departamento de Ciencias Básicas, Universidad del Bío-Bío, Chillán, Chile

Araucaria araucana (Mol.) Koch (commonly known as Monkey-puzzle tree or Chilean Pine) is an evergreen conifer, native to South America, and has been listed as vulnerable from a conservation viewpoint. Their distribution was strongly affected during the last glacial maximum (LGM), so it is important to know whether populations of this species show a genetic structure derived from that event. Until now, several studies in araucaria have been focused on *a priori* hierarchical determination of levels of genetic variation. In order to determine the genetic diversity in populations of *A. araucana*, in this study, an analysis of molecular variance using two criteria for ranking the levels of variation was carried out. First, three hierarchical levels were *a priori* established: i) among regions (Coastal-Andean), ii) among populations, iii) within population, based on geographic location. Secondly, three hierarchical levels were established too, but the highest level was defined *a posteriori*, through the determination of genetically homogeneous groups. Young leaves were collected from eight populations (two populations of coastal distribution and six of Andean distribution). DNA extraction was performed by CTAB method. Sixteen combinations of pairs of selective primers were tested. Three combinations of selective primers showed optimal AFLP band patterns. Bayesian clustering, via Gibbs sampling, showed the conformation of three groups, one consisting of two coastal populations and the Andean sector of Conguillío and the remaining two formed only by Andean localities. The first analysis did not detect variation between regions, while the latter registered 12.66% of the variation between groups of genetically homogeneous population. In addition, the *a priori* analysis found that within each region, the interpopulation variability was greater in the Andean region (19.07%) versus 4% in Costa. These genetic variation patterns are in agreement with the processes affecting the species during the LGM.

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P 22

MOLECULAR CHARACTERIZATION OF SWEET CHERRY CULTIVARS (PRUNUS AVIUM L) USING MICROSATELLITE MARKERS

Verónica Guajardo[1], Gonzalo Ravest[2], Boris Sagredo[1], Carlos Muñoz[3], Patricio Hinrichsen[2]
phinrichsen@inia.cl

[1]Centro de Estudios Avanzados en Fruticultura (CEAF). INIA Rayentué. Rengo, Chile
[2]INIA La Platina, Santiago, Chile
[3]Facultad de Ciencias Agronómicas, Universidad de Chile

Sweet cherry (*Prunus avium*, *Rosaceae*, 2n=16) is an economically important species for Chile. The accurate identification of existing cultivars is essential for the germplasm management, genotypes selection for breeding programs, establishment of commercial orchards, fruit commercialization, and other purposes. Microsatellites or simple sequence repeats (SSR) are locus-specific co-dominant markers showing a high degree of polymorphism, making them very useful for a variety of genetic studies. In this case, we performed the molecular characterization of 47 sweet cherry genotypes cultivated in Chile, using eight microsatellite markers from sweet cherry (3) and peach (5). Of the analyzed genotypes, 37 correspond to unique cultivars, with unique allelic pattern and they could be differentiated using only four markers, while the remaining would be synonyms or somaclonal mutations of other cultivars, since they have identical allelic pattern with the set of markers used. A group of these genotypes, previously described as sports, could not be differentiated from the original genotypes from which they came. The cultivars had between 3 and 8 alleles per locus, with a mean of 5.5, while the expected heterozygosity over the eight polymorphic loci averaged 0.69, ranging from 0.62 in UDP96-001 to 0.76 in PMS-30 markers. These results demonstrate the usefulness of inter-species transferability of markers and they are the basis for the development of a fingerprinting protocol using microsatellite markers for cultivars identification.

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P 23

USE OF SSR MARKERS IN RED RASPBERRY CULTIVARS IDENTIFICATION AND BREEDING

Pamela F. Rojas[1], Marina Gambardella[2], José San Martín[1], Boris Sagredo[1]
projas@inia.cl

[1]Instituto de Investigaciones Agropecuarias (INIA), CRI Rayentue-Raihuen, Rengo, Chile.
[2]Departamento de Fruticultura y Enología Facultad de Agronomía e Ing. Forestal, Pontificia Universidad Católica de Chile (PUC), Santiago, Chile.

Polymorphic codominants microsatellite markers are been widely used in DNA fingerprinting studies for cultivars identification, population genetics and phylogenetic analyses. With the objective to apply this technology to assist the Raspberry breeding programs developed in Chile by PUC and INIA, we selected 30 simple sequence repeats (SSR) markers previously reported to evaluate 16 raspberries genotypes from a germplasm collection of our project. Twenty-six SSR showed up scoreable products. In addition, a protocol of DNA extraction was improved to avoid PCR inhibitors present in most of Rubus DNA samples. Each marker was characterized for scoring quality, polymorphic information content (PIC) and discrimination power. Our results will be useful to generate the DNA fingerprinting of cultivars and advanced selected genotypes. In the future SSRs will be integrated as a tool for molecular assisted selection (MAS) in the raspberry breeding program.

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P 24

MOLECULAR CHARACTERIZATION OF PISCO CULTIVARS (*VITIS VINIFERA* L.) BY MICROSATELLITE MARKERS.

María Alejandra Montoya[1], Antonio Ibacache[2], Andrés Zurita-Silva[1]
andres.zurita@ceaza.cl

[1]Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile
[2]Instituto de Investigaciones Agropecuarias (INIA Intihuasi), La Serena, Chile

According to current regulations of Pisco Origin Appellation (from 1931), the elaboration and viticulture should be performed only from grape musts of *Vitis vinifera* L. grown in Atacama and Coquimbo Regions. In fact, SAG (1979) specified a list of cultivars for this purpose, which includes Muscat of Alexandrie, Pink Muscat, Austria Muscat, Yellow Muscat, White Early Muscat, Muscat Hambourg, Muscat de Frontignan, Black Muscat, Musque Vrai, Orange Muscat, Moscato Canelli, Torontel and Pedro Jiménez. To determine genetic relationships and variability between these materials, we performed a molecular SSR analysis among these cultivars, which were genotyped with six nuclear microsatellite loci (VVMD7, VrZAG47, VVS2, VrZAG79, VrZAG62 and VMC5GT) from gDNA. The data were calculated by multiple correspondence analyses, thus allowing the generation of a similarity tree using the 1-Jaccard distance and Ward average linkage as hierarchical method. The number of alleles detected per locus varied between 6 and 10, with a total of 51 alleles. The allelic frequency varied from 0.016 to 0.46. The expected heterozygosity was 0.804 and the observed heterozygosity was 0.618. Cluster analysis by Ward method differentiated notably Pisco cultivars from wine cultivars used as controls. Once established genetic relationships and similitude, a recovery of ancient cultivars will be performed to support industry diversification.

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P 25

DEVELOPMENT OF *LUPINUS LUTEUS* GENETIC LINKAGE MAP

Paula Mora[1], Annally Rupayán[1], Cristián Ortiz[1], Lorena Parra[1,2], Iván Maureira[1], Haroldo Salvo[1,2]
haroldo.salvo@cgna.cl

[1]Centro de Genómica Nutricional Agroacuícola (CGNA), CONICYT-REGIONAL, GORE LA ARAUCANIA, R10C1001, Unidad de Genómica y Bioinformática, Temuco, Chile
[2]Instituto de Investigaciones Agropecuarias, Unidad de Biotecnología, Carillanca, Chile

High cost of feed ingredients, lower availability, and an increasing demand of fish meals have forced the salmon industry to increase the use of alternative protein sources, such as plant proteins. *Lupinus luteus* (2n=2x=52) in one of the lupine species with the highest grain protein content; however, its semi-domesticated condition requires the improvement of agronomic and yield traits before becoming a valid alternative source of protein. The Agriaquaculture Nutritional Genomic Center (CGNA) has started several initiatives to study this species at genomic and the agronomic level. This poster shows an ongoing investigation, for which the main goal is to construct the first *L. Luteus* genetic linkage map. This map will allow the discovery of genes and/or QTLs related to nutritional quality and agronomic traits. We have developed a RILs-F7 mapping population of 215 recombinant inbred lines. Ninety one genomic SSR, EST-SSR, INDEL, and SNP markers, specifically developed for this species, were mapped using JoinMap® version 4. So far, seventeen out of 26 genetic linkage groups have been recovered with 64 loci mapped. This first map draft expanded 454.6 cM with an average marker density of 7.1 cM. We are currently developing and mapping new molecular markers to allow full recovery of all hypothetical 26 linkage groups. It is important to point out that six markers used in this research have being already associated to nutritional traits in a parallel association mapping study at CGNA.

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P 26

ASSOCIATION MAPPING OF SEED PHYTIC ACID CONTENT IN A WIDE RANGE DIVERSITY YELLOW LUPIN (*LUPINUS LUTEUS*) CORE COLLECTION

Gabriela Aravena Abarzúa[1], Lorena Parra González[1,2], Véronique Amiard[1], Javiera Aravena Calvo[1], Fernando Fuentes[1], Katherine Giraldo Velásquez[1], Joshua Udall[3], Jeff Maughan[3], Iván Maureira Butler[1]
ivan.maureira@cgna.cl

[1]Centro de Genómica Nutricional Agroacuícola (CGNA), CONICYT-REGIONAL, GORE LA ARAUCANIA, R10C1001, Unidad de Genómica y Bioinformática, Temuco, Chile

[2]Instituto de Investigaciones Agropecuarias, Unidad de Biotecnología, Carillanca, Chile

[3]Plant and Wildlife Science Dept., Brigham Young University, Provo, UT, USA

Phytic acid is probably one of the best known plant antinutritionals due to its strong capacity to chelate important minerals such as Fe2+/3+, Ca2+, Mg2+ and Zn2+, and mainly for reducing the bioavailability of phosphorus (Pi). This later condition increases Pi animal waste; thereby contributing to Pi water pollution and nutritional deficiencies. Phytic acid is also the most abundant source of P in seeds where is stored to be released during germination by the action of phytases. Yellow lupin is one of the four cultivated species of the genus *Lupinus*. Its levels of protein and sulfur amino acids seed content have made it an attractive protein source for animal feeding. However, despite its nutritional quality, yellow lupin possesses most of the antinutritionals typically associated to grain crops. The main goal of this research was to evaluate the amount of phytic acid seed content in a wide diversity range *L. luteus* core collection and to uncover genomic regions associated to this trait. A total of 161 yellow lupin accessions were genotyped using a set of SSR (genomic and ESTs) and SNP molecular markers and seed phytic acid was evaluated by HPTLC technology. Phenotypic-marker associations were carried out after estimating and incorporating the genetic population structure into the association model. Analysis of variance for phytic acid seed content showed significant differences among yellow lupin accessions and preliminary association analyses suggested the existence of several putative genomic regions affecting this trait.

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P 27

GENETIC STRUCTURE AND LINKAGE DISEQUILIBRIUM ANALYSES: TWO CRITICAL FACTORS FOR ASSOCIATION MAPPING STUDIES OF FLAX (*Linum usitatissimum* L.)

Braulio Soto-Cerda[1,2], Axel Diederichsen[3], Scott Duguid[4], Sylvie Cloutier[1]

sylvie.j.cloutier@agr.gc.ca

[1]Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba, Canada
[2]Centro de Genómica Nutricional Agroacuícola (CGNA), CONICYT-REGIONAL, GORE LA ARAUCANIA, R10C1001, Unidad de Genómica y Bioinformática, Temuco, Chile
[3] Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada
[4] Morden Research Centre, Agriculture and Agri-Food Canada, Morden, Manitoba, Canada

Flax (*Linum usitatissimum* L.) is a self-pollinated annual crop species, cultivated for its seed oil and stem fiber. It is the third largest natural fiber crop and its seed is the richest source of a-linolenic acid (ALA), which is an essential ω-3 fatty acid. In addition, flax is an attractive source of bio-compounds present in the seed such as lignans and mucilage suitable for functional food production. To make advances in flax breeding through association mapping (AM) it is pivotal to understand the genetic structure and linkage disequilibrium (LD) of flax germplasm. One advantage of AM is that it presents high mapping resolution accounted for by the historical recombination accumulated in natural populations and germplasm collections. However, population structure creates false-positive associations and LD pattern defines the optimum marker density and the most suitable AM approach. Thus, these factors are critical to perform successful AM studies. In this study, 408 flax accessions capturing the breadth of diversity present in the flax collection of the Plant Gene Resources of Canada were assessed to infer population structure and LD with the aim of conducting AM studies for agronomic, seed and fibre composition traits. The STRUCTURE and similarity analyses based on 370 mapped neutral microsatellite markers revealed the presence of two main subpopulations consistent with fiber and oil flax types. Further analysis within each main industrial group revealed the presence of six sub-clusters corresponding to their geographic distributions. The average LD block between linked markers measured as the square of the correlation coefficient (r^2) extent to 2 cM (FDR < 0.05), suggesting it should be possible to perform fine mapping of traits of interest. The assessment of population structure and LD in flax provides critical and novel information for future AM studies and for the application of marker assisted selection in flax breeding.

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P 28

MODERATELY SATURATED LINKAGE MAP OF TABLE GRAPE AS A SUPPORT FOR THE IDENTIFICATION OF QUANTITATIVE TRAIT LOCI (QTL) AND GENES UNDERLYING.

Maribel Mamani[1], Gonzalo Ravest[1], Gabriela Pastor[1], Marco Guerrero[1], Braulio Soto[1], Nilo Mejía[1], José Correa[2], Denisse Laborie[1], Patricio Hinrichsen[1]
phinrichsen@inia.cl

[1]Instituto de Investigaciones Agropecuarias, CRI La Platina.
[2]Universidad de Chile, Facultad de Ciencias Agronómicas.

In species such as grapevine, the availability of genetic maps is an important tool for breeding, since it is the first step to succeed in the identification of QTLs for traits of agronomic and commercial interest. The construction of genetic maps needs appropriate number of progenies to follow the genetic marker segregation, a large number of markers to uniformly cover the genome, altogether with the proper segregation of the traits under study. Here we report the saturation of a genetic map for table grapes using a full-sibling F1 population of 144 individuals from a cross of 'Ruby Seedless'× 'Sultanina'. At the beginning of this initiative, we had a map based on ca. 200 markers. The first step in the development of the currently consensus genetic map, based on 382 markers (262 SSRs, 97 AFLPs, 18 gene-based SNPs and five SCARs), was the saturation of specific linkage groups (LGs) where we had preliminarily detected QTLs for traits such as seedlessness, berry size (and its response to gibberellic acid treatment), cluster structure and sugar content. For this purpose, we first mapped SSR markers already described in other reference maps. Then, we identified SSRs on BAC-ends, derived from the contigs used to build the reference physical map, and finally we designed new SSRs directly from the genome sequence available after the French-Italian Consortium (clone 40,024, a back-crossing line derived from 'Pinot noir'). Right now, we have a map covering 1,247 cM with a minimal density of 10 markers per LG, uniformly covering the 19 linkage groups of the species, becoming a genetic tool essential to map QTLs for traits such as those related to post-harvet performance of the fruit.

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P 29

GENETIC DETERMINISM OF SUGAR CONTENT IN TABLE GRAPE BERRIES

José Correa[2], Cristian Valdés[1], Denisse Laborie[1], Maribel Mamani[1], Manuel Pinto[1], Patricio Hinrichsen[1]
phinrichsen@inia.cl

[1]Instituto de Investigaciones Agropecuarias, CRI La Platina.
[2]Universidad de Chile, Facultad de Ciencias Agronómicas.

Sucrose is the major transported sugar in grapevines but glucose and fructose make up the bulk of the sugar in the grape berry at all stages of development. Furthermore, the sweetness of berry juice depends more on amount of fructose than glucose at the same soluble solids content. Therefore, those varieties or genotypes with higher proportion of fructose cause a greater sensation of sweetness. The sugar content expressed as the ratio of fructose to glucose (fru/glu) was investigated on a progeny 'Ruby Seedless' × 'Sultanina' (n=140). Samples were harvested differentially from berry-thinned cluster in February and March of 2009-10 season when the berries of each genotype were in 18°Brix (grams of sugar per 100 ml of juice). The fru/glu ratio, based on HPLC detection, ranged from 1.02 to 1.58 g/L with a mean of 1.24 g/L and a variation coefficient of 12.90% (the latter is considered a low value for field conditions). The heritability of this trait calculated by Restricted Maximum Likelihood variance was 87.33%, what is considered as a high value. No significant correlation between sugar content and berry size was found. In contrast, there was a high phenotypic correlation between fructose and glucose content in g/L (r=93.6%, p=0.00). QTL analysis revealed a putative genetic position at linkage group 17 for the glucose and fructose content, showing a pleiotropic effect of co-localized QTLs on these traits which could be implicated in the correlation between these traits. In addition, these QTLs explained ~16.5% of phenotypic variance. This study show that the sugar content expressed as the fru/glu ratio hence sweetness of ripen berry (18°Brix) have a clear genetic determinism. Therefore, these results would be useful for future research on the biochemistry, genetics and breeding of the grape berry palatability.

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P 30

QTL FOR GIBBERELLIC ACID (GA3) RESPONSE OF BERRY SIZE IN TABLE GRAPE (VITIS VINIFERA L.)

José Correa[2], **Denisse Laborie**[1], Maribel Mamani[1], Manuel Pinto[1], Carlos Muñoz[2], Patricio Hinrichsen[1]
phinrichsen@inia.cl

[1]Instituto de Investigaciones Agropecuarias, CRI La Platina.
[2]Universidad de Chile, Facultad de Ciencias Agronómicas.

Bioactive gibberellins are commonly used to increase the size of berries in seedless table grape varieties. With the aim of identifying the genetic determinants for GA3 response, the interaction between genotype and GA3 (g×GA3) was investigated in a progeny (n=140) of 'Ruby Seedless'×'Sultanina'. Samples were harvested differentially from ripen and berry-thinned clusters sprayed with or without GA3 during the 2009-10 and 2010-11 seasons. GA3 applications of 10, 20 and 20 ppm were practiced at pre-bloom and on berries of 2-4 and 6-8 mm of diameter. Seed dry weight, berry fresh weight and volume were measured at harvest. Correlation between seasons for each trait and phenotypic distribution revealed a strong influence of g×GA3. According to a linear mixed model approach, the effect of g×GA3 on the phenotype of these traits was calculated as the best linear unbiased predictor (BLUP). Multiple QTL analyses based on the BLUP value showed the importance of the linkage group 18 on these traits giving a co-localized QTL. A single marker QTL analysis programmed in R was used to verify the participation of this QTL in the g×GA3. This analysis was based on a model selection through Bayesian criterion and using as factors the genotype of closest marker to QTL, GA3 treatment, season, segregants, and their interactions. A conservative level of significance (p=0.001) according to a permutation test (with 10,000 permutations) was used. Several GA3-interacting markers were detected, among which VvAGL11 gene was found explaining circa 27% of the g×GA3 variance. This gene has been recently shown to be responsible for seedlessness in grapevine (Mejía et al., 2011), indicating the complex role of g×GA3 at genetic level on the regulation of the berry size.

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P 31

EXTREME PLANT METAGENOMICS ALONG AN ELEVATIONAL SURVEY IN THE HYPERARID ATACAMA DESERT

Bernardo Pollak[1], Tatiana Kraiser[1], Francisca Díaz[2], Claudio Latorre[2], Claudio Latorre[3], Rodrigo Gutiérrez[1]
bpollakw@gmail.com

[1]FONDAP Center for Genome Regulation. Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, Chile.
[2]CASEB & Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile.
[3]Instituto de Ecología y Biodiversidad (IEB), Santiago, Chile.

The Atacama Desert is one of the driest deserts of the world, characterized by strong thermal oscillations(reaching 20-30°C in the day and often falling to below zero at night) and an average relative humidity of <20%. Yet, many different plant species manage to survive in these extreme environmental conditions along the western slope of the Andes. Here, different environmental factors(relative humidity, temperature and precipitation) vary systematically with elevation with pronounced effects on the distribution of the flora. The environmental conditions present throughout this transect define distinct ecosystems or "elevational belts"(prepuna, tolar, high tolar) each with its characteristic plant species. We argue that this range of conditions represents a natural laboratory for testing mechanisms of plant adaptation to extreme climates on different conditions(hyperaridity at low elevations or extreme cold at high altitude). We collected a total of 52 different species in an elevational survey from the edges of the Atacama Salar(S23.27385, W67.99930) at 2470 masl to Lake Lejia(S23.50305, W67.72371) at 4480 masl with the purpose of obtaining insights into the genetic composition of plants living in these environments. Collected species represent six distinct order-level taxa. By analyzing the distribution of these orders along our survey, we found that some orders are preferentially distributed to specific elevational belts whereas plant species show a wide range of preferences. Through paired-end SOLID and IonTorrent sequencing technologies we aim to partially sequence the genome of the collected species. Integrative network bioinformatics tools will then be used to identify potential genes involved in abiotic stress tolerance. We propose that characterizing the genetic composition of plant species adapted to survive the extreme environmental conditions in the Atacama Desert will provide insights into abiotic stress tolerance mechanisms. Understanding the mechanisms plants employ to adapt to these extreme environments is the first step towards developing biotechnological applications for crop improvement.

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P 32

NEW PLANT MOLECULAR BIOLOGY LABORATORY IN REGIÓN DE ARICA Y PARINACOTA

Wilson Huanca-Mamani[1], Bernarda Ventura[1], Victor Rojas[1], Mercelo Vargas[1], Karen Alache[2], Elizabeth Bastías[1]
whuanca@uta.cl

[1]Lab. Biotecnología Vegetal Depto. Producción Agrícola. Fac. Cs. Agronómicas. U. de Tarapacá.
[2]Syngenta S.A. Estación Arica.

The objective of this poster is show to scienticic community a new Plant molecular biology Laboratory of the Universidad de Tarapacá. New Laboratory was soported by CONVENIO DE DESEMPENO-MESESUP-2, a performance agreement signed with the Ministry of Education. One CD goals is stimulate a scientific regional integration with research centers from Perú and Bolivia. The CD support included human resources and lab equipments. Our research is focusing in two topics: Abiotics stress and reproductive development. Abiotic stress. Is our principal topic research and our objective is to identify and molecularly characterize salt o boron tolerance genes using local races of maize ("Lluteño") and tomato ("Poncho Negro"). Both races are adapted to grown in the Lluta valley, which presents soil and irrigation water with high salt and boron concentrations, around four to six times greater than soils used for commercial production respectively being these characteristics the major limiting factor in the diversification of commercial crops in this region coupled with the low quality of irrigation water and inadequate drainage systems. To reach this goal, a global gene expression profile of leaves and roots of "Lluteño" maize and "Poncho Negro" tomato under conditions of salt or boron stress will be obtained. With this information it will be possible to identify specific stress responsive-genes and obtain a functional categorization of those genes differentially regulated in both tissues. Up-regulated candidate genes will be selected either by stress (salt and boron) or tissue (leaves and root) for further characterization. Reproductive development. Is our minor topic research and basically our objective is to determine the molecular bases of the early embryo development using *Arabidopsis thaliana* like a model. We are characterizing to *Athena* a mutant screened in a cDNA-based RNA interference library aproach. *Athena* embryos show morphological defect that consist in a cellular overproliferation in the suspensor cells given rise to an embryo-like structure (or secondary embryo).

Convenio de Desempeño-Mesesup2. FONDECYT 11100492

P 33

GENIUS: GENE FUNCTIONAL NETWORK INFERENCE USING LOCAL EXPRESSION SIGNATURES

Tomas Puelma[1], Alvaro Soto[1], Rodrigo Gutierrez[2]
tfpuelma@uc.cl

[1]Departamento de Ciencia de la Computación, Pontificia Universidad Católica de Chile, Santiago, Chile.

[2]Centro de Regulación del Genóma FONDAP, Centro de Genómica Funcional de Plantas Núcleo Milenio, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, Chile

DNA microarray technology is currently the most widely used approach for profiling gene expression changes in model organisms. There are thousands of publicly available microarray data, which provides information on the expression of thousands of genes under many experimental conditions. This tremendous resource can be used to understand gene expression and to predict new properties of Arabidopsis genes. In this work we present GENIUS, a novel machine learning method, designed specifically to integrate large microarray datasets and predict gene functional networks for a biological processes of interest. By using the existing knowledge available in Gene Ontology, GENIUS is trained to find expression signatures: expression patterns found in subsets of datasets that discriminate the biological process of interest. The method then uses these signatures to predict new genes linked to the process and form a functional network. In contrast to state-of-the-art classification algorithms such as support vector machines or coexpression networks, GENIUS exposes the datasets that are useful to make functional predictions for the specific processes in study. Our results using an *Arabidopsis thaliana* dataset with more than 2,000 arrays show that GENIUS outperforms the predictions of previous works based on support vector machines or coexpression networks. Moreover, by using GENIUS, we were able to identify new and relevant components of the nitrogen response and use efficiency in *A. thaliana* that we validated then experimentally. We encourage plant biology community to use GENIUS web application, which can be accessed at http://networks.bio.puc.cl/genius.

P 34

IDENTIFICATION OF NOVEL MOLECULAR FACTORS AFFECTING NITROGEN USE EFFICIENCY IN ARABIDOPSIS THALIANA

Viviana Araus[1], Tomás Puelma[1], Rodrigo A. Gutiérrez[1]
evaraus@gmail.com

[1]Pontificia Universidad Católica de Chile, Facultad de Ciencias Biológicas, Departamento de Genética Molecular y Microbiología, Núcleo Milenio en Genómica funcional de Plantas, Centro FONDAP de Regulación del Genoma

Nitrogen (N) is an essential macronutrient for plants and quantitatively the most important for their development. As a major constituent of essential macromolecules (such as nucleotides, amino acids and chlorophyll), its availability is a key factor determining plant growth and productivity. To meet the increasing food demand, one of the main agricultural practices to increase yield is touse of nitrogen fertilizers. However, their massive use is limited by both their detrimental environmental impacts, including leaching and eutrophication, and high cost. In addition, bacterial denitrification leads to the generation of nitrogen oxides, which are released to the atmosphere and contribute to the greenhouse effect. As the global population and food demand continue to increase, a major challenge involves indentifying and characterizing the key factors determining crop nitrogen use efficiency (NUE). Despite the importance of understanding the basic processes involved in plant NUE, little is known about the molecular mechanisms regulating NUE. Toward this goal, we developed GENIUS, a novel bioinformatics tool for gene function prediction for complex traits. GENIUS uses gene ontology annotations and gene expression data to identify functional connections based among genes. We used GENIUS with a group of biological process that are important for NUE in plants and obtained a list of 260 genes functionally connected a potentially related to NUE. We applied a number of criteria to select 18 candidate genes for further functional analysis. Evaluation of NUE revealed changes in mutants and/or overexpressors in these candidate genes in Arabidopsis. These genes represent interesting targets for improving NUE in crops.

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P 35

NITROGEN REGULATORY NETWORKS CONTROLLING FLOWERING TIME IN *ARABIDOPSIS THALIANA*

Diana E. Gras[1], Yarela Mancilla[1], Elena A. Vidal[1], Rodrigo A. Gutiérrez[1]
grasdiana@gmail.com

[1]FONDAP Center for Genome Regulation. Millennium Nucleus Center for Plant Functional Genomics. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.

Nitrogen (N) is an essential macronutrient and its availability is one of the primary factors limiting plant growth and agricultural productivity. N nutrient/metabolites can have profound impact on root development, flowering time and other developmental programs. Some of the regulatory gene networks mediating root developmental responses to N availability have been identified. However, despite the economic importance of understanding the relationship between plant N nutrition and flowering, relatively little is known about the molecular mechanisms that control flowering time in response to N supply. To investigate how plants sense and respond to N at the molecular level to coordinate flowering time, we integrated known floral gene networks with N-networks obtained from public databases. Our systems analysis identified important floral genes regulated by N. Among them, several repressors of flowering time are regulated by nitrate including, two *TARGET OF EAT* 1 and 2 (*TOE1* and *TOE2*, respectively), *SCHLAFMUTZE* (*SMZ*) and *SCHNARCHZAPFEN* (*SNZ*). These genes are targets of microRNA172 (miR172), a regulatory factor that controls flowering time by the photoperiod pathway. To analyze the possible role of these transcription factors in the N-response, we evaluated the effect of nitrate treatments on the expression of these genes. *TOE1*, *TOE2*, *SMZ* and *SNZ* RNA accumulated quickly after KNO3 treatments but not after KCl treatments (as control) indicating they are nitrate responsive genes. We also found that miR172 was down regulated by nitrate treatments. To understand the functional role of this N-regulatory module for plant development, we analyzed the flowering time response to nitrate in toe1, toe2, smz and snz insertional mutants and in miR172 overexpressor lines. Our work defined a model for N control of flowering time in *Arabidopsis thaliana* that involves miR172 and their targets *SNZ* and *SMZ*.

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P 36

ESTABLISHMENT OF A GEL-BASED PROTEOME REFERENCE MAP OF YELLOW LUPIN SEEDS.

Takahiro Ogura[1], Ivan Maureira Butler[1], Michio Sunairi[2]
takahiro.ogura@cgna.cl

[1]Centro de Genómica Nutricional Agroacuícola (CGNA), CONICYT-REGIONAL, GORE LA ARAUCANIA, R10C1001, Unidad de Genómica y Bioinformática, Temuco, Chile

[2]Department of Applied Biological Sciences, College of Bioresource Sciences, Nihon University, Kanagawa, Japan

Seed storage proteins play important roles in the seedling growth. On lupin species, four classes of seed storage proteins have been reported, which are named as alpha-, beta-, gamma-, and delta-conglutin. There is, however, little information on the roles of each class of conglutin in the seedling growth. To elucidate the roles, metabolic network in the seedling should be understood at the protein level by proteomic profiling during the seedling growth. Yellow lupin contains a high amount of protein in the seed. Therefore, the seedling is useful to investigate the metabolic network. For the first step of the investigation, we established a proteome reference map of yellow lupin seeds using two-dimensional gel electrophoresis to clarify protein components in the seed. The experiments were conducted as follows. A protein sample was prepared from defatted flour of yellow lupin kernel by the TCA/acetone method. Iso-electric focusing was performed on a 24 cm Immobiline DryStrip gel (pH 4-7). SDS-PAGE was performed by the conventional method. The spots of protein on the gel were stained by CBB G-250. The image of the gels was analyzed by Image master 2D platinum. The detected proteins were digested by trypsin, and analyzed with LC-MS/MS. A database search was performed by Mascot web based search engine. By this study, 340 spots of the proteins were mapped on the gel image. Furthermore, 300 of these proteins were identified. More than 80% of the identified protein was classified in conglutin family. Among the conglutin family, beta conglutin was the richest protein. Alpha- and gamma-conglutin were also identified, whereas delta conglutin was not identified in this study. Each conglutin was shown diversity at a point of molecular mass and iso-electric point. Meanwhile, chaperone proteins such as heat shock proteins and many metabolic relational proteins also identified.

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P 37

IDENTIFICATION AND CHARACTERIZATION OF POLLEN-SPECIFIC PROMOTERS IN *ARABIDOPSIS THALIANA*.

Daniela Muñoz[1], Gabriel León[1]
danimunozs@gmail.com

[1]Laboratorio de Reproducción y Desarrollo de Plantas, Centro de Biotecnología Vegetal, Universidad Andrés Bello (UNAB).

The transition from a vegetative to a reproductive program in plants is accompanied by a massive transcriptional remodeling, evidencing the beginning of the gametophyte genetic program. It has been determined that about 14,000 genes are expressed during the development of the male gamete (pollen), and 5% of these genes are thought to be pollen specific. With the aim to identify pollen-specific promoters, we have identified genes that are expressed exclusively in pollen. To this, we use microarrays databases to identify genes that accomplish two criteria: i) they are not expressed in vegetative tissues and ii) their transcripts are detected only in developing pollen after the first mitosis. Using these searching criteria we identify the best 10 candidate genes and confirm the microarray data using RT-PCR for five of them. On the other hand, the putative promoter regions (500 bp upstream the start codon) of these genes were analyzed *in silico*, searching for overrepresented *cis* elements, which were characteristic in the promoter region of pollen-specific genes. Analysis of plants expressing a reporter gene (GFP/GUS) under the transcriptional control of these putative promoters will allow us to identify promoter sequences highly specific for pollen.

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P 38

THE PIP5K1 AND 2 ENZIMES ARE REQUIRED FOR THE NORMAL REPRODUCTIVE DEVELOPMENT IN *ARABIDOPSIS THALIANA*.

José M. Ugalde V.[1], Ricardo Tejos[2], Jiri Friml[2], Gabriel León[1]
jm_uv@hotmail.com

[1]Laboratory of Plant Development & Reproduction, Center of Vegetal Biotechnology, Universidad Andrés Bello, Chile

[2]Department of Plant Systems Biology, Flanders Institute for Biotechnology and Department of Molecular Genetics, Ghent University, Belgium

The Phosphatidyl Inositols (PtdIns) are membrane lipids located on the cytoplasmic side of animal and vegetal cells, 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*. Single mutant plants for these genes 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 in all stages of its development, 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 the Auxin sensitive promoter, DR5 associated to GUS we'll analyze the dynamic of this hormone in the pollen and pollen tubes of wild type and mutant plants. These results suggest an important role of Auxins in the reproductive development trough the PtdIns in Arabidopsis.

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P 39

CHARACTERISATION OF *ATSDL*, A PUTATIVE SORBITOL DEHYDROGENASE IN *ARABIDOPSIS THALIANA*

Roberto Parada[1], Diego Ampuero[1], María Francisca Aguayo[1], Valentina Castillo[1], Michael Handford[1]
mhandfor@uchile.cl

[1]Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile

In several plant families, polyols are the main product of photosynthesis. In the *Rosaceae* family, sorbitol is the polyol that is produced. This compound is synthesised in the source organs (mature leaves) by the action of sorbitol-6-phosphate dehydrogenase and phosphatases, and is then loaded into the phloem and translocated into the sink organs (young leaves, roots and fruits) where by the action of sorbitol dehydrogenase (SDH), it is converted to fructose and stored or metabolised. On the other hand, in the majority of plant families, sucrose is the main photosynthesis product and carbon form transported. Nevertheless, the presence of sorbitol and SDH activity have been detected in many of these species. In *Arabidopsis thaliana* (*Brassicaceae*), we have identified an open reading frame codifying for a peptide with high identity (>75%) with previously known SDHs, named AtSDL (At5g51970). Expression analysis in transgenic lines harboring *AtSDL* promoter::GUS fusions revealed ubiquitous expression during plant development, and a bioinformatic study showed possible regulation by many factors. Recombinant AtSDL is being produced in order to purify the protein and determine its biochemical constants. It was possible to purify the protein from bacterial and yeast systems, and using *in vitro* crude extracts, it was demonstrated that AtSDL is capable of reducing NAD+ in the presence of zinc and sorbitol or xylitol. In order to determine the role *in vivo* of AtSDL, plants with altered expression levels are being generated. Specifically, *AtSDL-His*-expressing tobacco lines and *atsdl-Arabidopsis thaliana* mutants are being analysed, the latter of which have diminished or abolished levels of *AtSDL* mRNA.

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P 40

IDENTIFICATION OF NUCLEOTIDE SUGARS TRANSPORTERS INVOLVED IN THE UPTAKE OF UDP-GLUCURONIC ACID IN THE GOLGI APPARATUS USING A BIOINFORMATIC APPROACH

Macarena Araya-Tapia[1], Carol Moraga[1], Hernán Salinas-Grenet[1], Ariel Orellana[1]
macarena.araya.tapia@gmail.com

[1]FONDAP Center for Genome Regulation, Núcleo Milenio en Biotecnología Celular Vegetal, Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andres Bello.

In plants, the synthesis of non-cellulosic polysaccharides in Golgi apparatus requires UDP-sugars as substrate. Nucleotide sugar transporters (NST) mediate the translocation of nucleotide sugars from the cytosol into the lumen of the Golgi apparatus. Using a bioinformatic approach more than 40 putative NSTs have been identified in *Arabidopsis thaliana*. Nevertheless, only a few of them have been characterized at biochemical level including their substrate specificity. Since several nucleotide sugars such as UDP-glucoronic acid (UDP-GlcA), UDP-galacturonic acid (UDP-GalA), UDP-xylose (UDP-Xyl) and UDP-arabinose (UDP-Ara) are required as substrates for the biosynthesis of non-cellulosic polysaccharides, we expect that several of the identified NSTs could play a role in this process. Since several enzymes involved in the conversion of UDP-GlcA into other substrates are predicted to be localized in the Golgi apparatus, we focused our research in the identification of a NST capable to transport UDP-GlcA. To this end, we first analyzed the uptake of UDP-[14C] glucuronic acid in enriched Golgi fractions of Arabidopsis. Our results show uptake of UDP-GlcA at 25°C, which decreased at 0°C. Additionally, when enriched fractions are heat-inactivated the uptake activity is abolished. These results suggest that a protein mediated process is involved in the uptake of this substrate. In parallel, we performed co-expression analysis using public transcriptome data and several public bioinformatic tools. From this analysis we obtained strong candidates for the transport of UDP-GlcA based in their coexpression with enzymes involved in the synthesis of non-cellulosic polysacharides.

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P 41

ANALYSIS OF THE EXPRESSION OF ATUTR1 AND ATUTR3 GENES ENCODING NUCLEOTIDE SUGAR TRANSPORTERS IN ARABIDOPSIS THALIANA

Gonzalo Cisternas[1], Adrián Moreno[1], Ariel Orellana[1]
go.cisternas@uandresbello.edu

[1]FONDAP Center for Genome Regulation, Núcleo Milenio en Biotecnología Celular Vegetal, Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andrés Bello.

Nucleotide sugar transporters (NSTs) are responsible of the incorporation of nucleotide sugars into organelles. In *Arabidopsis thaliana*, AtUTr1 and AtUTr3 share a high degree of similarity at amino acid level and both protein transport UDP-glucose into the endoplasmic reticulum (ER). The transcripts of both genes are up regulated during the unfolded protein response (UPR) suggesting that UDP-glucose incorporated by these NSTs is a substrate for glycoprotein re-glucosylation mediated by the UDP-glucose: glycoprotein glucosyltransferase (UGGT). UGGT is a key enzyme in the ER protein quality control mechanism and it has the capacity of reglucosylate misfolded glycoproteins in a UDP-glucose dependent manner. In this way, AtUTr1 and AtUTr3 appears has essential components of the ER protein quality control. Since the only two transcription factors described to regulate ER-QC mechanism components during UPR are AtbZIP60 and AtbZIP28, we analyzed whether AtUTr1 and AtUTr3 transcripts were regulated by these transcription factors by means of qPCR. We used samples obtained from insertional mutants in these transcription factors. In addition, in silico analysis of promoter regions of these genes reveal a conserved UPR response element. Also, we determined the expression pattern of AtUTr1 and AtUTr3 during plant development and under ER stress conditions that trigger UPR using GUS reporter lines harboring the promoter of AtUTr1 or AtUTr3 transcriptionally fused to the B-glucuronidase gene. A high degree of B-glucuronidase activity was observed in plants treated with DTT and tunicamycin in both reporter lines. Finally, both reporter lines showed an overlapped pattern of expression across several tissues during plant development with certain exception that comprise lateral roots and seeds.

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P 42

THE NUCLEOTIDE SUGAR TRANSPORTERS ATUTR10 AND ATUTR11 ARE INVOLVED IN CELL WALL BIOSYNTHESIS

Daniela Doñas[1], Ariel Orellana[1]
danieladc80@gmail.com

[1]FONDAP Center for Genome Regulation, Millennium Nucleus in Plant Cell Biotechnology, Center of Plant Biotechnology, Universidad Andrés Bello

Nucleotide sugar transporters (NSTs) are membrane protein located on the ER and Golgi apparatus, where they transport nucleotide sugars from the cytosol to the lumen of these organelles, for glycosylation reactions by specific glycosyltransferases. In plants, most of these reactions in the Golgi apparatus are involved in the synthesis of cell wall material, such as pectins and hemicelluloses, but until now no evidence of NST involved in pectin biosynthesis has been obtained. We have identified two putative NSTs in Arabidopsis thaliana, AtUTr10 and AtUTr11, which are probably involved in pectin synthesis during seed and male gametophyte development respectively, and characterized its expression pattern, sub cellular localization and phenotype of insertional mutants of these genes. We found that these genes are expressed differentially in critical stages of development, AtUTr10 being more important during seed development, while AtUTr11 in pollen development. The phenotypic analysis of insertional mutants of these genes corroborates the expression pattern analysis since mutants in AtUTr10 show alterations in the mucilage seed coat, while mutants of AtUTr11 posses pollen grains that are unable to elongate a normal pollen tube when germinated in vitro. Taken in consideration that the mucilage seed coat and the pollen tube are mostly composed by pectins, the physiological evidences presented here indicate a possible role for these NSTs in the synthesis of pectic wall material. In addition, the recombinant proteins AtUTr10-GFP and AtUTr11-GFP show a Golgi apparatus localization when transiently expressed in tobacco cells, organelle in which they would have to reside to be directly involved in pectin biosynthesis. All together, these results suggest that AtUTr10 and AtUTr11 are important for the synthesis of pectic wall material, indicating an overall importance of NSTs in the synthesis of the vegetal cell wall.

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P 43

DISRUPTION OF THE IRE1 SIGNALING PATHWAY IN ARABIDOPSIS THALIANA INVOLVES CHANGES IN THE SEED PROTEOME ASSOCIATED TO SEED FILLING

Adrián A. Moreno[1], Ricardo Nilo[1], Ariel Orellana[1]
a.moreno@uandresbello.edu

[1]FONDAP Centro de Regulación del Genoma, Núcleo Milenio en Biotecnología Celular Vegetal, Centro de Biotecnología Vegetal, Facultad de Ciencias Biologicas, Universidad Andrés Bello.

IRE1 is a transmembrane sensor located in the endoplasmic reticulum (ER). It is activated under stress conditions that trigger accumulation of unfolded protein inside ER lumen, evoking a cellular response widely known as the unfolded protein response (UPR). During this biological process IRE1 can participe in the uncoventional splicing of a mRNA substrate in the cytosol. In yeast and mammals this substrate is know as HAC1 and XBP1. Recently, in plant the substrate of IRE1 has been identified as AtbZIP60. The processing of these mRNA substrates lead to the synthesis of a new transcription factor that up-regulate the expression of several ER related genes in order to overcome this folding situation. After the identification of IRE1/bZIP60 pathway in plants, a important question that remains is where this signaling pathway is endogenous require? Since seed filling is a high demanding process involving an active synthesis of proteins and lipids that transit across the secretory pathway, it is possible to rationalize that the ER could be overload during this process and perphaps the IRE1/bZIP60 is activated to overcome the ER overload. To understand if the IRE1 signaling pathway is required for completing seed filling, we performed a seed proteome analysis using 2D gel electrophoresis using samples from wild type and ire1a ire1b mutant seeds. Surprisingly, ire1a ire1b mutant seeds accumulate more seed storage proteins (SSP) that wild type seeds challenging the hypothesis of an overloading of the ER during seed filling. In order to support our observation, we actually are measuring the splicing of AtbZIP60 as a product of IRE1 activity during seed development. Due SSP accumulation is mainly regulated by abscisic acid (ABA), we are measuring the content of this hormone in the ire1a ire1b mutant seeds. Finally, the role of unspliced form of AtbZIP60 in the mentioned process will be evaluated because it is counterpart in yeast never comes to be synthetized and in mammals is rapidly degradated by proteosome.

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P 44

ANALYSIS OF THE ROLE OF THE IRE1/BZIP60 BRANCH OF THE UNFOLDED PROTEIN RESPONSE DURING SALT STRESS IN ARABIDOPSIS THALIANA

Omar A. Sandoval[1], Adrian A. Moreno[1], Ariel Orellana[1]
om.sandoval.i@gmail.com

[1]FONDAP Center for Genome Regulation, Núcleo Milenio en Biotecnología Celular Vegetal, Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andrés Bello

The gene AtbZIP60 encodes an unconventional splicing-activated transcription factor, that is involved in the unfold protein response (UPR). Under endoplasmic reticulum (ER) stress conditions the mRNA of AtbZIP60 is processed by the ER membrane attached protein IRE1. In mammals, the ortholog of AtbZIP60 is Xbp1, which during UPR is processed by IRE1 to produce a transcription factor that is translocated into the nucleus, activating genes involved in UPR. On the other hand, the Xbp1 produced by the non-spliced mRNA is quickly degraded by the proteasome. In plants the spliced form of AtbZIP60 acts in a similar way to the spliced form of Xbp1. Data from the literature suggest that AtbZIP60 could be implicated in the salt stress. Moreover, overexpression of AtbZIP60 in *Arabidopsis thaliana* has a salt resistant phenotype. Results from our laboratory demonstrated that AtbZIP60 mRNA is not processed under salt stress conditions. Based on these results we propose that the unspliced form of AtbZIP60 could be involved in salt stress but not in the UPR. In this work we analyzed the expression levels of genes involved in salt stress and UPR in wild type, *ire1a ire1b* and *bzip60* mutant *Arabidopsis thaliana* plants under salt stress conditions. Our results suggest that the IRE1/bZIP60 branch of the UPR is not activated under salt stress.

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P 45

IDENTIFICATION OF STRESS-INDUCIBLE PROMOTER IN ARABIDOPSIS WITH THE AIM OF IMPROVING TOLERANTE TO SALINITY AND OSMOTIC STRESS.

Marcela Salazar[1], Luis León[1], Loreto Holuigue[1]
msalazari@uc.cl

[1]Laboratorio de Biología Molecular Vegetal, Departamento de Genética y Microbiología. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile

Salt stress is one of the major abiotic stresses that affect plant development. High concentrations of sodium in soils are deleterious to the growth and development of non-halophytes. To improve stress tolerance of crops, many genes have been tested in transgenic plants using either constitutive or stress-inducible promoters. Although several constitutive promoters have been successfully used to obtain stress-tolerance transgenic crops, these plants often suffer undesirable phenotypes. It is therefore desirable to generate transgenic plants that accumulate transgenic products only under stress conditions. With the objective to identify promoters that are induced specifically in roots under salt treatments, we made an in *silico* analysis of the Arabidopsis transcriptome by using public available microarray data and analysis tools (link virtual plant). We identified three genes highly induced by salt treatments in roots, verified their salt-inducible pattern of expression by real time RT-PCR and isolated the corresponding promoter sequences. The activity of these promoters to confer salt-inducible and root-specific expression of GUS reporter gene was evaluated by using histochemical assay in transformed Arabidopsis Plants. Each promoter possesses a distinct pattern of fold-induction, tissue-specificity, and kinetics of induction under salt stress, supporting their potential for crop biotechnology.

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P 46

SPATIO-TEMPORAL GENE EXPRESSION RESPONSES TO NITRATE IN ARABIDOPSIS ROOTS

Orlando Contreras-López[1], Elena A. Vidal[1], Rodrigo A. Gutiérrez[1]
locontre@uc.cl

[1]Núcleo Milenio en Genómica Funcional de Plantas (GFP), FONDAP Centro de Regulación del Genoma (CRG), Departamento de Genética Molecular y Microbiología. Pontificia Universidad Católica de Chile

Our long-term goal is to understand how plant perceive and respond to nitrogen (N) to modulate plant physiology, growth and development. Genome-wide transcriptional analyses have provided an impressive catalog of N-responsive genes participating in a wide range of processes. Despite this solid groundwork, the molecular mechanisms involved in regulating and coordinating N-responses at the organism, organ or cellular level are largely unknown. In addition, the majority of these genome-wide studies were performed at defined time points in whole plants or organs impairing our understanding of cell-specific regulatory gene networks and how they interact to coordinate organ responses over time. In this research, we propose to map and characterize dynamic N-regulatory networks acting within and/or between cell types in Arabidopsis roots. We want to understand how are cell-specific genome-wide responses orchestrated to produce coherent organ responses over space and time in response to nitrate treatments. We propose that root response to nitrate is dependent on the coordinated spatio-temporal regulation of regulatory networks in Arabidopsis. To address this question, we combined cell-sorting, transcriptomics analysis and integrative network bioinformatics to identify cell type-specific regulatory gene networks controlling root responses to nitrate over time. We have been able to characterize N-responsive genes with specific regulation patterns at cell-type level over time. This finding suggests that exists a fine tune control over gene response to N at both cell-type specific and temporal levels.

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P 47

ARABIDOPSIS THALIANA ENERGY METABOLISM GENES CONFER HEAVY METAL TOLERANCE IN *SACCHAROMYCES CEREVISEAE*

Matías Freire[1], Alexander Vergara[1], Lorena Norambuena[1]
matias.freire@gmail.com

[1]Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias Universidad de Chile, Santiago, Chile

In our lab we are studying mechanisms that regulate ionic homeostasis in order to develop organisms are able to resist ionic stress. Since plants have developed very efficient mechanisms of tolerance we have chosen *Arabidopsis thaliana* to select novel genes that may confer ionic tolerance. We performed a search and selection of genes analyzing a co-expression network, 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 had been obtained. Out of those genes, we have focused on three genes that have been annotated as involved in energy metabolism. The network neighbors of these three genes have high representation of ion transport-related GO. In order to investigate their capability to confer tolerance to salts and heavy metals we are using *Saccharomyces cerevisae* as an experimental model. We have cloned these genes to express them in yeast using two different episomal expression vectors, one with an inducible promoter and other with constitutive expression promoter. Their proper molecular design and subsequent expression have been checked. The plant genes were transformed in yeast to evaluate tolerance given by these genes to resist high concentrations of salt and metals. We have analyzed the tolerance of yeast through the growth of this microorganism in presence of sodium, zinc, copper, cadmium and cobalt. The results show that the expression of these Arabidopsis genes confers tolerance to different concentrations of all evaluated metals. However differential gene expression due to two different promoter results in opposites results suggesting that gene doses is an important determinant. The relationship of energy metabolism and ionic stress tolerance will be discussed.

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P 48

GENE EXPRESSION PROFILING ANALYSIS OF COPPER HOMEOSTASIS IN ARABIDOPSIS THALIANA

Talía T. Del Pozo[1], Mauricio M. González[2]
tdelpozo@inta.uchile.cl

[1]Laboratorio de Bioinformática y Expresión Génica (LBEG) / INTA. Universidad de Chile
[2]Centro de Regulación del Genoma (CRG).

As a result of copper essentiality for life, plants and most other organisms have developed a conserved and complex network of proteins to handling Cu in order to prevent its deficit and to avoid its potentially toxic effects. To better understand regulation of Cu homeostasis in plants, we use adult plant of Arabidopsis thaliana to provide an integrated view of how Cu status affects the expression of genes involved in cellular Cu homoeostasis. In doing so, we use real-time RT-PCR to compare shoot and roots transcriptional responses to Cu. We measure changes in the abundance of transcripts encoding transporters, chaperones and P-type ATPases and correlated those changes with variation of Cu content in both tissues. Our results indicated that in both tissues transcript levels of COPT2, 4, and ZIP2 transporters and CCH chaperone were significantly down-regulated comparing to controls plants in response to Cu excess. In contrast, Cu chaperones ATX1, CCS, COX17-1 including two putative mitochondrial chaperones (At3g08950; At1g02410) were up-regulated under similar conditions. Regarding P-type ATPases, a reduction of HMA1, PAA1, PAA2, and RAN1 transcript levels in shoot after Cu exposure was observed, while HMA5 transcripts increased exclusively in roots. In plants growing under Cu-deficient conditions, COPT2, ZIP2, HMA1, and PAA2, were significantly up-regulated in shoots. Thus, our results indicated a common transcriptional regulation pattern of transporters and chaperone components, in particular transcriptional changes of COPT2, ZIP2, and CCH showed an inverse relation with Cu content suggesting that these proteins are required to avoid excess and deficit of Cu.

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P 49

PLANT GROWTH PROMOTING BACTERIA ARE NATURALLY ASSOCIATED WITH *ARABIDOPSIS THALIANA*

Tatiana Kraiser[1], Bernardo González[2], Rodrigo Gutiérrez[1]
tdkraise@uc.cl

[1]Pontificia Universidad Católica de Chile
[2]Universidad Adolfo Ibáñez

Plants have functional association with bacteria. Bacteria can enhance plant growth by regulation of the phytohormones levels and by increasing nutrient bioavailability. *Arabidopsis thaliana* is one of the most favorite model system in plant biology and has been utilized extensively to understand plant:pathogen interactions. However, there are few reports that document *Arabidopsis* interactions with plant growth promoting bacteria. Our goal was to evaluate the naturally occurring interactions of *Arabidopsis thaliana* with beneficial bacteria. Using both culture independent and dependent methods, we detected the presence of bacteria in *Arabidopsis* plants grown under standard conditions and in surface sterilized seed. Some of the isolated bacteria from *Arabidopsis* tissues showed plant growth promoting activity. Because of the importance of nitrogen as a nutrient for plant growth, we investigated the effect of bacteria on plant nitrogen nutrition. We evaluated the importance of biological nitrogen fixation for plant growth under limiting nitrogen conditions. Our results indicate *Arabidopsis* is intimately associated with many different bacteria species, some of which enhance plant nitrogen nutrition and growth.

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P 50

STRUCTURAL CHARACTERIZATION AND EXPRESSION PATTERN ANALYSIS OF LLP PROTEIN UNDER BIOTIC STRESS CONDITIONS IN ARABIDOPSIS

Consuelo García Mardones[1], Grace Armijo[1], Loreto Holuigue[1]
consu.garcia@uandresbello.edu

[1]Laboratorio de Biología Molecular Vegetal. Departamento de Genética Molecular y Microbiología . Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile

Salicylic acid (SA) is a phytohormone described as essential for gene activation during the plant defense response induced by biotrophic pathogens. Using transcriptomic analysis we identified early genes expressed under SA treatments in Arabidopsis thaliana, where LLP showed the highest activation level. Its role in the defense response to avirulent strains of Pseudomonas syringae is suggested by SA-mediated activation after bacterial inoculation and also by a decreased growth of this pathogen in LLP-overexpressor lines compared to wild type plants. Additionally, LLP protein is located in the plasma membrane, but no transmembrane domains seem to be present in its structure. Due to its unknown biological function, we aim to characterize LLP structure and expression patterns in bacterial inoculated tissues, in order to understand its role in biotic stress. For this purpose, we evaluated the functional relations of LLP to the lectin family in Arabidopsis. We found that LLP codes for a carbohydrate binding protein from the legume lectin family. Moreover, according to homology modeling strategies LLP shows a legume lectin fold similar to describes for legume lectins. A putative signal peptide and also two possible N-glycosylation sites might be contained in its sequence, suggesting that LLP goes through the secretory pathway, which could explain its subcelular localization. Experimentally, we demonstrated by enzymatic deglycosylation the presence of glycans attached to LLP. On the other hand, different treatments with detergents, pH or ionic changes indicate that LLP is an external peripheral protein highly attached to the plasma membrane or with a probable location in a microsomal domain. Currently we are evaluating LLP accumulation patterns during Pseudomonas syringae infections, by transiently expressing the LLP-GFP fusion protein under its own promoter transiently in tobacco leaves and stably transforming in Arabidopsis plants.

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P 51

FUNCTIONAL PROMOTER ANALYSIS OF GRXC9, A GENE ACTIVATED BY A NON-CANONICAL SALICYLIC ACID-DEPENDENT PATHWAY IN ARABIDOPSIS

Ariel Herrera[1], Eva Villarroel[1], Grace Armijo[1], Francisca Blanco[1], Loreto Holuigue[1]
asherrer@uc.cl

[1]Laboratorio de Genética Molecular Vegetal. Facultad de Ciencias Biológicas, Departamento de Genética Molecular y Microbiología. Pontificia Universidad Católica de Chile.

Salicylic acid (SA) is one of the key signals involved in defense responses against biotic and abiotic stresses. GRXC9 gene, coding for a glutaredoxin with antioxidant function, is one of the genes rapidly activated by SA in Arabidopsis, independently of NPR-1 protein (a master co-activator of SA-induced gene). We are interested in identifying the mechanism of transcriptional activation of this gene. An in silico promoter analysis of the GRXC9 gene identified two putative SA-responsive as-1-like elements in its proximal region. In this work we used a combination of tools to elucidate the function of these elements in the SA-mediated transcriptional activation of the GRXC9 gene. Mutants in the TGA 2/5/6 subclass of transcription factors showed impaired GRXC9 activation by SA. In vivo reporter assays, using constructs containing the full length, deletions and mutants of the GRXC9 promoter controlling the expression of GUS reporter gene, indicated requirement of both as-1-like elements for SA-mediated activation of GRXC9 gene. In a physiological context, we have showed the transcriptional activation of GRXC9 in plants treated with two stress conditions associated to SA production, inoculation with *Pseudomonas syringae* AvrRPM1, a biotrophic pathogen and treatment with UV-B light. To test SA dependence in GRXC9 induction, we used sid-2 and NahG plants, deficient in SA production and accumulation, respectively. Our results indicate that SA activates transcription of GRXC9 gene by a mechanism involving TGA transcription factors and as-1-like elements found in the promoter.

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P 52

ANALYSIS OF THE INTERACTION OF UPR INVOLVED BZIP28 AND BZIP60 TRANSCRIPTION FACTORS WITH THE NPR1 COACTIVATOR IN *ARABIDOPSIS THALIANA*.

Macarena Greve-Muñoz[1], Ariel Orellana[1], Francisca Blanco[1]
mblanco@unab.cl

[1]FONDAP Centro de Regulación del Genoma. Núcleo Milenio en Biotecnología Celular Vegetal. Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andrés Bello.

Salicilyc acid (SA) is a key player of the defense response in plants. SA activates a wide number of genes involved in defense response were an important part encodes proteins with antimicrobial activities called PRs. The expression of these genes is mediated by the NPR1 coactivator and the TGAs transcription factors. Nevertheless, several endoplasmic reticulum (ER) stress responsive genes are upregulated in the presence of SA and this regulation is dependent on NPR1. Surprising, this process is independent of TGAs transcription factors revealing the involvement of other unknown TFs in the defense response. Since bZIP28 and bZIP60 are involved with the unfolded protein response (UPR), regulating the expression of several ER stress responsive genes and these genes overlap with the genes upregulated by SA, we propose that these TFs could be interacting with NPR1 during pathogen infection. To evaluate the interaction of these TFs with NPR1, we perform a double hybrid assay (Y2H) using these proteins. Preliminary results indicate that interaction of these TFs with NPR1 could be possible. Also, we use a bimolecular fluorescent complementation assay in Arabidopsis protoplast to rule out the possible false positive interaction that can take place in yeast. As additional strategy, a double hybrid system in Arabidopsis protoplast will be evaluated.

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P 53

THE SALICYLIC ACID-INDUCIBLE *LLP* GENE FROM ARABIDOPSIS ENCODES A PLASMA MEMBRANE PROTEIN THAT PLAYS A ROLE IN THE DEFENSE RESPONSE TO *PSEUDOMONAS SYRINGAE* AVR-RPM1 INOCULATION

Grace Armijo[1], Aldo Seguel[1], Consuelo García[1], Paula Salinas[1], David Leiva[1], Loreto Holuigue[1]
giarmijo@uc.cl

[1]Laboratorio de Biología Molecular Vegetal, Departamento de Genética Molecular y Microbiología, Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago de Chile.

The plant response to biotic stress is mainly regulated by hormones such as salicylic acid (SA). The main role of SA has been characterized in the defense response induced by biotrophic pathogens that are specifically recognized by the plant. We identified a group of genes early-activated by SA in *Arabidopsis thaliana*, of which *LLP* gene (from lectin-like-protein) had the highest level of activation. This gene codes for a protein with structural similarity to the legume lectin family and so far has not been associated to any biological function. To evaluate the role of this gene in the plant defense response, we inoculated wild type and sid2 plants (mutants in SA biosyntesis) with different strains of *Pseudomonas syringae* pv tomato (Pst), and we found that *LLP* is activated by avirulent strains of Pst by a SA-dependent mechanism. We also developed Arabidopsis lines transformed with 35S::LLP-GFP construct and analyze the localization of the fusion protein by confocal microscopy. LLP-GFP was located in the plasma membrane of the plant cell. Later, we isolated homozygous mutant lines null for LLP and developed transgenic lines overexpressing LLP fused to c-Myc epitope. Using these lines, we made a loss or gain of function analysis, by quantifying the proliferation of Pst in plant leaves and also measuring the cell death in this tissue, as a consequence of the specific response to biotrophic pathogens. We found that LLP overexpression produces a decrease in Pst Avr-Rpm1 proliferation and an increase in cell death in the inoculated tissues. These results strongly suggests that LLP is a plasma membrane protein involved in the defense response to Pst Avr-Rpm1. Currently we are looking for specific interactors of this protein, to gain further insights in its specific function in the defense response.

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P 54

FUNCTIONAL CHARACTERIZATION IN PEACH FRUITS OF PRUNUS PERSICA TRANSCRIPTION FACTORS PREDICTED TO REGULATE THE EXPRESSION OF CO-EXPRESSED GENES

Paula Vizoso[1], Elena Barindelli[2], Lee Meisel[3]
paula.vizoso@gmail.com

[1]Plant Molecular Genetics Lab, Plant Biotechnology Center, Andrés Bello University, Santiago, Chile

We have previously reported the in silico analyses of differentially expressedand co-expressed genes in peach fruitsunder different postharvest conditions (Cold and ripening processes), as well as conserved putative cis-regulatory elements in these co-expressed genes. We have selected four putative transcription factors predicted to play a role in the regulation of these co-expressed genes to characterize functionally. Transient over-expression of these transcription factors (ILR3, NAC/BT3, WRKY75, CBF1) in peach fruits, and subsequent quantification by qPCR of predicted target genes, confirms that these transcription factors regulate the expression of these predicted target genes in peach fruits.

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P 55

DEVELOPMENT OF TRANSGENIC PLUMS USING AN SIRNA CONSTRUCT FOR IMPROVING PLUM POX VIRUS RESISTANCE

Manuel Acuña[1], **Julia Rubio**[2], Evelyn Sánchez[1], Paola Barba[3], Álvaro Castro[4], Carolina Toro[1], Humberto Prieto[1]
hprieto@inia.cl

[1]Laboratorio de Biotecnología, Centro Regional de Investigacion La Platina, Instituto de Investigaciones Agropecuarias (INIA), Santiago, Chile.

[2]Programa de Doctorado en Ciencias Silvoagropecuarias, Facultad de Agronomía, Universidad de Chile, Santiago de Chile.

[3]Grapevine Breeding and Genetics Doctoral Program, Cornell University, Geneva Agricultural Experiment Station, New York, U.S.A

[4]Programa de Doctorado en Biotecnología, Facultad de Química y Biología, Universidad de Santiago, Santiago, Chile.

Plum pox virus (PPV) is one of the pathogens that have the most serious impacts on stone fruits. This virus is the causal agent of "sharka disease" and mainly infects apricots, peaches and plums. Sharka has been detected in locations around the world, most notoriously Mediterranean Europe and the United States. In Chile, PPV infection was first detected in 1994, and the specific isolate Diderot (PPV-D) was first characterized in 1996. A variety of strategies have been used to fight the disease, the most successful of which is post-transcriptional gene silencing (PTGS). The in vivo generation of double-stranded RNA hairpin structures as inducers of specific gene silencing was described in 2003. Since then, the use of "hairpin small interfering RNAs" (or hsiRNA) has made it possible to degrade a target RNA (i.e., the viral coat protein), allowing for the generation of virus-resistant plant lines. In 2009, we described the successful establishment and improvement of a genetic transformation platform for Prunus spp. using the Japanese plum as a model. In this article, we present the generation and evaluation of several transgenic plum lines that have been genetically modified with an hsiRNA construct (PPV-iRNA) directed at silencing highly sensitive regions of the PPV coat protein gene that were arranged in tandem. GM plants were grafted onto PPV-D infected Adesoto-101 (*Prunus insititia*) plants. The results, which are based on the analysis of plants' symptomatology and virus loads measured by ELISA and qRT-PCR, allowed us to identify two plum lines that are resistant to the virus after two seasons of evaluations.

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P 56

EVALUATION OF GRAPEVINE TRANSGENIC ROOTSTOCKS FOR VIRUS RESISTANCE IN GRAFTED PLANTS

Daniela Muñoz[2], Elizabeth Torres[1], María Consuelo Medina[1], Patricio Arce-Johnson[1]
daniemunoz@udec.cl

[1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

[2]Departamento de Ciencias y Tecnología Vegetal, Escuela de Ciencias y Tecnología, Universidad de Concepción, Los Ángeles, Chile

Grapevine (*Vitis vinifera*) is a fruit species of great importance and the largest in planted area around the world. Its production is affected by diverse pathogens including the viruses. Viral diseases spread and accumulate in plants due to the multiplication of infected material and they can not be chemically treated. However plants have developed mechanism to defense themselves from viruses like gene silencing. We are using the same strategy to induce silencing of the infecting viruses, transforming grapevine rootstocks with constructions containing viral sequences in sense and antisense separated by an intron, to obtain a double RNA molecule inside cells of the transformed plant. We have transformed and regenerated plants of a grapevine rootstock with a construction to induce silencing against the Grapevine Fan Leaf Virus (GFLV), one of the most severe and widespread vine viruses. It is expected that the silencing mobile signal spreads through the graft to the scion inducing silencing in the whole plant. We have obtained more than thirty transgenic lines verified by PCR for the genetic construct. The positive plants were acclimated *ex-vitro* and then, one year old plants were multiplied by cuttings in the greenhouse. Ten lines are being tested for virus resistance. Three plants of each line were grafted with small shoots containing one bud from a GFLV infected plant. Preliminary results obtained in greenhouse controlled conditions show that GFLV is not detected in the developed shoot growing from the scion in some grafted lines

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P 57

THE PROMOTER OF AN ANKYRIN-LIKE PROTEIN GENE FROM VITIS VINIFERA IS HIGHLY INDUCED BY BOTRYTIS CINEREA INFECTION.

María De Los Angeles Miccono[1], Christian Montes[1], Julia Rubio[1], Hugo Peña-Cortés[2], Humberto Prieto[1]
hprieto@inia.cl

[1]Biotechnology Lab., INIA-La Platina Station
[2]D. Alkalay Biotechnology Center, UTFSM

Constitutive promoters such as CaMV35S (from the 35S RNA of the Cauliflower Mosaic Virus) and nos (*A. tumefaciens* nopaline synthase gene) have frequently been used as experimental tools to assess the effects of transgene expression in many plant species. Even though they may be suitable for proof-of-concept experiments, these constitutive promoters present a number of potential drawbacks for use in genetically improved crops, including a negative public perception. Thus, gene expression under the control of inducible intragenic promoters has become the preferred strategy to develop new plant varieties, including improvements in pathogen resistance. Grey mold, caused by *Botrytis cinerea*, is a prominent grapevine disease in Chile. *B. cinerea* is a necrotrophic filamentous fungus of very difficult agronomical management due to its broad host spectrum and abundant sources of inoculation. Several strategies have been used to improve fungal tolerance in grapevine, however, the lack of long-lasting tolerant phenotypes and remarkable increased resistance in the fungus to these strategies represent an obstacle to achieve a full pathogen resistance. In this study we present evidence for a novel necrotrophic pathogen and hormone inducible promoter from grapevines. We identified relevant cis-acting elements in a 1000 base pair upstream region from an ankyrin-like protein gene, dissected into five different sections and fused to the green fluorescent protein (GFP) reporter gene. Transient GFP expression assays using agro-infiltration of grapevine leaves were carried out and evaluated under UV radiation in order to discriminate the effect of two different necrotrophic pathogens (*B. cinerea* and *Alternaria spp.*) and the treatments with the hormones salicylic acid, methyl jasmonate and ethylene. Results showed differential expression depending on deletion in the fragments and the treatment used, allowing the confirmation of minimal signals for promoter specificity and activation.

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P 58

GENETIC TRANSFORMATION OF GRAPE ROOTSTOCKS TO INCREASE SODIUM TOLERANCE

Carlos Aguirre[3], Blanca Olmedo[1], Catalina Alvarez[1], Andrés Zurita[2], Humberto Prieto[1]
caguirre.d@gmail.com

[1]Laboratorio de Biotecnología, INIA La Platina, Chile.
[2]Centro de Estudios Avanzados en Zonas Áridas- CEAZA, Chile.
[3]Programa de Doctorado en Cs. Silvoagropecuarias y Veterinarias, Universidad de Chile.

Chile is one of the main table grape producers and exporters. However, this activity has serious challenges regarding the environmental conditions and desertification process of important areas of cultivation. Salinity is a major problem, with soil conductivities over 4 dS/m; these conditions lead to yield reduction of about 10%. To solve this problem, plants have different mechanisms to overcome salinity stress, maintaining low cytosolic Na+ concentrations and high K+/Na+ rates. Over-expression of genes involved in vacuolar sodium compartmentalization, i.e. *A. thaliana* NHX1 (Na+/H+ antiporter) and AVP1 (H+-Pyrophosphatase, EC 3.6.1.1), have been evaluated to obtain salinity tolerant phenotypes in a number of plant species. In this work, we describe the genetic transformation of the grape hybrid ´Harmony´ (Dog Ridge, Vitis champinii X C1613) and the cultivar 'Thompson seedless', with the *A. thaliana* vacuolar pyrophosphatase AVP1 gene. The generated transgenic plants and the primary physiological evaluations will be shown and discussed.

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P 59

GREEN FLUORESCENT PROTEIN EXPRESSION DIRECTED BY THE 35S CAULIFLOWER MOSAIC VIRUS PROMOTER IN TRANSGENIC PEACH TREES

Julia Rubio[1], Christian Montes[2], Carolina Toro[2], Manuel Acuña[2], Humberto Prieto[2]
hprieto@inia.cl

[1]Agricultural Sciences Doctoral Program, Universidad de Chile
[2]Biotechnology Lab., La Platina Station, Instituto de Investigaciones Agropecuarias

Several regeneration and transformation strategies have been reported in peach (*Prunus persica* L.); however, none of these procedures have resulted reproducible. Transformation events have been reported using particle bombardment or *Agrobacterium*-mediated transformation of immature embryos and other explants such as embryo sections of mature seeds. However, the regeneration of plants from transgenic tissues is still difficult and the recovery of non-chimeric plants has not been well described to date. Since 2004, several transformation protocols have been evaluated in order to define a system that reproducibly leads to the generation of transgenic peaches. In this work we describe a reliable transformation and regeneration system to produce transgenic peach plants using cotyledons sections as starting materials. *A. tumefaciens* strain GV3101 expressing the Green Fluorescent Protein (GFP) reporter gene under the regulation of the constitutive promoter 35S RNA from the Cauliflower Mosaic Virus was used to evaluate these transformation systems. In the same construct, the "Nos pro-nptII-Nos ter" cassette as a selectable marker was used. In vitro cultured cotyledon segments were *Agrobacterium*-co-cultivated and, after selection, transgenic shoots were regenerated, rooted, and acclimatized. Transgenic status was confirmed by PCR, Southern blot and GFP expression evaluation by UV epifluorescence microscopy and UV stereoscopic imaging. Results showed that the 35-S promoter-directed expression of GFP was observed in all of analyzed tree tissues, including leaves, radicles, flowers and fruits. The use of this system has opened the possibility of introducing new important traits into peach genome, and PPV resistance based on the gene silencing strategy using constructs previously evaluated in the diploid Japanese plum model is our current focus.

This is a Biofrutales S.A. work

P 60

PHYTOENE SYNTHASE GENES (PSY1 AND PSY2) OF *DAUCUS CAROTA* EXERT DIFFERENTIAL RESPONSE TO HORMONES AND FUNCTIONALITY IN A HETEROLOGOUS PLANT.

Orlando Acevedo[1], Paulina Fuentes[1], Claudia Stange[1]
cstange@uchile.cl

[1]Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile

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 synthesis of phytoene. PSY is 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. Here we show a phylogenetic analysis and an amino acidic alignment that show the principal domains of the proteins, as the differences in both carrot PSYs. We observed that PSY1 grouped together with monocots PSY1 while PSY2 is grouped together with PSYs of dicot origin. Moreover, both proteins share conserved sequence motifs found in squalene synthases and phytoene synthases of other plant species and microorganisms. Expression analysis showed that psy1 is expressed mostly in mature leaves while psy2 is expressed preferably in young leaves and in mature storage carrot roots. In addition, psy2 and not psy1 expression is induced in the presence of abscisic acid treatment and repressed by auxin (2,4D), while psy1, and not psy2, is induced by giberelic acid. We evaluated the functionality of both genes by means of over-expression in *Nicotiana tabacum*. The expression of psy1 produced a slightly increment in total carotenoids and b-carotene, but tobacco lines transformed with psy2 produced over 70% of increment in total carotenoids and b-carotene. This study let us to conclude, that carrot psy1 and psy2 are functional *in planta* and that psy2 is a good candidate to be used in metabolic engineering of commercially relevant plants.

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P 61

LYCOPENE B-CYLASE1 GENE (LCYB1) FROM *DAUCUS CAROTA* REGULATES CAROTENOID BIOSYNTHESIS IN TOBACCO AND CARROT.

Juan Camilo Moreno[1], Fernando Valenzuela[1], **Claudia Stange**[1]
cstange@uchile.cl

[1]Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile, Chile

Carotenoids are isoprenoid pigments involved in photosynthesis, photo-protection and hormone synthesis in plants. They are produced in plastids and one of the key regulatory steps of the biosynthesis is mediated by the lycopene b-cyclase enzyme (LCYB) that cycles lycopene to give rise to the orange pigment, b-carotene, a vitamin A precursor with strong antioxidant properties. In carrot (*Daucus carota*) two lcyb genes (lcyb1 and lcyb2) have been reported. Dclcyb1 expression presents the highest increase throughout the plant development and post-transcriptional gene silencing of Dclcyb1 decreases total carotenoids. In addition, through heterologous complementation in *E. coli*, we prove that Dclcyb1 and Dclcyb2 genes codify for functional LCYB enzymes and that DcLCYB1 is more efficient than DcLCYB2. In this study, we over expressed Dclcyb1 in tobacco and carrot to evaluate its functionality in planta. *N. tabacum* transgenic plants express between 2.5-300 fold the Dclcyb1 gene and also the DcLCYB protein determined through western blot. Besides, the expression analysis of the endogenous Ntpsy1, Ntpsy2 and Ntlcyb1 in tobacco indicates that these genes increase between 2-14, 2-13 and 2.5-8 fold, respectively. HPLC analysis showed that transgenic tobaccos exert an increment in total carotenoids (1.5- 9 fold) and b-carotene (1.6-5 fold). In transgenic carrots that over-express Dclcyb1 between 2-8 fold, Dcpsy1, Dcpsy2 and Dclcyb2 are over-induced between 2-5 fold. Almost all carrot lines have a significantly increase in total carotenoids and b-carotene in leaves and roots. Taken togheter, Dclcyb1 gene is functional in plants and produces an effective modification of the carotenogenic pathway, increasing the carotenoid content. Therefore, it can be applied in metabolic enginnering in order to reach a greater carotenoid accumulation in plants with high comercial value.

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P 62

A CANDIDATE GENE APPROACH TO STUDY THE *BRASSICA OLERACEA* TO1000DH3 WHITE FLOWER PHENOTYPE

Daniela Quezada[1,2], Jacqueline Rilling[1,2], Pablo Cárdenas[1], Takahiro Ogura[1], Wayne Clark[3], Isobel Parkin[3], Federico Iñiguez-Luy[1], María L. Federico[1]

maria.federico@cgna.cl

[1]Centro de Genómica Nutricional Agroacuícola (CGNA), CONICYT-REGIONAL, GORE LA ARAUCANIA, R10C1001, Unidad de Genómica y Bioinformática, Temuco, Chile

[2]Universidad de La Frontera (UFRO), Carrera de Biotecnología, Temuco, Chile

[3]Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, Canada

Plant carotenoids can accumulate in flowers and fruits where they serve as pigments and precursors to a range of scents that attract pollinators and secure seed dispersal. The present study was conducted to investigate the genetic basis underlining the white flower (*wf*) mutant phenotype exhibited by the *Brassica oleracea* L. double haploid (DH) rapid cycling line TO1000DH3. Previous work in our research group mapped the flower color mutation (FC) to *B. oleracea* chromosome C3 using the BolTBDH mapping population, which segregates for this flower color trait. During early stages of flower development, *wf* petals are yellow suggesting that genes involved in carotenoid biosynthesis are present and functional in TO1000DH3. In addition, the *wf* allele is dominant over the *wt* (yellow) allele and no intermediate phenotype (cream) is observed in heterozygous plants. Hence this mutation could likely affect carotenoid degradation rather than biosynthesis. Based on synteny information with Arabidopsis, a candidate gene (Carotenoid Cleavage Dioxygenase 4, CCD4) was identified in C3. Using primer pairs targeted to conserved regions, two CCD4 genes (*BolC.CCD4.a* and *BolC.CCD4.b*) were mapped using the BolTBDH population. Remarkably, the TO1000DH3 *BolC.CCD4.b* allele co-segregated with the *wf* phenotype and mapped to the previously identified C3 location. In contrast, the TO1000DH3 *BolC.CCD4.a* allele segregated independently of the *wf* phenotype and mapped to C1. This indicates that TO1000DH3 *BolC.CCD4.b* could be involved in the generation of the *wf* phenotype. Interestingly, genomic sequence information revealed that this gene contains repetitive element sequences in its 5´and 3´ non-coding regions; these elements are absent in TO1000DH3 *BolC.CCD4.a.* We are currently performing gene expression studies to compare allele-specific mRNA levels at different stages of petal development in yellow and white flowers.

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P 63

METABOLIC ENGINEERING OF CAROTENOID CONTENT IN *BRASSICA NAPUS* L. THROUGH SEED-SPECIFIC OVEREXPRESSION OF AN ENDOGENOUS *PSY* GENE

Ada E. López[1], Humberto A. Gajardo[1], Mónica A. Schmidt[3], María L. Federico[1]
maria.federico@cgna.cl

[1]Centro de Genómica Nutricional Agroacuícola (CGNA), CONICYT-REGIONAL, GORE LA ARAUCANIA, R10C1001, Unidad de Genómica y Bioinformática, Temuco, Chile

[2]Universidad de Talca, Programa de Doctorado en Cs. Mención Ingeniería Genética Vegetal, Talca, CHILE

[3]Donald Danforth Plant Science Center, 975 North Warson Road, Saint Louis, MO 63132, USA

Phytoene synthase (PSY) has been shown to catalyze the first committed and rate-limiting step of carotenogenesis in several crop species, including *Brassica napus* L. Due to its pivotal role, PSY has been a prime target for breeding and metabolic engineering the carotenoid content of seeds, tubers, fruits and flowers. In addition, pioneer work in "Golden Rice" has demonstrated that the source of *PSY* transgene has a major impact on carotenoid accumulation since different PSY proteins differ in their ability to form fully functional protein complexes. In *B. napus*, ovexpression of a bacterial phytoene synthase (*CrtB*) increased seed carotenoid levels 50-fold, however, overexpression of a plant or endogenous *PSY* has not been reported to date. In this context, our work aims at enhancing carotenoid content in *B. napus* seeds with the potential of reaching higher levels than previously reported overexpressing *CrtB*. First, we used an *Escherichia coli* heterologous complementation system that carries an *Erwinia uredovora* gene cluster for the production of □-carotene. Using this system, we demonstrated that *BnaC.PSY.a* was capable of complementing a defective *CrtB* gene, and thus, has functional phytoene synthase activity *in vivo*. Secondly, we placed *BnaC.PSY.a* coding sequence under the control of the soybean *lectin* promoter and added a hexa histidine tag (6XHis-tag) to be able to distinguish the overexpressed endogenous protein from its naturally occurring homologues (pCambia2300-*Lec::BnaC.PSYa-6XHis*). We also constructed a pCambia2300-*Lec::CrtB* vector to be used as a control in our transformation experiments. We have currently transformed a total of 8560 cotyledon explants using an *Agrobacterium tumefaciens* transformation method. The first five *Lec::BnaC.PSYa-6XHis* and *Lec::CrtB* transgenic events have been confirmed by PCR and several putative transgenic lines are currently in our pipeline. Future studies will evaluate and compare the achieved levels of transgene expression, PSY protein and carotenoids in seeds of these *B. napus* transgenic lines.

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P 64

ANTIOXIDANT CAPACITY AND POLYPHENOL CONTENT OF LEAVES AND FRUIT OF NATIVE CHILEAN TREES

Lida Fuentes[1], Juan Pablo Martínez[2], Darcy Ríos[3], Carlos Figueroa[4]
lfuentes@creas.cl

[1]Centro Regional de Estudio en Alimentos Saludables (CREAS) - Instituto de Investigación Agropecuaria La Cruz, Blanco 1623 of 1402, Valparaíso, Chile

[2]Instituto de Investigación Agropecuaria La Cruz - Centro Regional de Estudio en Alimentos Saludables (CREAS), Chorrillos 86, La Cruz, Casilla 3, Chile

[3]Centro Regional de Estudio en Alimentos Saludables (CREAS) - Universidad Técnica Federico Santa María, Blanco 1623 of 1402, Valparaíso, Chile

[4]Facultad de Ciencias Forestales - Centro de Biotecnología, Universidad de Concepción, Casilla 160-C, Concepción

Many native plant species are in vulnerable condition due to their habitat loss. One of the conservation strategies for native plants is their sustainable use in healthy food industry. Nevertheless, few studies about the healthy food property of these plants have been made. The objective of this work was to characterize the antioxidant capacity (AC) and polyphenol content (PC) of leaves and edible fruit of three native species of Chile: Peumo (*Cryptocarya alba*), Arrayan (*Luma apiculata*) and Lleuque (*Prumnopitys andina*). Plant tissues were collected from Biobio and Araucania Regions. The AC and PC was measured by FRAP and Folin-Ciocalteu method, respectively. The values were compared with those from leaves and fruit of raspberry (*Rubus idaeus* cv. Heritage). The AC in leaves of Peumo, Arrayan and Lleuque was higher than that observed in fruit due to an association with great PC. Arrayan and Lleuque fruit showed an AC similar to that exhibited by raspberry fruit. Interestingly, Peumo fruit showed four-fold increase in AC compared with that of raspberry fruit. However, all native fruit showed a similar PC with that of raspberry fruit, therefore other antioxidant molecules should contribute to the high AC observed in Peumo fruit. These results indeed the high antioxidant activity of native species from Chilean forest compared to high value crop as raspberry. Further studies are underway to elucidate the presence of other antioxidant molecules in native fruit and to reveal AC and PC in other native Chilean trees.

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P 65

ANALYSIS OF XYLOGLUCAN ENDOTRANSGLYCOSILASE GENE IN TILTING *PINUS RADIATA* D. DON SEEDLING

Claudio A. Valenzuela C.[1], Patricio Ramos[1], Nicolás Cruz[1], Raúl Herrera[1]
cvalenzuela@utalca.cl

[1]Laboratory of Plant Physiology and Molecular Genetic, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca

The gravitropic response in gymnosperms induces physiologic signal, promoting stem reorientation to gravity vector, the change affect the structural component of cell wall like lignin and cellulose. From both components hemicellulose participation in gravistimulation is not well reported in gymnosperm species. Nevertheless, it is well known the importance for maintenance of cell wall architecture and remodelling, allowing cellular expansion. Xyloglucan endotransglycosylase (XTH) was found in a SSH libraries of tilting radiata pine. This enzyme takes part in the enlargement and shortener xyloglucan (XG) chains, being responsible for the incorporation of newly synthesized XG into the wall matrix. The aim of this study is the characterization of XTH gene from radiata pine in response to gravitropic effect and its hormonal regulation. The *PrXTH1* gene sequence was obtained using RACE-PCR, and promoter zone with Genome Walker. Sequences obtained were analyzed by bioinformatics tools. 60 seedlings from radiata pine were leant with an inclination of 45°. The samples were taken at 2,5, 10, 24 hours of treatment to evaluate XTH gene expression by qRT-PCR. *PrXTH1* full-length showed an ORF the 894 bp and a deduced polypeptide sequence of 298 amino acids classified in-group I in the phylogenetic Tree of related protein. A sequence of 1500 bp was obtained from the promoter region showing cis elements related to hormonal and light control. *PrXTH1* was differentially expressed across the stem, and up-regulated at 2,5 hours. Cell wall remodelling is intricate and requires several biochemical players. The key rol of XTH is discuses in this work.

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P 66

CHARACTERIZATION OF TWO TRANSCRIPTION FACTOR MADS-BOX INDUCED IN RESPONSE TO BENDING IN *PINUS RADIATA* D. DON

N. Cruz[1], P. Ramos[1], C. Valenzuela[1], R. Herrera[1]
nicruz@utalca.cl

[1]Laboratory of Plant Physiology and Molecular Genetic, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca

In nature, conifer trees develop cell lignification during the gravitropic response in the lower side of the stem. There have been described transcription factors involved in lignification but the molecular mechanisms underlies the response to lost of verticality is still unknown. The study of genes expression at late times from bend-induced radiata pine seedlings was carried out. Total RNA was extracted at late times of induced gravitropic stimuli and samples from the upper and lower half of the stem through a longitudinal cut was collected. Suppressive substractive libraries (SSH) was built, containing a total of 1453 clones with a fragment size between 300 and 1500 pb. Expression studied from SSH libraries of radiata pine have showed that several of these sequences corresponded to transcription factor with MADS-box and K-box domain. It is well known that transcription factors trigger the response at early time, therefore total RNA was extracted from the apical zone of stem at 15, 30, 60 and 120 minutes of induced gravitropic stimulus. Samples from the upper and lower half of the stem through a longitudinal cut were considered, and relative genes expression was studied by RT- qPCR. The two transcription factors showed to be differentially expressed. Full lengths of the two MADS-box using RACE 5´ y 3´ technique were obtained. Blastp analysis showed that sequences have high identity with other MADS-box proteins available in Genbank. Both PrMADBJ and PrMADBT have a full length of 166 and 193 amino acids respectively. These two MADS-boxes include 56 amino acids located in the N-terminal sequence, 9 of which are identical to all family members described so far.

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P 67

ANALYSIS OF DIFFERENTIAL GENE EXPRESSION SOS3 INDUCED BY SALT STRESS IN *DESCHAMPSIA ANTARCTICA*

Margarita Avilés[1], Daisy Tapia[1], Alejandra Sandoval[1], Manuel Gidekel[2], Ana Gutiérrez[1]
margarita.avilesmoncada@gmail.com

[1]Laboratorio de Fisiología y Biología Molecular Vegetal, Facultad de Ciencias Agropecuarias y Forestales, Departamento de Producción Agropecuaria. Universidad de La Frontera, Temuco, Chile.
[2]Venturel@b, Escuela de Negocios. Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibanez, Santiago, Chile.

Deschampsia antarctica is a poacea, one of two vascular flowering plants native to the Antarctic, known as grass antarctic or grass hairy antarctic. He lives in extreme weather conditions such as low temperatures, high salt concentrations and high UV radiation, he developed coping mechanisms to the surrounding hostile environment. These different types of stresses have an effect on gene expression, causing inhibition or overexpression of these. Such is the case the way SOS (Salt Overly Sensitive) specific route of plants in response to salt stress, varying the level of expression of the genes SOS1, SOS2 and SOS3 as a measure of resistance to salt. From the gene sequence SOS3 obtained from cDNA *Deschampsia antarctica* of Bank in Antarctic conditions, therefore seeks to assess the SOS3 gene expression by qRT-PCR technique in Deschampsia antarctica plants stressed by salt. For this analysis the plants were exposed to different concentrations of NaCl (0.25M, 0.5 M, 0.75 M) in different time periods (3, 6, 12 hours), taking samples of roots and leaves, contrasted with a control group without application of NaCl. The results show a differential expression of SOS3 gene under different conditions of stress and in different tissues tested. This differential expression allows us to understand the possible mechanism of plant response to salt stress compared in order to maintain balance and homeostasis in their natural habitat.

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P 68

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN *Deschampsia antarctica* DESV., AGAINST OXIDATIVE STRESS BY UV RADIATION

Alejandra Sandoval[1], E. Fernández[2], Manuel Gidekel[3], Ana Gutiérrez[1,3]

[1]Universidad de La Frontera

[2]Universidad Católica de Córdova

[3]Universidad Adolfo Ibañez

asandova@ufro.cl

Overexposure to ultraviolet (UV) promotes the formation of highly toxic substances called Reactive Oxygen Species (ROS). To reduce the damage produced by ROS plants have developed in the course of evolution a series of defense mechanisms. When these mechanisms are overwhelmed oxidative stress occurs which can cause serious damage even leading to cell death.

Since the discovery of so-called "hole" in the ozone layer over the Antarctic, the interest in studying the effects of UV radiation on plants has increased considerably. *Deschampsia antarctica* Desv. is the only grass specie adapted to extreme environmental conditions of the Antarctic Peninsula. Therefore, this plant can be used a model organism to study and identify the mechanisms of tolerance against UV-B radiation.

The aim of this study was to identify genes that regulate the response and tolerance of *D. antarctica* against oxidative stress induced by UV radiation. The gene expression analysis was done by means of microarray, and was validated by real-time qRT-PCR. A group of 158 differentially expressed genes (97 induced genes and 62 repressed genes) was obtained in *D. antarctica* plants growing in the Antarctic. Among the induced genes, including transcription factors, proteases, membrane proteins, defense and detoxification enzymes. Also present protein factors involved in signal transduction. On the other hand, to avoid photoinhibition Deschampsia genes suppresses the expression of components of the PSI, which is an essential process in order to grow in high light. A high percentage of the sequences analyzed (42%) corresponded to hypothetical proteins, or with unknown function. The results suggest that these genes are involved in protection against oxidative stress.

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P 69

A ROLE FOR AN ABSCISIC ACID REGULATED TRANSCRIPTION FACTOR IN THE MODULATION OF METABOLISM IN TOMATO FRUITS

Adriana Bastías[1], Rosa Argamasilla[2], Vicent Arbona[2], Aurelio Gómez-Cadenas[2], José A. Casaretto[1]
jcasaretto@utalca.cl

[1]Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Talca, Chile.
[2]Departament de Ciències Agràries i del Medi Natural. Universitat Jaume I. Castelló de la Plana, Spain.

Tomato is not only the most important horticultural crop but also is the most widely used model to study different aspects of fruit development. Phytohormones modulate growth and several changes during fruit development, thus are considered essential for the adequate completion of every stage of development. An important aspect of fruit development is the modulation and regulation of its metabolism, however, a link between hormonal signals that participate during this process and the control of gene expression involved in the modulation of metabolism is still incipient. Among plant hormones, abscisic acid (ABA) has been described to increase during maturation of several fruits. In tomato, ABA accumulates just prior the production of ethylene, suggesting that it is required for normal initiation of tomato fruit ripening. We have previously shown that a bZIP transcription factor, *SlAREB1*, which mediates ABA- and stress-regulated gene expression in tomato is also expressed during fruit development. Metabolite profiling indicated that over-expression of *SlAREB1* caused an increment of several amino acids, sugars and organic acids. To investigate other metabolic changes influenced by *SlAREB1*, a LC-MS-based metabolite profiling was performed. Compounds such as glycoalkaloids, flavonols and phenylpropanoids annotated by combining literature survey and tomato metabolite databases such as MoToDB were detected. Tomato immature green (30 DPA) and red ripe (68 DPA) fruits of wild type and trangenic plants with different expression levels of *SlAREB1* were also used to analyze changes in gene expression of putative target genes. Our results suggest that ABA signaling may be important for processes that take place during fruit ripening.

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P 70

CHARACTERIZATION OF *SLNAP1* AND *SLNAP2*, NAC-LIKE TRANSCRIPTION FACTORS ENCODING GENES DURING ABIOTIC STRESS IN *SOLANUM LYCOPERSICUM*.

Andrés Leiva[1], Camila Arellano[1], **Andrea Vega**[1]
avegac@puc.cl

[1]Departamento de Ciencias Vegetales. Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica de Chile.

The NAC family of transcription factors is an essential component in the regulation of different developmental processes, including embryo and shoot meristem development, lateral root formation and hormone signaling. In addition, these transcription factors have been associated with traits of agronomic importance such as senescence and plant response to both biotic and abiotic stress. Although the NAC family is widespread in plants but not in other eukaryotes, few *NAC* genes have been functionally 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* contain NAC-family conserved domains in their corresponding predicted proteins. Phylogenetic analyses suggest a putative orthology with NAP (NAC like activated by PI/AP3) from *Arabidopsis thaliana*. 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 different plant tissue and during senescence, as it has been previous described in other plant species. Additionally, these transcription factors were induced in leaves under salt and drought stresses. All these data indicate that these transcription factors may have a putative role in abiotic stress response inducing plant senescence. 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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P 71

EFFECT OF NACL ON PLANT GROWTH AND SOD AND APX ENZYMATIC ACTIVITY OF WILD AND CHERRY CROPPED TOMATO

María Del Pilar Acosta[1], Alejandro Antúnez[1], Ricardo Pertuzé[2], Aníbal Ayala[1], Lida Fuentes[3], Héctor Araya[4], Stanley Lutts[5], Juan Pablo Martínez[1]
jpmartinez@inia.cl

[1]Instituto de Investigaciones Agropecuarias (INIA-La Cruz/INIA-La Platina), Chorrillos Nº 86, La Cruz, Quillota, Chile/Santa Rosa 11610, La Pintana, Casilla 3, Santiago, Chile

[2]Universidad de Chile, Facultad de Ciencias Agronómicas, Santa Rosa 11315, Santiago, Chile

[3]Centro Regional de Estudios en Alimentos Saludables (CREAS), Blanco 1623, Of. 1402. Edificio Torres Mar del Sur II. Valparaíso, Chile

[4]Facultad de Química y Farmacia, Universidad de Valparaíso, Avda. Gran Bretaña 1093, Valparaíso, Chile

[5]Université catholique de Louvain, Laboratoire d'Ecologie des Grandes Culture, 2 (bte 11) Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

According to FAO, approximately 20% of the current 230 million ha of irrigated land is salt-affected. Human activities and climate change of last decades may accelerate soil salinization with broad impact on vegetable cropping. Therefore, the study of wild related species had become a modern tool to improve the genetic of traditional crops. The aim of this work was to elucidate the effect of saline stress (NaCl) on growth and superoxide dismutase (SOD) and ascorbate peroxidase (APX) enzymatic activities in vegetable tissue (roots, shoot and leaves) of two tomato genotypes: wild (*Solanum chilense Dun.*) and Cherry (*Solanum lycopersicum var. cerasiforme L.*). Both tomato species were grown in greenhouse and hydroponic system at two NaCl concentrations: 80 and 160 mM, representing two different saline stress treatments, contrarested with the control at NaCl 0 mM. Plant growth (shoots) and SOD and APX enzymatic activities were evaluated in order to understand the response of free radical oxygen detoxification within the plant. Vegetative tissues of wild and cherry tomato plants were analyzed for their antioxidant capacity by FRAP method. Cherry tomato plants showed a decrease of relative growth rate (RGR) under salinity treatments (80 and 160 mM) compared to wild genotype. Antioxidant capacity was more elevated in wild than cherry tomato. Tolerance responses to oxidative stress, evaluated by SOD and APX enzymatic activities were higher in wild tomato than in cherry under saline stress condition. In addition, the expression of SOD and APX are being evaluated. Therefore, characters related with tolerance to oxidative stress from *S. chilense* under salinity stress are interest tool to improve the commercial tomato genetic breeding programs.

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P 72

ANTIMICROBIAL PROPERTIES OF PHENOLIC COMPOUNDS FROM CHILEAN BERRIES

Víctor Polanco C[1], Loreto Espinoza S[1], Camila Martínez CH[1], Miguel Jordán Z[1], Nicole Trombert[1], Patricio Manque M[1], Noelle Blanc S[1]
victor.polanco@umayor.cl

[1]Laboratorio de Biotecnología, Centro de Genómica y Bioinformática, Instituto de Biotecnología, Universidad Mayor, Santiago, Chile

Research on the berry phenolics is a rapidly increasing area, because of the high content and diversity of phenolic compounds present in berries and their central role in diet. In fact, wild Chileans berries contain more flavonols than commonly used fruit and vegetables. Flavonoids are potent antioxidants and they inhibit lipid peroxidation and exhibit various physiological activities including anti-inflammatory, antiallergic, anticarcinogenic, antihypertensive and antiarthritic. However, little is known about your antimicrobial activities for plant and human pathogens. Therefore, the general objective of this proposal is to investigate the effects of Chilean berries (blueberry, Chilean guava, maqui, strawberry) and berry phenolics on plant and human pathogens. Antimicrobial activity of chileans berries and their phenolic extracts and purified phenolic fractions were measured against selected plant and human pathogens. Pathogenic bacterial strains, both Gram-positive and Gram-negative, were selectively inhibited by bioactive berry compounds. Berries and their phenolics selectively inhibit the growth of human pathogenic bacteria. In addition, we evaluated the effect of phenolic extraction on miceral growth and conidial morphology of Botrytis cinerea fungus. Antimicrobial properties of berries could be utilized in functional foods and plant protection. Furthermore these compounds would be of high interest for further evaluation of their properties as natural antimicrobial agents for food and pharmaceutical industry.

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P 73

FUNCTIONAL CHARACTERIZATION OF FCAAT1 (ALCOHOL ACYLTRANSFERASE) FROM CHILEAN STRAWBERRY (FRAGARIA CHILOENSIS L.(MILL).

Miriam González Rojas[1], Wilfried Schwab[2], Raúl Herrera[1], María Alejandra Moya-León[1]
mzgonzal@puc.cl

[1]Laboratorio de Fisiología Vegetal y Genética Molecular, IBVB, Universidad de Talca. Talca, Chile.
[2]Biomolecular Food Technology, Technische Universität München. Freising, Germany.

Volatile esters are flavor components of the majority of fruits. The last step in ester biosynthesis is catalysed by alcohol acyltransferase (AATs), a member of the BAHD superfamily. This enzyme transfers an acyl group from a donor to the hydroxyl, amino, or thiol group of an acceptor molecule to yield an acyl ester derivative. Due to their key role in ester biosynthesis, the activity of AAT enzyme was investigated on extracts of various fruit species, showing that AAT activity is ripening induced, and the substrate specificity of AATs towards different substrates, both alcohols and acyl-CoAs, appears to be broad. Aroma is an important attribute of the Chilean strawberry fruit. A novel alcohol acyltransferase (*FcAAT1*) gene has been identified that plays a crucial role in flavor biogenesis in *F. chiloensis*. The coding region of FcAAT1 was sub-cloned in frame with a polyhistidine affinity tag into the expression vector pET29 a(+), and introduced into *E. coli* for heterologous expression. Recombinant expression in *Escherichia coli* and assays on their ability to use different alcohols as substrates provided evidence of its functionality. The substrate specificity of FcAAT1 recombinant protein was tested in vitro by supplying a range of alcohols and acyl-CoAs and then analyzing the volatiles produced, using GC-MS. These studies have found that FcAAT1 have the ability to utilize a broad range of substrates, and that the formation of esters in fruit is subject to the availability of acyl-CoA molecules and alcohol substrates.

P 74

ISOLATION AND BIOINFORMATIC CHARACTERIZATION OF PUTATIVE PROMOTER SEQUENCES OF CELL WALL MODIFYING GENES IN *FRAGARIA CHILOENSIS*

Cristóbal Concha[1], María C. Opazo[2], Alejandra Moya-León[2], Carlos R. Figueroa[1]
carfigueroa@udec.cl

[1]Facultad de Ciencias Forestales - Centro de Biotecnología, Universidad de Concepción, Casilla 160-C, Concepción, Chile

[2]Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Casilla 747, Talca, Chile

Fruit softening in *Fragaria* species is concomitant to an increase in expression level of genes associated to cell wall modification like polygalacturonase 1 (*PG1*), pectate lyase (*PL*), endoglucanase 1 (*EG1*) and expansin 2 (*EXP2*). Regulation of gene expression is not well determined in those ripening-associated genes. With the aim to find certain *cis*-acting DNA elements located in the vicinity of these genes, we have proposed to isolate and characterize at the bioinformatic level the putative promoter sequences of *PG1*, *PL*, *EG1* and *EXP2* genes in *F. chiloensis*. A comparison between *F. chiloensis*, *F.* x *ananassa* and *F. vesca* sequences was also performed. Unknown genomic DNA sequences adjacent to the known cDNAs sequences of *F. chiloensis*' genes were amplified by PCR reactions using the GenomeWalker Kit and Advantage 2 Polymerase (both from Clontech). PCR products were cloned and sequenced. Putative *cis*-acting elements in promoter sequences were analyzed using the PlantCARE bioinformatic tool. Putative promoter sequences of 1038, 339, 837 and 847 bp were obtained for *PL*, *PG1*, *EG1* and *EXP2* genes, respectively. The bioinformatic analysis reported the presence of putative responsive promoter elements to several signals such as hormones, light and stress-related. The abscisic acid response element and a MYB binding site were abundant motifs found in *PL*, *EG1* and *EXP2* promoter sequences. The comparison of putative promoter regions isolated in *F. chiloensis* with equivalent *F.* x *ananassa* and *F. vesca* sequences revealed differences in some hormone-responsive elements. Further research is needed to improve the characterization of these promoter regions in *F. chiloensis*.

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P 75

EFFECT OF POSTHARVEST TREATMENT OF CALCIUM AND AUXINS ON EXPRESSION OF CELL WALL-MODIFYING GENES IN THE *FRAGARIA CHILOENSIS* FRUIT

Mariana Díaz[1], Patricia Vera[1], Osvin Arriagada[1], María C. Opazo[2], Alejandra Moya-León[2], Carlos R. Figueroa[1]
carfigueroa@udec.cl

[1]Facultad de Ciencias Forestales - Centro de Biotecnología, Universidad de Concepción, Casilla 160-C, Concepción, Chile

[2]Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Casilla 747, Talca, Chile

Chilean strawberry (*F. chiloensis*) is a non-climacteric fruit with a high softening rate that contributes to its perishability during storage. Previous reports have indicated the repressor role of the synthetic auxin naphthalene acetic acid (NAA) in the expression of cell wall-modifying genes during ripening of strawberry fruit. On the other hand, the conservation of cell wall integrity of fleshy fruit by calcium treatment is well documented, although its role at the gene expression level has not been well elucidated until now. In order to determine the effect of calcium and auxins on gene expression during postharvest, ripe fruit was dipped in solutions containing $CaCl_2$, NAA, or a combination of both. Fruit was cold stored and samples were evaluated at 0, 2, 5 days, and after 8 days plus two days at room temperature. We evaluated the transcriptional levels of six cell wall-modifying genes and two genes related to calcium and auxin response. Our results indicated that after cold storage, the combined treatment produced a low expression level in almost all genes. This was particularly evident in polygalacturonase (*PG1*), pectate lyase (*PL*) and endoglucanase (*EG1*) genes suggesting that both signals could repress expression of important genes related to strawberry fruit softening. Conversely, after cold storage calcium-treated fruit showed up-regulation of pectin methylesterase (*PE1*) and calcium sensing receptor (*CAS*) genes, as well as auxin with xyloglucan endotransglucosylase/hydrolase (*XTH1*) gene. We conclude that complex alterations on gene expression could be observed in calcium- and auxin-treated fruit after eight days of cold storage plus two days of shelf life at room temperature, suggesting that the down-regulation of key softening genes like *PG1*, *PL* and *EG1* could be important in maintaining the quality of strawberry fruit during shelf life.

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P 76

ISOLATION AND CLONING OF CDNA HOMOLOGOUS TO CALMODULIN GENE DIFFERENTIALLY EXPRESSED UNDER ALUMINUM-STRESS IN HIGHBUSH BLUEBERRY

Claudio Inostroza-Blancheteau[1], Felipe Aquea[2], Marjorie Reyes-Diaz[2], María De La Luz Mora[3], Miren Alberdi[3], Patricio Arce-Johnson[2]
parce@bio.puc.cl

[1]Center of Plant, Soil Interaction and Natural Resources Biotechnology, (BIOREN) Universidad de La Frontera, Temuco, Chile.

[2]Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.

[3]Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Temuco, Chile.

Aluminun (Al) toxicity is one of the major factor that limit the productivity and quality of crops in acid soils of Southern Chile. In these soils the major symptom of Al phyto-toxicity is a rapid inhibition of root growth. Currenlty, the most preponderant fruticultural activity in this region is the blueberry production (*Vaccinium corymbosum L.*). Several Al-regulated genes have been identified in the roots of different plant species. To investigate the molecular bases of Al toxicity and Al tolerance of blueberry, cDNA-amplified fragment length polymorphism (cDNA-AFLP) was used for identifying Al-regulated genes in roots of an Al-resistant genotype, Brigitta, and an Al-sensitive, Bluegold. One year old plants were transferred to hydroponic medium supplemented with 0 and 100 µM of AlCl3. Root samples were taken between 0 to 48 h of treatment. The transcript-derived fragments (TDFs) were named VCAL by (*Vaccinium corymbosum Aluminum*). The TDF-VCAL19 was selected, sequenced and their homologies compared in the databases. The expression this TDF was confirmed by real time (qRT-PCR). VCAL19 of 511 nucleotides and with BLAST score of 2e-138, was homologous to calmodulin gene (*CaM*). We cloned and characterized this gene using Rapid Amplification of cDNA ends (RACE-PCRs) to obtain full length cDNAs of 790 pb and an Open Reading Frame (ORF) 447 pb, encoding a protein of 140 amino acid. Alignment of the deduced amino acid sequence was compared with other plant species and the phylogenetic tree was performed, being showed an 99% identity with *CaM 202* of *Daucus carota*. Molecular characterization might explain the involvement of this gene in resistance to Al in the roots of blueberry.

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P 77

EPSPS EXPRESSION ANALYSIS IN A CHILEAN L. MULTIFLORUM BIOTYPE RESISTANT TO GLYPHOSATE HERBICIDE, USING REAL-TIME PCR.

R Galdames G[1], Javier Río A[1], Jorge Días S[1]
rgaldame@inia.cl

[1]Unidad de Biotecnología, Instituto de Investigaciones Agropecuarias (INIA)

Since its commercial introduction in 1974, glyphosate has become the dominant herbicide worldwide. In plants the glyphosate is toxic because inhibits the enzyme 5 enolpyruvylshikimate-3-phosphate synthase (EPSPs), resulting in shikimate accumulation and reduced production of aromatic amino acids. In glyphosate resistant-weeds two main resistance mechanism are the reduced glyphosate translocation (nontarget site–based) and/or the mutation in the EPSPs gene (target site-based). Recently, gene amplification has been identified as an additional resistance mechanism in *Amaranthus* palmeri biotype which is positively correlated with increase in EPSPs cDNA expression level and EPSPs protein activity. Several glyphosate resistant ryegrass biotypes (*L. multiflorum*) have been identified in Chile. Gene amplification has not been explored as a possible mechanism to explaining herbicide resistance in this weed. Seeds from resistant (R biotype) and susceptible (S biotype) plants were germinated and transplanted into pots for growth in a greenhouse. At 3-5 leaf stage (5 weeks post emergence), plants were sprayed with 540 g a.e./ha of glyphosate. Similar number of plants (R and S biotype) were sprayed with water and used as a control. At 24, 48, 72 and 96 hours after treatment (HAT), leaf tissue were sampled for shikimate measurement and total RNA extraction to measure EPSPS cDNA expression level. When treated with glyphosate, susceptible (S) plants at 24 to 96 HAT accumulated 4 to 16 fold more shikimic acid than resistant (R) plants, indicating that the EPSPs was inhibited, whereas the R plants did not accumulated shikimate, indicating that EPSPs was still functioning. Using quantitative RT-PCR, preliminary data shows that S and R biotypes did not differ in EPSPs expression relative to the housekeeping genes eEF1A and YT521-B, indicating that the gene amplification mechanism is not operating in the R biotype studied.

P 78

MOLECULAR CHARACTERIZATION OF *VVMYBA1* IN THE NEW TABLE GRAPE VARIETY PINK GLOBE

Claudia Santibáñez-Orellana[1], Elizabeth Torres[2], Alfredo Chimenti[3], Patricio Arce-Johnson[1]
ctsantib@uc.cl

[1]Pontificia Universidad Católica de Chile
[2]Universidad de Chile
[3]AGRIFRUTA S.A

Vitis vinifera is one of the most important fruit species in the country used for wine and table grapes production. Berry color results from the biosynthesis and accumulation of anthocyanins in the berry skin, processes that are commonly regulated by transcription factors belonging to the MYB and bHLH families in plants. *VvmybA1* gene is a major determinant of berry color variation in table grape and its instability is the major cause of somatic variation for this trait. The accumulation of anthocyanins in the berry skin is very important for the production of red wine and black and pink table grapes. Pink Globe variety was generated by spontaneous mutation of Red Globe variety in field. This new variety is characterized by reduced accumulation of anthocyanins in the berry skin producing red/pink fruits, very popular in Asian markets. Molecular markers based on DNA analysis described for grapes did not allow differentiation between both varieties, suggesting punctual mutations involved in the generation of this new variety. In the present study we characterized *VvmybA1* gene of Pink Globe variety and compared it with the same gene from Red Globe. By DNA sequencing we found two types of *VvmybA1* genes in Pink Globe: one is similar to the Red Globe gene, presenting SNPs in its sequence, which are part of the inherent variability of myb genes. The other type has a base insertion in exon 3 of *VvmybA1*, resulting in a change in the reading frame which generates an early stop codon, leading to the translation of a truncated protein. These results suggest a possible explanation for the differential accumulation of anthocyanins in the fruit. Sequencing of the promoter region of *VvMybA1* from both varieties may also explain this differential accumulation of anthocyanins, which will be discussed in this work.

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P 79

USE OF *ACO* GENE EXPRESSION AS A MARKER FOR THE DETECTION OF VERAISON STAGE IN TABLE GRAPES

Bruno G. Defilippi[1], **Pablo Muñoz-Robredo**[1], Mauricio González-Agüero [1], Christian Chervin[2]
bdefilip@inia.cl

[1]Instituto de Investigaciones Agropecuarias, INIA-La Platina, Unidad de Postcosecha, Casilla 439-3, Santiago, Chile.

[2]Université de Toulouse, UMR990, Genomique et Biotechnologie des Fruits, INRA/INP-ENSAT, BP 32607, 31326 Castanet-Tolosan, France

Veraison is a key developmental stage in grapes, as most of the compositional changes that determine quality are triggered in this stage. However, due to the large number of grape varieties and the effect of internal and external factors, it is a challenge to identify when veraison occurs during development. Recently, it was demonstrated that ethylene biosynthesis was up-regulated at veraison. In this work, we evaluated the potential use of the expression of ACO genes as an indicator of veraison. Experiments were performed using three varieties of table grapes (Vitis vinifera L.) that were sampled weekly, from the early stages of fruit development until commercial maturity. Three *VvACO* genes were characterized and their expression was quantified. In three table grape cultivars (Thompson and Crimson seedless and Red Globe), the *VvACO1* accumulated at higher levels, in comparison to *VvACO2* and *VvACO3* , and in all three cultivars the *VvACO1* transcripts accumulated around the veraison stage, at levels four to five-fold higher than at the harvest stage. This *VvACO* transcript accumulation around veraison has also been observed by other teams in France and Australia. However, the *VvACO1* transcript peak at veraison was more obvious in Thompson and Crimson seedless grapes and concomitant with the characteristic changes observed at veraison, especially in terms of the sugar accumulation rate. Therefore, *VvACO1* expression seems to be a suitable measurement for identifying veraison in table grape; however, there are differences at the genotypic level that need to be investigated further

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P 80

FACTORS AFFECTING L-IDONATE DEHYDROGENASE GENE EXPRESSION: A KEY STEP IN TARTARIC ACID BIOSYNTHESIS DURING TABLE GRAPE (*VITIS VINIFERA L.*) DEVELOPMENT

Cinthya Araneda[1], Bruno G. Defilippi[2], Mauricio González-Agüero[2], Pablo Muñoz-Robredo[2]
bdefilip@inia.cl

[1]Facultad de Ciencias Biológicas, Escuela de Ingeniería en Biotecnología, Universidad Andrés Bello.
[2]Instituto de Investigaciones Agropecuarias, INIA-La Platina, Unidad de Postcosecha, Casilla 439-3, Santiago, Chile.

Flavor is the most important quality attribute determining consumer preference in fruit, including table grapes. This organoleptic property is directly influenced by the content and composition of sugars and organic acids that are determined by the interaction between the genetic background of the cultivar and environmental factors. In grapes, tartaric acid is one of the most abundant organic acid in the berry, and it has been observed that an important step in the synthesis of tartaric acid is related to the L-idonate dehydrogenase (*L-idnDH*) enzyme; however, the molecular changes underlying this process for table grapes are unknown. 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 grown under different environmental conditions. To correlate the gene expression levels with tartaric acid content in the berry, we quantified the organic acid profile from early stages of fruit during development. To determine the influence of ethylene in tartaric acid biosynthesis we performed a trial by applying an ethylene enhancer and an ethylene inhibitor at veraison. As previously shown, within the organic acid profile malic acid was the predominant one close to the veraison stage, only been overcome in later stages of development by tartaric acid. In addition, it was possible to define that the expression profile of VvL-idnDH was concomitant to the concentration of tartaric acid. Relative to ethylene, the concentration of tartaric acid was decreased by stimulating ethylene production through ethephon applications. The significance of these results from table grape is discussed in this work.

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P 81

EXPRESSION ANALYSIS OF NUCLEOTIDE-SUGAR TRANSPORTERS IN GRAPEVINES (*VITIS VINIFERA* L.)

Daniella Utz[1], Michael Handford[1]
daniutz@ug.uchile.cl

[1]Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

All plant cells are surrounded by the cell wall, mainly composed of cellulose and non-cellulosic polysaccharides. Cellulose synthesis occurs at the plasma membrane, whereas the non-cellulosic polysaccharides are synthesised in the Golgi apparatus. In this organelle, the glycosylation reactions are catalysed by glycosyltransferases, that recognise specific nucleotide-sugars (NDP-sugar) and transfer the sugar molecule to glycan acceptors. Most nucleotide-sugars, are synthesised in the cytosol and their mechanism of entry into the Golgi lumen is via nucleotide-sugar transporters (NSTs). In *Arabidopsis thaliana*, the GONST1-5 family of NSTs specific for GDP-sugars and localised in the Golgi. Therefore, the GONST family is involved in the import of GDP-sugars into the Golgi lumen for the subsequent decoration of glycans. In grapevine (*Vitis vinifera* L.), it has been determined that the non-cellulosic polysaccharides contain sugars derived from GDP-sugars. To determine the conservation of the mechanism involved in the synthesis of non-cellulosic polysaccharides, the grapevine genome was analysed bioinformatically for the presence of GONST orthologues. Two sequences with 78% identity at the amino acid level to GONST proteins were identified, both of which possess the molecular characteristics of NSTs of GDP-sugars. We have called these orthologues VvGONST-A and VvGONST-B. The Cloning of both NSTs and their expression pattern in different grape organs using RT-PCR was performed successfully. Finally, *Nicotiana tabacum* leaves will be transiently transformed with VvGONST-A and VvGONST-B promoter::GFP fusion construct in order to determine the cellular localization.

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P 82

STUDIES ON THE EXPRESSION REGULATION OF A PUTATIVE TONOPLAST MONOSACCHARIDE TRANSPORTER VVTMT2 IN *VITIS VINIFERA*

Elizabeth Torres[1], Claudia Santibañez-Orellana[2], Patricio Arce-Johnson[2]
elixita.tp@gmail.com

[1]Universidad de Chile
[2]Pontificia Universidad Católica

Berries development in grapevine (*Vitis vinifera*) is characterized by a massive accumulation of sugars in the mesocarp and phenolic compounds in the skin, two processes that occur principally from veraison to maturity. During maturation, monosaccharides are transported from apoplast to the cytoplasm of mesocarp cells by hexose transporters (VvHTs) and then stored in the vacuole. Several of the hexose transporters localized in the plasmatic membrane have been studied, they are functional and their expression is highly regulated by sugars. However very little is known about the monosaccharide transporters localized in the tonoplast. Three ORFs of putative tonoplast monosaccharide transporters were found in the Vitis genome and called VvTMTs. These three show an extended middle loop between the putative trans-membrane helices six and seven in a similar way as *Arabidopsis thaliana* transporters AtTMTs. VvTMT1 has been cloned and characterized and it was observed that its expression decrease with berry development, while VvTMT2 remains uncharacterized. Macroarrays experiments show that VvTMT2 expression increases during fruit development, at the same time that accumulation of sugars starts, therefore we hypothesize that VvTMT2 expression is regulated by sugars. We collected berries at three stages of development and extracted RNA from the pulp. We perform a RT-qPCR analysis and confirm that VvTMT2 gene expression increases with maturity. We performed a bioinformatics analysis of 2kb of VvTMT2 promoter sequence. Cis-elements involved in sugar regulation were over-represented in this sequence: MYBGAHV, SURE1, W-Box, AMYBOX1 and PIRYMIDINEBOX. To check the functionality of these elements we have cloned 1.8Kb of VvTMT2's promoter to direct gus expression. This construction will be transformed in *Arabidopsis thaliana* and then different concentration of sugars will be tested in hydroponic culture. We expect to see an increase in gus expression with sugars concentration.

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P 83

CLONING AND PARTIAL CHARACTERIZATION OF TWO PUTATIVE SODIUM TRANSPORTERS FROM VITIS VINIFERA CV. THOMPSON SEEDLESS.

Carlos Aguirre[3], Andrés Zurita[2], Humberto Prieto[1]
caguirre.d@gmail.com

[1]Laboratorio de Biotecnología, INIA La Platina, Chile.
[2]Centro de Estudios Avanzados en Zonas Áridas- CEAZA, Chile.
[3]Programa de Doctorado en Cs. Silvoagropecuarias y Veterinarias, Universidad de Chile.

Salinity and drought are major limiting factors for crop productivity. Although sodium is required by some plants, high concentrations result harmful due to osmotic and ion-specific effects. Plants have different mechanisms to overcome these stresses, including low cytosolic Na+ concentrations maintenance and high intracellular K+/Na+ rates. These housekeeping functions involve Na+ extrusion and vacuolar compartmentalization coupled to a H+ electrochemical gradient. In that way, most of the incoming Na+ into the root cells is pumped back out by plasma membrane Na+/H+ antiporters like *A. thaliana* SOS1. The sodium fraction that cannot be excluded is compartmented into the vacuole by tonoplast Na+/H+ antiporters, such as the *A. thaliana* NHX1. In grapes, cation/H+ VvNHX1 has been the only cloned and characterized antiporter from the NHX family. This K+/Na+ transporter has low affinity and its expression partially complements the salt sensitive phenotype of the *ena1-ena4 nhx1* yeast mutant. Meanwhile, there are not reports about the SOS family in grapes. In this work cloning and partial characterization by bioinformatics tools of two new putative grape sodium transporters, which are possibly involved in sodium vacuolar compartmentalization (VvNHX2) and extrusion (VvSOS1) are described. Characterization is additionally discussed in order to use these genes as biotechnological tools to generate new grapevine materials with enhanced tolerance against these abiotic stresses.

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P 84

SEARCHING FOR THE MOLECULAR BASIS OF "CHILLING REQUIREMENT" CONCEPT IN TEMPERATE CLIMATE DECIDUOUS FRUIT-TREES: EXPRESSION ANALYSIS OF ENZYMES INVOLVED IN STORAGE CARBOHYDRATE METABOLISM IN GRAPEVINE-BUDS.

Amanda Donoso[1], Ricardo Vergara[1], Francisco Pérez[1]
frperez@uchile.cl

[1]Universidad de Chile, Facultad de Ciencias, Laboratorio de Bioquímica Vegetal.Casilla 653. Santiago. Chile.

Temperate zone deciduous fruit trees need a period of exposition to winter chilling for blooming well in spring. This is known as chilling requirement and is genetically determined for each species. This chilling accumulation period has also been linked to the release of buds from endodormancy. However, when buds of deciduous fruits are exposed to low temperatures also suffer a process of acclimation and deacclimation to freezing. Previous reports indicate that carbohydrate metabolism is associated with acclimation process of buds to chilling. In this work, we studied the expression pattern of genes coding for isoforms of amylase from endodormant buds, under different times of exposure to cold. The results indicated that at least two of the amylase genes expressed in grapevine-buds, VvAMY2 and VvAMY4 (beta- and iso- amylase, respectively), are strongly induced by chilling during the first 15 days of exposure, but later at 21 and 44 days of exposure, their expressions are strongly suppressed. The drastic change in the expression of these genes in grapevine-buds during chilling accumulation period, suggests that it could be related to the satisfaction of the chilling requirement, necessary for subsequently resuming growth in *Vitis*.

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P 85

HYPOXIA, HYDROGEN PEROXIDE AND ETHYLENE INDUCE DIFFERENTLY THE EXPRESSION OF THE TRANSCRIPTION FACTORS VVERF1, VVERF2 AND VVEIN3 WHICH IN TURN CAN MEDIATE THE RESPONSE OF ANTIOXIDANT AND ALTERNATIVE RESPIRATORY GENES IN GRAPEVINE-BUDS

Francisca Parada[1], Ricardo Vergara[1], Francisco Pérez[1]
frperez@uchile.cl

[1]Universidad de Chile, Facultad de Ciencias, Laboratorio de Bioquímica Vegetal. Casilla 653 Santiago. Chile

In grapevine buds (cv. Thompson seedless), transcription factors linked to the ethylene signaling pathway VvERF1, VvERF2 and VvEIN3 were differentially induced by hypoxia, hydrogen peroxide (H2O2) and ethylene. So while VvERF1 was induced by the three stimuli, VvERF2 was mainly induced by hypoxia and VvEIN3 by ethylene. Moreover, these three stimuli strongly induced the expression of genes encoding antioxidant enzymes such as ascorbate peroxidase (VvAPX), glutathione peroxidase (VvGLPX), superoxide dismutase (VvSOD) and catalase (VvCAT) and enzymes of the alternative respiratory pathway, type II NAD(P)H-oxidase resistant to rotenone (VvaND) and alternative oxidase (VvAOX), all of which are part of the defense mechanisms of plants against oxidative stress. Surprisingly, gamma-amino butyric acid (GABA), repressed the expression of genes coding for the transcription factors mentioned above and for antioxidant and alternative respiratory enzymes. Based on these results, we suggest that the above transcription factors may be important regulators of the antioxidant response in grapevine buds.

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P 86

IDENTIFICATION AND CHARACTERIZATION OF GENES RELATED TO EMBRYO DEVELOPMENT IN TABLE GRAPE (VITIS VINIFERA L.)

Miguel García-Rojas[1], Patricio Hinrichsen[1], Mauricio González-Agüero[1]
miguel.garcia.rojas@gmail.com

[1]Instituto de Investigaciones Agropecuarias (INIA) – CRI La Platina

Table grape (Vitis vinifera L.) is the most important species exported by the Chilean fruit industry. Seedlessness and berry size are key quality parameters for fresh consumption. Although progress has been made in understanding the molecular basis of stenospermocarpy, the biochemical and molecular basis underlying this process are poorly understood. To contribute to this understanding, several genes were identified by an integrative approach that includes a quantitative trait locus (QTL) mapping and a high-throughput sequencing of cDNA (RNA-Seq). For both analysis we used a reference population originated from a 'Ruby Seedless' x 'Thompson Seedless' crossing, which included contrasting phenotypes, i.e. seeded and seedless segregants, with large and small berries and different stages of development (flowering, fruit setting and 6-8 mm), treated or not with gibberellic acid. In this work, we cloned and characterized the expression pattern of five genes encoding putative proteins related to embryo development, such us: embryonic factor 1 (FAC-1), leafy cotyledon 1 (LEC-1) and embryo defective (EMB), dicer-like 1 (DCL-1) and altered meristem program gene (AMP-1). The expression profile was characterized by real-time PCR assays, performed in three different development stages and in four contrasting phenotypes (three individuals each) related to seed and berry size. As berry development progressed, larger changes in the transcript levels were observed for most of the analyzed genes; the significance of these changes during table grape berry development will be discussed.

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P 87

STUDY OF IN VITRO GERMINATION OF *UGNIMOLINAE* TURCZ SEEDS

Arturo Morales[1,2], Ivette Seguel[2], Lorena Díaz[2], Elizabeth Carihuentro[2], Mercedes Castro[2]
iseguel@inia.cl

[1]Instituto de Investigaciones Agropecuarias (INIA) Carillanca, Temuco, Chile
[2]Escuela de Agronomía, Facultad Recursos Naturales, Universidad Católica de Temuco (UCT), Temuco, Chile

The process of domestication of *Ugnimolinae* T., has meant among other things develop a genetic improvement program. One of the difficulties of this program is to ensure adequate germination for seeds from crosses made. The objective of this research is optimizing through in vitro methods, the germination rate seeds. Using seeds from three genotypes fruits of the last season, an experiment was made with different basal mediums (Murashige & Skoog, 1962 (MS); MS + Fluridone (FLU); Agar-Water, Agar Water + FLU) and different dark incubation periods (0, 1, 2, 3 and 4 weeks). Agar 7g l-1 was added to all the media. pH of media was adjusted at 5,8 prior to sown. Each treatment had 4 repetitions with twenty five seed each. After the dark incubation period the seeds were incubated at photoperiod of 16/8 (Light/Darkness) and at 22 °C for 30 days. The utilization of Fluridone (1-methyl-3-phenyl-5-[3-triflurомethyl (phenyl)]-4-(1H)-pyridinone) as supplement for the basal mediums, it was made for studied the effect as inhibitor of the hormone that has a role on the induction and maintenance of seed dormancy, absisic acid (ABA). The differences on seeds germination rates obtained with the different basal mediums and dark incubation periods are highly significant, the treatment which has the highest rates of germination is the basal medium Agar- Water + FLU under 1 week of darkness, 40, 43 and 77% for genotypes 1,2 and 3 respectively.

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P 88

EFFECT OF MATERNAL NUTRIENT ENVIRONMENT OF LITHOSPERMUM ARVENSE ON OFFSPRING GERMINABILITY

Mercedes Longás[1], Guillermo Chantre[1], Mario Sabbatini[1]
mmlongas@criba.edu.ar

[1]Universidad Nacional del Sur, Departamento de Agronomía, CERZOS

Lithospermum arvense is a weedy annual species of winter cereal crops of the south-west area of Buenos Aires, Argentina. Environmental factors may change maternal influence on seed development having consequence on the variance of fitness components such as seed dormancy. The aim of this work was to investigate the effect of soil nitrogen level on the maternal environment on seed dormancy of the progeny. Plants of L. arvense were fertilized with 0, 50 and 100 Kg N/Ha (urea 46%N) applied at emergence and vegetative stages. Freshly matured seeds (F1) were stored until 3720°C.day and 7719°C.day of after-ripening. Germination trials were carried out in a growth chamber under a constant thermal regime (15°C). Seeds were incubated in 9-cm Petri dishes containing soil with different nitrogen enrichment levels (equivalent to 0, 25, 50, 100 and 200 Kg N/Ha). Final germination percentages were recorded after a 21-day incubation period. No interaction was found (p=0.96) among the evaluated factors (level of maternal fertilization*nitrogen incubation medium enrichment level*after ripening time). The effect of nitrogen fertilization level on the maternal environment produced a closely significant effect on the germinability of the progeny (p=0.06), irrespective of soil nitrogen level of the incubation medium. No statistical differences were observed on seed germinability between after ripening time periods. Further studies should be conducted in order to evaluate possible fitness effect of nutrient maternal environment on futures generation (F1, F2, F3).

P 89

IDENTIFICATION AND MOLECULAR CHARACTERISATION OF NATIVE FUNGAL *TRICHODERMA* SPECIES AND THEIR AFFECT ON PLANT GROWTH

Romina Almasia[1], Gabriel Pérez[2], Pedro Castillo[2], Margarita Carú[3], Michael Handford[1]
mhandfor@uchile.cl

[1]Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile

[2]Biopacific, La Cisterna, Santiago y Laboratorio de Microbiología y Ecología Microbiana, Facultad de Ciencias, Universidad de Chile

[3]Laboratorio de Microbiología y Ecología Microbiana, Facultad de Ciencias, Universidad de Chile

Some species of the fungal genus *Trichoderma* establish biological interactions with various micro and macroorganisms. Some of these interactions have potential biotechnological applications, such as in the biostimulation of plants. The objective of our study was to prospect, identify and molecularly characterise a set of native strains and then, using *Arabidopsis thaliana*, analyse the effect of each strain as a biostimulating agent. To prospect native strains, samples of *Trichoderma*-like fungi were isolated from rhizosphere plant material and soil from south-central Chile and Patagonia. Initially, the fungi were identified morphologically, resulting in 15 strains with asexual characters consistent with those described for *Trichoderma*. For a more precise identification of isolates, molecular markers such as ITS1 and ITS2, tef1, cal1 and chi18-5 (formerly chi42) were sequenced and the phylogenetic relationships were analysed using the following programs; Mr. Bayes, MEGA 5, GeneDoc and Dendroscope. Furthermore, the sequences were analysed by DNA barcode tools (www.isth.info). These studies confirmed that the isolates were indeed *Trichoderma* species. To determine their promoting effect on plants, *Arabidopsis* seeds were grown on inclined agar plates containing MS medium, and 4 days after germination, the seedlings were exposed to each strain. The fungal spores were placed at 5 cm from the primary root tip. After 6 days of growth in presence of the fungus, it was observed that the inoculation of each strain stimulated lateral root formation and biomass production (fresh and dry weight) in a strain-specific manner. Summarising, we identified a set of native strains of *Trichoderma* with a variety of biostimulating properties. It has been previously described that IAA-related indoles produced by *Trichoderma* have a stimulatory effect on plant growth. Therefore, in order to investigate the process involved, the fungal culture extract will be evaluated, as well as the expression of genes related to an auxin response.

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P 90

SEED GERMINATION RESPONSES OF FOUR ALGARROBO (PROSOPIS CHILENSIS (MOL.) STUNTZ) ACCESSIONS UNDER DROUGHT CONDITIONS.

Rodrigo Muñoz[1], Paloma Gachón[1], Claus Westphal[1], Carlos Navarrete[2], Jaime Bravo [3], Cristian Ibáñez[1]
cibanez@userena.cl

[1] Departamento de Biología. Facultad de Ciencias. Universidad de La Serena. La Serena. Chile
[2]Departamento de Matemáticas. Facultad de Ciencias. Universidad de La Serena. La Serena. Chile
[3]Centro de Estudios Avanzados en Zonas Áridas (CEAZA). La Serena. Chile

In the unirrigated regions, shortage of rain is one of the major problems in the development of agriculture. This climatic condition prevents plants to have a normal development and makes it harder for farmers to improve productions. Our research is focused in searching for genes associated with this abiotic stress in order to transfer them to important agriculture crops of arid zones. Our model plant is Algarrobo (Prosopis chilensis (Mol.) Stuntz), a tree adapted to this arid conditions. Previous to gene selection, relevant physiological aspects of the tree must be known in order to determine the best accession. In this work, we show seed germination performance of four (4) accessions of Algarrobo's under drought stress conditions. Two accessions represent the most northern distribution and the others two represent the most southern distribution. To generate drought stress, we supplied MS media with several concentrations of PolyEthylene Glycol (PEG) and Mannitol. For PEG, petri dishes contained 0,006M; 0,009M; 0,012M; 0,015M or 0,018M. For Mannitol, concentrations tested were 0,1M; 0,2M; 0,3M and 0,4M. Germination rates and roots elongation were measured daily. Preliminary results show that during first three weeks for both PEG and Mannitol, no significant differences on seed germination rates among accessions were observed. However, after the third week, drought stress generated by higher PEG and Mannitol concentrations provoked a slowing-down in root elongation and root thickness compared with controls, for almost all accessions. Further methodological approaches, results and conclusions obtained in this genetic background under drought stress conditions are discussed.

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P 91

SEED GERMINATION RESPONSES OF TWELVE CHILEAN ALGARROBO (PROSOPIS CHILENSIS (MOL.) STUNTZ) ACCESSIONS UNDER IN VITRO SALINE CONDITIONS

Claus Westphal[1], Paloma Gachón[1], Rodrigo Muñoz[1], Carlos Navarrete[2], Jaime Bravo [3], Cristian Ibáñez[1]
cibanez@userena.cl

[1]Departamento de Biología. Facultad de Ciencias. Universidad de La Serena. La Serena. Chile
[2]Departamento de Matemáticas. Facultad de Ciencias. Universidad de La Serena. La Serena. Chile
[3]Centro de Estudios Avanzados en Zonas Áridas (CEAZA). La Serena. Chile

Arid zones are the most difficult environment for plants development. In this habitat, abiotic stresses (mainly salinity, water shortage and poor soils) can limit plant growth and reduce vegetal production. Among abiotic stresses, salinity (high content of salts in the soil) is one of the most harmful. Salinity reaches almost 50% of the arable land in the world and in Chile, salinity is a latent problem between Arica/Parinacota to El Libertador Bernardo O'Higgins Regions. Among plants growing naturally in these saline environments, native trees represent an interesting model plant for research in this context because they have evolved many physiological adaptation mechanisms like deep root system, efficient methods to move salt from soil to surface and alternative seed germination strategies. For this study, we have chosen the Algarrobo (Prosopis chilensis (Mol.) Stuntz), an important ecologic, economic and cultural tree for the arid and semi-arid zones of Chile. As a first evaluation, we have studied one of the most sensitive stages in the life's plant which is particularly affected by saline environments, namely seed germination. We have analyzed twelve (12) Algarrobo accessions collected in a latitudinal transect ranging from Coquimbo to Metropolitan Regions. Experimental results under several saline concentrations ranging from 0 to 600mM NaCl have shown germination for all accessions up to 300 mM NaCl. However, at 450 and 600 mM NaCl, seed germination showed significant differences among accessions, suggesting that an important genetic component might be present among them. Further methodological approaches, results and conclusions obtained in this genetic background under saline environments are discussed.

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P 92

EFFECT OF LOW CONCENTRATION OF OXYGEN ON ROOT OF CHERRY ROOTSTOCKS.

Guillermo Toro[1], Michelle Morales[1], Alejandro Riquelme[1], Manuel Pinto[2]
mpinto@inia.cl

[1]Centro de Estudios Avanzados en Fruticultura. Rengo. VI Región.
[2]Instituto de Investigaciones Agropecuarias, CRI La Platina.

The anaerobiosis on rhizosphere affects drastically the plant respiratory metabolism causing oxidative stress in root plant. Also morphological modification and changes to chlorophyll content could be observed. It has been reported the existence of variability in the response of cherry rootstocks to anaerobiosis. However, it is unclear response of rootstocks against this stressful condition. The present work evaluated the respiratory response and oxidative damage of cherry rootstock in low oxygen condition. Two cherry rootstock varieties, Colt and Mazzard F-12, were subjected to 8mgO2 L-1 (normoxia) and 2mgO2 L-1 (hypoxia) for 5 days. The rootstocks were growth in a greenhouse under hydroponic system and with photoperiod 16/8 h. Low oxygen concentration was obtained through the N2 supplied (100mL min-1). Oxygen consumption was measured on root tips by Clark-type oxygen electrode. The chlorophyll content was obtained through SPAD index. Root tissue samples were stored at -80°C until the analyses of lipid peroxidation by MDA method and the observation of hypertrofic lenticels by using a stereoscopic microscope. Both rootstocks showed differences in oxygen consumption during stress condition. Oxygen consumption rate decreased in Colt and Mazzard during the hypoxic period (197 and 82,82 umolO2 min-1 g-1 DW, respectively). After 5 days the oxygen consumption of hypoxia-treated Colt was 90% lower than control rootstocks and for stressed Mazzard F-12 was 67%. After three days, the roots under hypoxic condition reached the maximum content of MDA, this suggests that they were in the peak of oxidative damage. Oxygen consumption correlated negatively with lipid peroxidation of rootstock for both cherry varieties, suggesting that the level of lipid peroxidation was involved in regulation of root respiration. Chlorophyll content was significatively lower in hypoxic-treated Colt respect to control rootstock. Hypertrofic lenticels were observed in both oxygen conditions, this result suggested that this morphologic modification is not associated to hypoxia.

CONICYT y GORE, VI región, Chile

P 93

COMPARISON OF EXPRESSION PROFILES OF ANTIOXIDANT SYSTEM IN *PRUNUS* ROOTSTOCKS UNDER HYPOXIA STRESS

Paula Pimentel[1], Rubén Almada[1], María José Arismendi[1], Boris Sagredo[1], Manuel Pinto[1]
ppimentel@inia.cl

[1]Centro de Estudios Avanzados en Fruticultura (CEAF_R08I1001), INIA CRI Rayentué, Rengo, Chile.
[2]Programa Doctorado en Cs. Mc. Biología Celular y Molecular Aplicada. Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera, Temuco, Chile.
[3]INIA CRI La Platina, Santiago, Chile.

A well-oxygenated root-zone environment is essential for a healthy root system, but frequently they experience root-zone hypoxia mainly due to soil waterlogging, soil compaction, over-irrigation or poor drainage. Exposure of plants to most adverse conditions causes oxidative stress, which affects plant growth due to the production of reactive oxygen species (ROS) such as superoxide radicals, singlet oxygen, hydroxyl radicals and hydrogen peroxide. These ROS are all very reactive and cause severe damage to membranes, DNA and proteins. To counteract the toxicity of ROS and protect the cells, a complex antioxidant defense system, composed of both antioxidants like ascorbate (AsA), glutathione (GSH), phenolic compounds and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), gluthatione reductase (GR) and ascorbate peroxidase (APX) has developed in all plant cells. In this work, we analyzed the expression profile of several genes that are part of the enzymatic antioxidant system, in two different *Prunus* rootstocks with contrasting response to hypoxia stress. The expression analyses of these genes suggest that the hypoxia-tolerant rootstock has a larger protective capacity against oxidative damage by maintaining higher expression pattern of antioxidant system than the hypoxia-susceptible rootstock.

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P 94

HEMOGLOBIN-LIKE GENES AND *PRUNUS* ROOTSTOCKS TOLERANCE TO ROOT HYPOXIA

Rubén D. Almada[1], Paula Pimentel[1], María José Arismendi[2], Patricio Hinrichsen [3], Manuel Pinto[1], Boris Sagredo[1]
ralmada@inia.cl

[1]Centro de Estudios Avanzados en Fruticultura (CEAF_R08I1001), INIA CRI Rayentué, Rengo, Chile
[2]Programa Doctorado en Cs. Mc. Biología Celular y Molecular Aplicada. Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera, Temuco, Chile.
[3]INIA CRI La Platina, Santiago, Chile

In poorly drained soils, water from heavy rains or excessive irrigation saturates the root environment displacing the air from the soil pockets and generating hypoxia (low oxygen level) around roots. The inability of stone fruit trees to withstand low oxygen condition in the root zone results in substantial yield loses, decreased fruit quality and plant mortality. In stone fruit trees, the yield and tolerance to many environmental stresses is mediated, in part, by the performance of the rootstock. The high variability in the physiological responses to hypoxia in *Prunus* species suggests that different molecular mechanisms could evolve within the genus for dealing with this stress. However, the molecular bases of such responses are scarcely understood. Molecular responses to low oxygen levels have been analyzed in *Arabidopsis thaliana* as well as in few crop species. In those species, *non-symbiotic* (*nsHb*) *HEMOGLOBIN*-like genes stand out among hypoxia related genes. In order to gain further understanding about the molecular responses of *Prunus* rootstocks to hypoxia, we cloned and analyzed the expression pattern of *Prunus spp*. *nsHb*-like and *truncated* (*trHb*) *Hb*-like genes in roots of *Prunus* rootstocks with contrasting response to this stress. We observed that the putative *Prunus nsHb* and *trHb* genes were higher expressed in roots of tolerant rootstocks under hypoxia caused by flooding. Furthermore, tolerant rootstock plants developed hypertrophic lenticels on the stems after 30 days of hypoxia. Our results suggest that these genes (*nsHb* and *trHb*) and morphological changes could be part of the adaptive mechanisms evolved within *Prunus* genus for surviving hypoxic situations.

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P 95

FERMENTATIVE PATHWAY GENES ARE UP-REGULATED IN ROOTS OF STONE-FRUIT ROOTSTOCKS (*PRUNUS SPP.*) EXPOSED TO HYPOXIA STRESS

María José Arismendi[1], Rubén Almada[2], Paula Pimentel[2], Patricio Hinrichsen[3], Manuel Pinto[3], Boris Sagredo[2]
bsagredo@inia.cl

[1]Programa Doctorado en Ciencias mención Biología Celular y Molecular Aplicada. Facultad de Ciencias Agropecuarias y Forestales. Universidad de La Frontera. Temuco, Chile.
[2]Centro de Estudios Avanzados en Fruticultura (CEAF_R08I1001). INIA CRI Rayentué. Rengo, Chile.
[3]INIA CRI La Platina, Santiago, Chile.

Soil flooding and waterlogging are environmental constraints that are increasingly important for stone-fruit orchard development. Under these situations, water saturates the root environment displacing the air from the soil pockets and generating hypoxia (low oxygen level) around roots. 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 Chile, most stone-fruit trees being planted are grafted on clonal rootstocks. Furthermore, rootstocks determine, in part, the stone fruit tree tolerance to many environmental stresses such as root hypoxia. Even though, most of the *Prunus* rootstocks are classified as hypoxia sensitive, 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*) gene expression was analyzed by qPCR in two *Prunus* rootstocks (Tolerant: Mariana 2624; susceptible: Mazzard F12/1) with contrasting response to hypoxia and exposed to flooding. In general, an increment in the *ADH*, *PDC* and *LDH* expression was observed in the rootstocks studied. It is important to highlight the *ADH* differential expression between genotypes analyzed. These results suggest a role of fermentative pathways in the adaptive responses of these species to hypoxia stress.

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P 96

EFFECT OF FLOODING ON THE PHYSIOLOGICAL RESPONSE OF GRAPEVINES (VITIS VINIFERA L.) CV. SULTANINA GRAFTED ON DIFFERENT ROOTSTOCKS

Michelle Morales[1], Guillermo Toro[1], Gabriel Selles[2], Raúl Ferreyra[2], Manuel Pinto[1]
mmorales@ceaf.cl

[1]Centro de Estudios Avanzados en Fruticultura (CEAF), Rengo, Chile.
[2]Instituto de Investigaciones Agropecuarias (INIA La Platina), Santiago, Chile.

The objective of the study was evaluated the effect of flooding on grapevine cv. Sultanina grafted on two different rootstocks: Harmony (S/H) and Freedom (S/F). For this, one year old plants (grafted and non grafted) were cropped in pots and the next measurements were practiced: CO2 assimilation rate (A), stomatal conductance (gs), chlorophyll content and dry matter accumulation (DMA). The flooding treatment was done by keeping the water 3 cm above the substrate surface during nine weeks. In control plants, the substrate was kept at field capacity using humidity sensors. In the flooded treatment, the oxygen diffusion rates (ODR) in the substrates was close to 0,2 ?g cm-2 min-1, while in the control values averaged 4,56 ?g cm-2 min-1. After 8 days of flooding (DF) the S/H plants showed significant reduction in A with respect to control, while in non grafted (NG) and S/F plants this decrease in A was significantly only after 17 DF. Under flooding the reduction in gs was significant at 7 DF in NG plants, at 12 DF in S/F plants and only 26 DF in S/H plants. In this last case gs values not related to early decline in A. In general, chlorophyll content was lower in flooded plants. Under flooding in S/H plants a lower DMA was associated with the early decline in A. In S/F flooded plants there also was a reduction in DMA, however in this case total DMA was higher with respect to the other flooded plants. This shows that Freedom rootstock induced a high vigor to the graft even under flooding. The high value of A observed in S/F plants during the first week under flooding suggest that this could correlate well with tolerance to short-term flooding. More research is needed to corroborate these results in cultivars more susceptible than Sultanina to flooding.

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P 97

ROOT-BASED ASSESSMENT OF BORON STRESS TOLERANCE IN NATURALISED *VITIS* GENOTYPES COLLECTED FROM ARID REGIONS FOR DEVELOPING GRAPEVINE ROOTSTOCKS.

Loreto Cavieres[1], Claudia Bavestrello[1], Antonio Ibacache[2], Marco Molina-Montenegro[1], Andrés Zurita-Silva[1]
andres.zurita@ceaza.cl

[1]Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile
[2]Instituto de Investigaciones Agropecuarias (INIA Intihuasi), La Serena, Chile

Boron along with salinity and sodicity are main abiotic stress constrains, commonly associated with arid environments or irrigation practices, which occurs in northern Chile where electric conductivity in irrigation water exceeds the optimal levels. Furthermore, Northern soils exhibit high boron content by its volcanic origin and gradual accumulation of this element from continued irrigation with boron-enriched waters. Boron is an essential micronutrient for plant development and growth and it's deficiency and toxicity ranges are very tight. *Vitis vinifera* is a sensitive species regarding high boron concentrations. Evaluation of Boron stress tolerance was performed by assessing five naturalised genotypes of *V. vinifera*. Assays were carried out by using mini-rhizotrons, where root growth was evaluated in parallel with morphometric, physiological and molecular analysis, along with collecting shoot tissues during the experiment. Plants were grown in controlled conditions (growth room at 24° C, relative humidity of 60% and photoperiod of 16/8 hrs. Day/night). Two irrigation treatments were assayed: control with distilled water and T1 with a solution of boric acid 15 ppm, with watering every week. Eight months plants from clonal in vitro cultures were used, coming from Grapevine germplasm bank (GermoVidNor) located in Vicuña during summer season. Clonal plantlets were acclimatized and transplanted to peat and perlite sustrate (3:1) when developed roots. Finally, 2 plants were re-transplanted to mini-rhizotrons. These were installed on closed plastic containers for avoiding excessive evapotranspiration at the growth room. Parameters included plant biomass measurements, growth and root architecture and net photosynthesis (IRGA). We found that accession 5 was the most tolerant to Boron toxicity, and the accession 146 was the less tolerant.

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P 98

CHARACTERIZATION OF NATURALISED *VITIS* COLLECTED FROM ARID REGIONS: A BORON TOLERANCE SOURCE FOR DEVELOPING GRAPEVINE ROOTSTOCKS IN NORTHERN CHILE.

Marisol Herrera[1], Alejandra Milla[1], Antonio Ibacache[2], Pedro León[2], Andres Zurita-Silva[1]
andres.zurita@ceaza.cl

[1]Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile
[2]Instituto de Investigaciones Agropecuarias (INIA Intihuasi), La Serena, Chile

Considering the current models of climate change, lands under salinization are expected to increase during this century, especially in those areas where a drastic decrease in precipitation events has been predicted, or is already occurring as in northern Chile. The main drawback that affects the productivity and quality of the grapevine production in arid zones is the poor root system development caused by factors such as salinity (toxicity by Cl, B, Na), alkaline soil rhizosphere (low efficiency in nutrient absorption), low organic matter content, and water availability, leading to reductions in productivity by 40%, increasing concomitantly the production costs to reach optimal quality demanded in global markets. Soil Boron concentrations are rising (over 1 mg/L boric acid) due to the higher evaporation rates and boron content in irrigation streams, thus damaging sensitive crops as Grapevine. Boron is an essential micronutrient for plant growth; it plays a structural role in the cell wall and membranes, synthesis of hormones, formation of chlorophyll, and reproductive functions. Boron generates toxicity and plants suffer alterations at morphological, physiologic and molecular level, affecting plant productivity. We collected ancient naturalised grapevines from Northern Chile to create a grapevine germplasm bank (GermoVidNor) with the rationale of characterizing plants that have been suffering extreme conditions, identifying plant materials adapted to abiotic stresses. Twelve naturalised genotypes were studied using a toxic boron concentration (10 ppm). Root development was evaluated by using mini-rhizotron, and we made physiological and molecular analysis to determine the degree of tolerance to stress. Parameters regarding root architecture and responses to Boron toxicity between genotypes are presented and discussed. The exploitation of current variability in naturalised germplasm and the use of integrated approaches are the foundations for rootstock breeding strategy towards enhancement of stress tolerance.

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P 99

ALUMINUM DETECTION AND LIPID PEROXIDATION IN BREAD WHEAT SEEDLINGS (TRITICUM AESTIVUM L.) GROWING IN AN ANDISOL

Ligia Pinilla[2], Gustavo Curaqueo[1], Pablo Cornejo[1], María De La Luz Mora[1], Fernando Borie[1], **Paula Aguilera**[2]
paguilera@ufro.cl

[1]Center of Amelioration and Sustainability of Volcanic Soils. BIOREN-UFRO. Universidad de La Frontera, P.O. Box 54-D, Temuco, Chile

[2]Doctorate Program in Science of Natural Resources, Universidad de La Frontera, Temuco, Chile

Wheat (Triticum aestivum L.) is the most important cereal produced in Chile, mainly due to the economic value of their production, acreage and mass consumption. Wheat is cropped in volcanic soils, characterized by low pH and high aluminum (Al) saturation. Aluminum is a highly phytotoxic metal due to it produces its negative effects on plant root growth, affecting the absorption of water and nutrients. Al-phytotoxicity tolerance in wheat has been associated with some of the properties of roots apices, because they have shown a differential behavior respect to Al accumulation among Al-tolerant and Al-sensitive varieties. The detection of Al in roots tissues has been traditionally based on various methods of staining. Such as the use of the hematoxylin staining, which forms a colored complex when reacts with Al3+. The aim of this study, was to visualize Al-hematoxylin complex in wheat roots and to relate with of the membranes in shoots. Wheat seedlings of six Al-tolerant varieties were grown in hydroponic conditions with and without Al. Hematoxylin solution (0.2%) for detecting location of Al was used. Moreover, lipid peroxidation was assayed by measuring thiobarbituric acid reactive substances (TBARS) as an indicator of oxidative stress. Our results indicated that the presence of Al-hematoxylin complex discriminates between Al-tolerant varieties, high to low intensity of color varieties presented the following order: BT> Crac>Otto>Bakan>Invento>Porfiado. Regarding lipid peroxidation, BT, Bakan and Crac reached the largest accumulation of TBARS, suggesting they would be more sensitive to the presence of Al. In conclusion, hematoxylin allowed us to differentiate between Al-tolerant varieties, and by TBARS was observed that varieties with the most intense staining corresponded to varieties with increased oxidative stress. These results give essential information regarding the selection of wheat plants tolerant to Al in natural conditions.

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P 100

DEVELOPMENT OF CRYOSCAN: A DEVICE TO STUDY LOW AND HIGH EXOTHERM IN PLANT TISSUES

Rubio Sebastián[1], Bravo Sebastián[1], Pérez Francisco[1]
frperez@uchile.cl

[1]Laboratorio de Biologia Vegetal, Facultad de Ciencias, Universidad de Chile

When buds of deciduous fruit-trees are exposed to low temperatures, evolves cold-hardiness, a physiological adaptation to deal with hard climatic conditions along the winter. Cold-hardiness or freeze resistance in plants is marked by the lowering in the freezing point of intracellular water. The freezing of tissues can be measured by differential thermal analysis (DTA), which measure the heat of fusion released during the freezing event (exotherm). Two distinct exotherms are normally observed in buds of temperate fruit-trees. The high exotherm (HTE) is the result of extracellular water freezing within the bud-axis and scales, while the low (LTE) is due to intracellular ice formation. While cold hardiness is developed in plant-tissues, LTE is shifted to lower temperatures, reaching a maximum value when cold-hardiness is maximum. Temperatures beyond LTE causes cellular damage, therefore acclimation of buds of temperate fruit-trees can be followed by measuring the LTE. Cryoscan, a device for the detection of HTE and LTE of buds of different fruit-trees, was developed and assembled in our laboratory. The device consists of a group of peltier elements which gradually cool in 8 h the cooper container from 10ºC to -33ºC. When the system reaches the samples freezing point, the sample releases the latent freezing heat, which leads to a temperature differences between the two faces of another peltier element, which is detected as a voltage peak. Here, we show preliminary results of acclimation and deacclimation experiments in grapevine-buds (cv Thompson Seedless), and in vegetative and reproductive buds of nuts (*Juglans regia*).

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P 101

COLD TOLERANCE EVALUATION OF CHILEAN RICE (*ORYZA SATIVA*) GENOTYPES AT THE SEEDLING STAGE

Gabriel Donoso[1], Mario Paredes[1], Viviana Becerra[1], Israel Díaz[2], Rodrigo Contreras[3]
gabriel.donoso@inia.cl

[1]Centro de Biotecnología de los Alimentos (CBA), Centro Regional de Investigación Quilamapu, Instituto de Investigaciones Agropecuarias (INIA), Chillán, Chile.

[2]Universidad de Concepción. Chillán. Chile.

[3]Universidad de Concepción. Concepción. Chile.

In Chile, low temperature is the most important abiotic stress affecting rice yield. Chilean rice is grown under the optimal temperature needed for rice cultivation, with a minimal temperature between 5ºC to 10ºC. This temperature induces a cold stress in rice plant, decreasing plant density in field. Therefore, the identification of cold tolerance genotypes at the seedling stage is important to increase the effectiveness of Rice Breeding Program of Chile. To identify cold tolerance genotype at the seedling stage, 42 genotypes were evaluated: 21 experimental lines from breeding program from INIA Quilamapu, Chile (Quila 154601, Quila 154804, Quila 156603, Quila 157302, Quila 159005, Quila 185007, Quila 213801, Quila 221801, Quila 225001, Quila 225101, Quila 225103, Quila 228603, Quila 230513, Quila 230603, Quila 231902, Quila 233008, Quila 235207, Quila 235501, Quila 237908, Quila 241309 and Quila 242104), and cultivars from Chile (Buli, Brillante, Oro, Ambar, Diamante and Zafiro), China (IRRI Yuhkara, IRRI Norin 9, IRRI Ji-Jing-60, IRRI Tepuke and IRRI LI Jina Xintuan Hegu), Spain (Susan, Guara, Euro, Hispagran, Guadiamar and Ranbali), India (Basmati, Basmati C621 and Sugandh-2) and Philippines (Korea 2). Seeds were germinated at 28ºC, transplanted in a rice soil (Vertisol) and grown in greenhouse. Seedlings of three leaves were treated for 7 days at 5ºC on night. Leaf damage was evaluated ten days after the end of the stress and a ranking of cold tolerance was made. Experimental lines from Chile (Quila 154804, Quila 156603 and Quila 241309) showed higher levels of cold tolerance than the cultivars from Spain, China, India, Philippines and Chile. However, others Chilean experimental lines (Quila 242104, Quila 225103 and Quila 228603) presented a lower cold tolerance than the check cultivars. These results indicate the importance to include this selection method to Rice Breeding Program of Chile to improve genotype selection accuracy.

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P 102

THE EFFECT OF WATER DEFICIT AND HIGH TEMPERATURE STRESS ON THE PHYSIOLOGY AND BIOCHEMICAL RESPONSES OF ALOE BARBADENSIS MILLER (ALOE VERA).

Carlos Salinas[1], Isabel Ramírez[1], Claudia Huerta[1], Matías Freire[1], **Liliana Cardemil**[1]
lcardemi@gmail.com

[1]Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile

Aloe vera is a CAM plant, adapted to arid environments and cultivated in the IV Region of Chile. The objective of this work was to investigate if the water and temperature conditions of this region would affect the plant physiology and the quality of the gel which has a commercial value. For this, we determined the water use efficiency (WUE) and gel production of plants under different water regimes and temperatures. We also quantified by semi quantitative RT-PCR the expression of genes encoding proteins associated with stress responses such as the HSP, ubiquitin, and superoxide dismutase, and the accumulation of these proteins by western blot analyses. The presence and concentration of sugars and polysaccharides responsible for the osmotic adjustment (fructans) of the plant and for the economical qualities of the gel (galactoglucomannan) were also evaluated. Our results indicate that Aloe vera increased the WUE under water deficit, due to an efficient osmotic adjustment. The plant showed an increase in gene expression and accumulation of the stress protection proteins. Total sugar increased and analysis of partially methylated alditol acetates by GC-MS of polysaccharides showed that the glycosidic linkages of the fructans and galactoglucomannan changed during drought. Our results indicate that heat stress and drought induce physiological and molecular responses in Aloe vera, changing the composition of the gel which could affect the commercial value of the plant.

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30 de noviembre • 2 de diciembre, 2011
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P 103

TOTAL PHENOLIC COMPOUNDS IN TWO HYMENOPHYLLACEAE WITH CONTRASTING HOST TREE DISTRIBUTION AND RESPONSES TO DESECCATION-REHYDRATION CYCLE

Karina I. Acuña [1], Katherin A. Aravena[1], María José Parra[1], León A. Bravo[2]
kacuna@udec.cl

[1]Departamento de Botánica. Facultad de Ciencias Naturales y Oceanográficas. Universidad de Concepción, Casilla 160-C, Concepción, Chile

[2]Departamento de Ciencias Agronómicas y Recursos Naturales. Facultad de Ciencias Agropecuarias y Forestales; Center of Plant, Soil Interaction and Natural Resources Biotechnology BIOREN, Universidad de la Frontera, Casilla 54-D, Temuco, Chile.

Phenolic compounds play major role in plants-environment interaction. Between its functions, they can act as scavenging molecules of ROS since they are effective donors of electrons or protons helping to stabilize free radicals that could cause oxidative damage. Environmental stresses, such as excess light and low water availability, may produce redox imbalances exacerbating ROS. Hymenophyllaceae are poikilohydrous epiphytes associated to humid and deep shade. However, it has reported species inhabiting the upper canopy strata of the temperate humid forest of Southern Chile. In the trunks of host trees there is an increase in light intensity and a decrease of water availability with height (vertical gradient). In desiccation experiments made with two Hymenophyllaceae of contrasting distribution in the host tree, *H. cruentum*, restricted to the base, was less desiccation tolerant than *H. dentatum* which is widely distributed toward the upper canopy. During rehydration, *H. cruentum* undergoes recovery problems such as oxidation of its fronds (brownish) and loss of chlorophyll, while *H. dentatum* fully recovers. We hypothesize that a higher content of phenolic compounds in *H. dentatum* could prevent oxidative damage during a desiccation-rehydration cycle. The aim of this study was to determine whether total phenolic contents are associated to desiccation tolerance in filmy ferns. For this purpose we evaluate possible differences in total phenolic contents of fronds between the above species subjected to desiccation and rehydration cycle under low, optimum and saturating light. The results shows interspecific significant differences in full hydrated, desiccated and rehydrated states at all light intensities and only intraspecific differences in *H. cruentum* in low and saturating light between hydrated and desiccated state. These results suggest that phenolic compounds could be important ROS scavengers in *H. dentatum*. Light may influence scavenging activity of phenols or ROS production because less damage occurs in *H. cruentum* under light.

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P 104

EFFECT OF FREEZING STRESS AND DROUGHT STRESS ON NON-TRANSGENIC POTATO AND TRANSGENIC POTATO HARBORING THE SCCBF1 GENE CLONED FROM SOLANUM COMMERSONII"

Pino M.T.[1], Balboa M.[1], Avila A.[1], Millaguir S.[1], Chen T.H.H.[2], Jeknić, Z.[2]
mtpino@inia.cl

[1]Instituto de Investigaciones Agropecuarias (INIA)
[2]Oregon State University, USA

The cultivated potato (Solanum tuberosum) is sensitive to freezing and drought stress, limiting their distribution and productivity. This research aimed to study drought and frost responses in non-transgenic (WT) and transgenic potatoes (Lines-Z45), genetically transformed with ScCBF1gene cloned from Solanum commersonii, a wild potato highly tolerant to abiotic stress. S. tuberosum cv Cardinal and S. commersonii DunPI243503 clone13 were genetically modified with 35Sp::ScCBF1gene. Freezing tolerance was studied by ion-leakage test in non-acclimated and cold-acclimated plants. Experimental design was a randomized complete block with three replications per experiment. Three independent experiments were performed in time. In S. commersonii, the comparative analysis of frost tolerance showed that Lines-Z45, significantly improved their frost tolerance. Moreover, the results showed that lines-Z45 improved frost tolerance after cold-acclimation. In relation to S. tuberosum, Lines-Z45 significantly improved frost tolerance compared with their WT. However, no significant differences were observed between cold-acclimated and non-acclimated plants. The same transgenic lines-Z45 were evaluated for drought tolerance both in Vitro and greenhouse conditions. For each genotype and drought treatment, three independent experiments were conducted using three replicates per experiment. In Vitro, different drought stress levels were induced by using PEG4000. Simulated drought stress in Vitro adversely affected plant growth in potato plantlets. Lines-Z45 in both Solanum species showed higher vegetative growth and plant survival. Also, Lines-Z45 showed significant best root development according to WinRhizo Pro-software. Greenhouse results, supported in Vitro testing, decreasing of stomatal conductance and photosynthetic parameters occurred in all genotypes during drought stress, WT were more adversely affected than Lines-Z45. QT-PCR analysis showed that ScCBF1 expression was associated with D1-Pyrroline-5-carboxylatesynthase gene expression and free proline synthesis in stems and leaves under stress. ScCBF1 gene was able to induce cold and drought tolerance, however the gain in drought tolerance was most relevant than freezing tolerance, in terms of agriculture needs.

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P 105

THE COMBINED EFFECTS OF DROUGHT AND WARMING ON PHOTOSYNTHESIS AND XANTHOPHYLL PIGMENTS CONTENT IN *PHACELIA SECUNDA*.

Carolina Hernández-Fuentes[1], Carolina Hernández-Fuentes[2], Lohengrin A. Cavieres[1], Lohengrin A. Cavieres[2], León A. Bravo[3], León A. Bravo[4]
carohernandez@udec.cl

[1]Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Chile.

[2]Instituto de Ecología y Biodiversidad (IEB), Santiago, Chile

[3]Departamento de Ciencias Agronómicas y Recursos Naturales, Facultad de Ciencias Agronómicas y Forestales, Universidad de La Frontera, Chile

[4]Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus. Universidad de La Frontera, Chile

Contrasting climate change scenarios have been proposed for the Andes of central Chile: one with increased temperatures combined with a decline in precipitations, exacerbating the summer drought, and a second scenario where the increases in temperature co-occur with increased rainfall. Our goal was to determine how warming and changes in summer precipitations will affect photosynthetic performance and xanthophyll content on *Phacelia secunda*, a widely distributed species along the Andes The warming was applied using OTC (open top chamber). A drip irrigation system was used to increase soil moisture during the growing season. We measured xylematic water potential (□x), photosynthesis (Amax) and xanthophyll content in plants from 1.600, 2.800 and 3.600 masl. Plants from1600 masl of OTC presented lower □x (between 16-60%) than other treatments and elevations. Plants from lower elevations exposed to irrigation presented □x close to zero. No differences were found in plants from 3600 masl. At lower altitudes the Amax was lower in OTC and control treatments (32 and 34%, respectively) than irrigated plants, however, in plants from 3600 masl Amax. was a 45% higher in OTC than control. In plants from 1600 masl exposed to drought and warming zeaxanthin was absent and they presented lower de-epoxidation index (between 0.06- 0.09). By contrast, plants from 2800 masl, presented a greater content of zeaxanthin in OTC than control plants. In plants from 3600 masl the zeaxanthin content was 34% greater in controls than OTC. At lower altitudes the lutein content was higher in plants from OTCs. However, in plants from 3600 masl the lutein was 48% higher in control than OTC plants. The combined effects of warming and drought caused a drastic decrease in photosynthetic performance. The xanthophyll cycle is an important photoprotective mechanism but only when drought is not very severe and under low temperature stress.

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P 106

A PROTEOMIC COMPARISON OF YELLOW LUPIN LEAVES BY 2-D FLUORESCENCE DIFFERENCE GEL ELECTROPHORESIS (DIGE) TO DETECT WATER-STRESS RESPONSIVE PROTEINS.

Takahiro Ogura[1], Véronique Amiard[1], Javiera Arvena Calvo[1], Iván Maureira Butler [1]
takahiro.ogura@cgna.cl

[1]Centro de Genómica Nutricional Agroacuícola (CGNA), CONICYT-REGIONAL, GORE LA ARAUCANIA, R10C1001, Unidad de Genómica y Bioinformática, Temuco, Chile

Yellow lupin is a leguminous plant that contains a high amount of protein in the grain. Therefore, lupin grain has been used as a protein source in feed production destined to livestock and aquaculture. Lupin yield are menaced by the effects of the coming global warming, and consequently, breeding of abiotic stress resistant varieties of yellow lupin is desired. For this breeding, elucidation of plant response systems against abiotic stress is important. Thus, we have conducted a 2-D Fluorescence Difference Gel Electrophoresis (DIGE) analysis of yellow lupin leaves using water-stressed leaves as an example of an abiotic stress to detect water-stress responsive proteins. The experiments were conducted as follows. The protein samples were obtained from the yellow lupin leaves that were treated for three weeks without water supply (the water-stressed group) or with water supply (the control group). Iso-electric focusing (IEF) was performed on Immobiline DryStrip with a mixture of Cy5 labeled protein sample and Cy3 labeled standard protein sample. These proteins were further separated by polyacrylamide gel electrophoresis. Then, the images of the gels of both groups were compared using the DeCyder software. It was observed that four spots of protein presented significant differences between the groups. One of these proteins was up regulated and the other three proteins were down regulated by water-stress. The up regulated protein may be involved in plant protection against water-stress. Meanwhile, the down regulation of the three other proteins may be a consequence of the decreasing metabolic activity of stressed leaves. The ongoing identification of these proteins by LC MS/MS will allow us to better understand lupin resistance to water-stress.

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P 107

INFLUENCE OF *IN VITRO* GROWTH CONDITIONS ON PHOTOSYNTHETIC PERFORMANCE AND SURVIVAL RATE OF *CASTANEA SATIVA* DURING *EX VITRO* TRANSFERENCE

Patricia L. Sáez[1], Leon A. Bravo[2], Manuel E. Sánchez[1], Mirtha I. Latsague[3], Darcy G. Ríos[1]
patrisaez@udec.cl

[1]Laboratorio Cultivo de Tejidos Vegetales, Facultad de Ciencias Forestales y Centro de Biotecnología, Universidad de Concepción, casilla 160-C, Concepción, Chile.

[2]Departamento de Ciencias Agronómicas y Recursos Naturales, Facultad de Ciencias Agropecuarias y Forestales; Center of Plant, Soil Interaction and Natural Resources Biotechnology BIOREN, Universidad de La Frontera. Temuco, Chile.

[3]Escuela de Ciencias Ambientales, Facultad de Recursos Naturales, Universidad Católica de Temuco. Temuco, Chile.

Malformations observed in traditional *in vitro* culture can be avoided through the management of culture conditions, which may promote the development of microshoots similar to grown in nursery. The culture under conditions that favor a better development, could involve a reduction in *ex vitro* acclimatization time, faster acquisition of autotrophic behavior and consequently higher survival rates, triggered mainly by a greater photosynthetic capacity (Pn). Thus, our goal was to evaluate the influence of *in vitro* conditions (mixotrophic, MP and photomixotrophic, PhP) on the physiology and growth of *Castanea sativa* during rotting (R) in growth chamber and after transfer to greenhouse (TG). Both, MP and in PhP decreased Pn at the beginning of R, but in MP this decrease was maintained until the end of R, and showed a low Pn in TG, even lower than that observed *in vitro*. This poor adaptation to new autotrophic conditions was accompanied by a high mortality rate (around 50%). On the contrary, in PhP, Pn was always higher than that observed in MP, showing a decrease at beginning of TG which coincided with the change in environmental conditions. However, at the end of the evaluation period in greenhouse, Pn was recovered and even exceeded the values achieved *in vitro*. Parameters associated to photochemical activity (maximal efficiency of PSII and electron transport rate) shown the same tendency. The increase in Pn at the end of the evaluation stage may be associated with an acquisition of autotrophy; due to a translocation of assimilates stored. This idea is supported by the survival rate at this stage, which exceeded 90% in PhP. The results suggest that photomixotrophic conditions during *in vitro* culture improves the photosynthetic performance in early stage after *ex vitro* transference, playing a key role in the acclimatization process, which may reduce the transference stress.

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P 108

NITRIC OXIDE INCREASES CHLOROPHYLL CONTENT AND THE EXPRESSION OF ELIPS PROTEIN IN GRAPEVINE (*VITIS VINIFERA* L.) AND WHEAT (*TRITICUM AESTIVUM* L.)

Alejandro Riquelme[1], Pamela Alvarez[2], Daniela Olivares[2], Manuel Pinto[3]
mpinto@inia.cl

[1]Centro de Estudios Avanzados en Fruticultura. Rengo. VI Región.
[2]Universidad de Chile, Facultad de Ciencias Agronómicas.
[3]Instituto de Investigaciones Agropecuarias, CRI La Platina.

Numerous studies suggest that nitric oxide plays a key role in plants. This molecule has the ability to emulate dependent effect of light on plants. Nitric oxide can inhibit the degradative pathway of chlorophyll and also to promote the synthesis of this pigment. The expression of early light induced proteins (ELIPs) also has been suggested that is promoted by this biomolecule. These proteins are able to bind chlorophylls and it is speculated that they participate in the formation of photosystems in young leaves. The aim of this study was to evaluate the effect of exogenous application of nitric oxide on the chlorophyll content and expression of ELIPs like protein in etiolated young leaves of grapevine and wheat. Buds of grape cv. Sultanina and wheat seedlings were kept in dark to obtain etiolated leaves. Nitric oxide treatment was performed directly spraying 100 µM S-nitroso-N-acetylpenicillamine (SNAP) solutions. Etiolate leaves sprayed with distilled water was used as control. Chlorophyll and ELIPs were induced by different light intensity treatments. The total chlorophyll content was measured by Arnon´s method and ELIPs expression was performed by western blots, using pea-ELIPs antibodies. The results indicate that nitric oxide increased the accumulation of total chlorophyll and the ELIPs expression in both grapevine and wheat etiolated leaves. This positive effect was demonstrated to be light dependent in both species; no induction was found either in chlorophylls and ELIPs when nitric oxide was sprayed in dark. Furthermore, this induction increased with the light intensity.

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P 109

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

Felipe Moraga[1], Antonia Yarur[1], Gabriel León[1]
moraga.fe@gmail.com

[1]Laboratory of Plant Reproduction & Development, Center of Plant Biotechnology, Andrés Bello University.

Flowering in plants is under a tight genetic and environmental control, and the most important pathways regulating this process are the photoperiod and the vernalization. Both converge in the master regulatory gene *FLOWERING LOCUS T (FT)*, which encodes a small protein produced in the leaf vasculature that travels to the shoot apical meristem (SAM), activating the expression of the floral meristem identity genes. *FRIGIDA* 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. proFLC:GUS transgenic plants show less GUS histochemical activity when grown on salicylic acid (SA), which indicate that this response is controlled at the transcriptional level. Therefore, is expected that levels of *FLC* transcripts decrease after the treatment with SA, allowing *FT* expression. Moreover, we propose that transient expression of *FT* should be enough to promote flowering without vernalization. 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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P 110

SUCROSE ACCUMULATION DUE TO SUCROSE PHOSPHATE SYNTHASE (SPS) ACTIVITY IN *COLOBANTHUS QUITENSIS* IS COLD AND PHOTOPERIOD REGULATED.

Marely Cuba[1], Kathleen S. Cid[1], Alejandro Navarrete[3], Cynthia Retamal[3], León A. Bravo[4]
mcubaster@gmail.com

[1]Laboratorio Biotecnología y Estudios Ambientales, Campus Los Ángeles, Universidad de Concepción, Los Ángeles, Chile

[2]Depto. Ciencias y Tecnología Vegetal, Escuela Ciencias y Tecnología, Campus Los Ángeles, Universidad de Concepción, Los Ángeles, Chile

[3]Laboratorio Fisiología Vegetal, Fac. Cs. Nat. y Oceanográficas, Universidad de Concepción, Concepción, Chile

[4]Lab. Fisiología y Biol. Mol. Vegetal, Depto. Cs. Agronómicas y Recursos Nat., Fac. Cs. Agropecuarias y Forestales, Universidad de La Frontera, Temuco, Chile

[5]Center of Plant, Soil Interaction and Nat. Resources Biotech., Scientific and Technological Bioresource Nucleus, Universidad de La Frontera, Temuco, Chile

In plants, the key enzyme in sucrose synthesis is sucrose phosphate syntase (SPS), SPS is transcriptional and post-translational regulated during development and specific environmental stimuli. Three SPS gene family (A, B and C) have been described in dicot species. *Colobanthus quitensis* has a wide distribution, from Mexico to southern Antarctic Peninsula, and is the only dicot plant living in Antarctic. Sucrose accumulation is related to high SPS activity during cold acclimation at laboratory conditions of *C. quitensis*, and this feature has been associated to its survival in the Antarctic's harsh conditions and made it an interesting model. Recently, we have reported two SPS isoform (A and B) in *C. quitensis*. We hypothesize that high sucrose accumulation and SPS activity observed in cold acclimated Antarctic ecotype of *C. quitensis* depend of differential pattern expression of SPS isoforms induced by low temperature, but this wills contrast with other continental ecotypes. Our aim is to study SPS isoforms expression pattern, enzymatic activity and their associate with sucrose accumulation, under laboratory conditions, using Antarctic populations; and to compare it with continental populations (Punta Arenas) under natural growing conditions. Plants from Antarctic populations were grown at 4 °C and 15 °C and at two photoperiods (21/3 and 8/16). Leaves and roots tissue from natural conditions were taken follow a daily course. *Cq*SPSA and *Cq*SPSB have differential organ expression and day length and low temperature differential regulation while *Cq*SPSA transcript expression is negatively regulated, *Cq*SPSB does not appear to be largely affected but its regulation, under natural conditions is not clear yet. Consistently, SPS transcripts were associated to higher SPS protein and activity, which resulted in sucrose accumulation under laboratory conditions, in contrast to plants from the field. Although, Antarctic populations showed association of transcripts expression and enzymatic activity. Currently, we are studying the sucrose contents on both populations' conditions.

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P 111

CHARACTERISATION OF *ATA6PR*, A PUTATIVE ALDOSE-6-PHOSPHATE REDUCTASE FROM *ARABIDOPSIS THALIANA*

María Sofía Zamudio[1], Joel Wurman[1], Roberto Parada[1], Michael Handford[1]
mhandfor@uchile.cl

[1]Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile

Sorbitol is the main product of photosynthesis in the *Rosaceae* family, which includes fruit trees of economic importance such as peach, pear, apple, loquat, etc. This polyol is synthesised in the source organs of these species from glucose-6-phosphate by aldose-6-phosphate reductase (A6PR) and sorbitol-6-phosphate phosphatase (S6PP). Sorbitol is then translocated via the phloem to sink organs (such as roots and fruits) and metabolised to glucose by sorbitol oxidase (SOX) or to fructose by sorbitol dehydrogenase (SDH). In non-*Rosaceae* sucrose-accumulating species, sorbitol and other polyols has been described, mainly associated with tolerance to drought, saline and cold stress. Using reverse genetics, we have identified *AtA6PR*, a putative A6PR from the non-*Rosaceae Arabidopsis thaliana* which possesses the main molecular characteristics of A6PRs described in other organisms. Our objective is to characterise this gene and determine its role in *Arabidopsis* metabolism. RT-PCR analysis shows that *AtA6PR* is ubiquitously-expressed, and fluorescent microscopy indicates that AtA6PR-GFP is localised in the cell cytosol. *AtA6PR* has been cloned and expressed in the heterologous system *E. coli*, and the purified protein will be used to generate a polyclonal mouse anti-AtA6PR antisera. Additionally, recombinant AtA6PR has been produced in an *in-vitro* expression system for activity assays. Advances in this work will be discussed. Finally, *AtA6PR*-over-expressing lines are being generated and multiple homozygous *ata6pr* knock down mutants have been identified in order to determine the role *in-vivo* of the A6PR enzyme in species that do not translocate and accumulate sorbitol (or polyols in general). Our results indicate that *ata6pr* mutants have diminished tolerance to salt stress.

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P 112

FOLIAR CA/AL RATIO EFFECTS ON PHOTOCHEMICAL PARAMETERS IN TWO BLUEBERRY CULTIVARS GROWN IN AL-SATURATED ANDISOL AMENDED WITH CALCIUM SULFATE

Cristian Meriño-Gergichevich[1], Patricia Poblete-Grant[3], Miren Alberdi[2], Marjorie Reyes-Díaz[2]
cmerino@ufro.cl

[1]Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile.

[2]Departamento de Ciencias Químicas y Recursos Naturales; Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN-UFRO), Universidad de La Frontera, Temuco, Chile.

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

Blueberry (*Vaccinium corymbosum* L.) is adapted to acid soils (pH ≤ 5.5), but is sensitive to phytotoxic aluminum (Al3+). Nonetheless, calcareous amendments as calcium sulfate (CaSO4) are used to ameliorate the harmful effect of Al3+ on crops physiology. Although blueberries have low calcareous requirements, this cation is an essential factor for processes such as photosynthesis. Healthy bushes contain up to 8000 mg kg-1 DW Ca and up to 400 mg kg-1 DW Al in leaf tissue. It has been reported that the degree of Al-stress on photosynthetic apparatus is correlated with Ca/Al molar ratio (Ca/Al), when ratio is below 6.2 would indicate 75% risk of Al toxicity. However, little is know about Ca/Al on photosynthetic apparatus in this commercial crop. In order to know foliar Ca/Al effects on photochemical performance, maximal quantum yield (Fv/Fm), effective quantum yield (ΦPSII), electron transport rate (ETR), and non-photochemical quenching (NPQ) we evaluated these features in an Al-tolerant (Legacy) and an Al-sensitive (Bluegold) blueberry cultivars. One year-old plants were grown in an Andisol (Al-saturation ~60%) amended with CaSO4 at 0 (control soil), 1000, 2000 and 4000 kg ha-1 during 60 days. Gypsum improved Ca/Al up to 75 and 53% in Legacy and Bluegold respectively, compared to the control. Foliar Ca/Al in both cultivars did not showed changes on Fv/Fm with respect to the control plants ($P > 0.05$), instead ΦPSII and ETR were improved in Legacy by an increased Ca/Al (≥5.0), whereas Bluegold showed a significant increase at 4000 kg ha-1 (Ca/Al = 16.3). A reduction up to 137% in NPQ of Legacy plants amended with 4000 kg ha-1 (Ca/Al = 7.7) were observed in comparison to control plants ($P \leq 0.05$). In conclusion, CaSO4 addition improved leaf Ca/Al, although this ratio did not show a positive effect on fluorescence parameter of Al-sensitive cultivar Bluegold.

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P 113

STUDY OF EFFECT OF MODIFIED ATMOSPHERE (AM) TREATMENTS ON POSTHARVEST QUALITY OF RACHIS OF FLAME SEEDLESS AND REDGLOBE TABLE GRAPES VARIETIES.

Claudia Huerta[1], Christian Silva[1], Iván Balic[1], Bruno Defilippi[2], Reinaldo Campos-Vargas[1]
reinaldocampos@unab.cl

[1]Universidad Andrés Bello, Fac. Ciencias Biológicas, Centro de Biotecnología Vegetal
[2]Instituto de Investigaciones Agropecuarias, INIA La Platina

Rachis green color is an important parameter in grape cluster quality. Modified atmosphere (AM) is a technology used to extend the postharvest life in table grapes. AM was studied in Flame Seedless and RedGlobe table grape varieties as a tool to reduce the loss of green color of rachis. The results indicated that AM helped to keep the rachis quality in both cultivars. We analyzed the amount of chlorophyll-a in order to understand the differences between rachis after AM and only air treatments. Control rachis stored in air showed higher content of chlorophyll-a than AM packaged. In addition, we analyzed by qRT-PCR the mRNA abundance of chlorophyllase (Chl1) and pheophorbide-a oxygenase (PAO) genes as markers of chlorophyll degradation. The results indicated that in both varieties after cold storage the Chl1 mRNA of rachis stored in AM was higher than control. PAO mRNA was reduced by cold storage in Flame and RedGlobe cultivar, but AM did not induce changes of this gene. Other senescence-associated genes have being studied to understand the rachis loss quality.

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P 114

A PRELIMINARY STUDY ON AVOCADO FLAVOR: CHANGES RELATED TO FATTY ACID AND AROMATIC VOLATILE METABOLISMS DURING RIPENING OF AVOCADO VAR. 'HASS'

Amaya Busto[1], Orianne Gudenschwager[2], Bruno G. Defilippi[2], Mauricio González-Agüero[2]
amayabusto@gmail.com

[1]Universidad Andrés Bello, República 217, Santiago, Chile.
[2]Instituto de Investigaciones Agropecuarias (INIA) – CRI La Platina

The organoleptic characteristics that determine the quality of many fruits are one of the main factors influencing on consumer acceptability. The ripening process of "Hass" avocado is triggered once the fruit is harvested, and it is under ethylene regulation. Flavor is a complex genetic trait that is manifested in ripe fruit through the interaction of many metabolic pathways and regulatory circuits, including sugar, organic acid, lipid and volatile components. However, in avocado little is known at the molecular, genetic and biochemical level of the pathways responsible for the synthesis, accumulation and regulation of the main compounds responsible of flavor. In order to understand the biological basis of avocado flavor, we characterized several stages during ripening -with and without an ethylene inhibitor (1-MCP)- in terms of maturity parameters, fatty acids and aroma-related volatile compounds, and gene expression. We cloned and quantified by qPCR genes that are key components of lipid biosynthesis: BCCP and BC subunits of Acetyl- CoA Carboxylase and stearoyl-ACP desaturase (SAD). On the other hand, we studied the genes of key enzymes that are involved in alcohol and aldehyde synthesis: lipoxygenase and three isoforms of alcohol dehydrogenase found on avocado (ADH1, 2 and 3). As fruit ripening progressed, a decrease in aldehydes (i.e. hexanal and trans-2-hexenal) was observed. In addition, changes in lipid content during ripening were correlated with transcriptional changes in BCCP subunit and SAD during the process. Further studies should be performed to identify genes and characterize the pathways that could allow to understand the flavor development during avocado ripening.

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P 115

DIFFERENCES OF MEALINESS INCIDENCE IN EARLY AND LATE SEASON VARIETIES OF PRUNUS PERSICA AND ITS MODIFICATIONS BY POSTHARVEST TREATMENTS

Dayan Sanhueza[1], Daniela Muñoz[1], Ariel Orellana[2], Bruno Defilippi[3], Reinaldo Campos-Vargas[1]
reinaldocampos@unab.cl

[1]Universidad Andres Bello, Fac. Ciencias Biológicas, Centro de Biotecnología Vegetal

[2]Universidad Andres Bello, Fac. Ciencias Biológicas, Centro de Biotecnología Vegetal; FONDAP CRG 15090007

[3]Instituto de Investigaciones Agropecuarias, INIA La Platina

Peaches and nectarines [Prunus persica (L.) Batsch] are one of the most important species of the genus Prunus. However these fruits are highly perishable after harvest. The most used method to increase the postharvest life of peaches and nectarines is storage and transport at low temperature (0°C). However, prolonged cold storage could induce chilling injury (CI) represented mainly by mealiness or woolliness. This chilling injury disorder induces a dried flesh with a grainy sand-like texture when fruits are eaten but with a normal external appearance. It is known that mealiness incidence depend on temperature and length of storage, but also genotype is an important factor on susceptibility to CI. In this context, we evaluated the mealiness incidence on P. persica fruits harvested early (December) or late (January-February) season and stored for 21 days at 4°C. In addition, we study the effects of postharvest treatments that could reduce the CI incidence such as controlled atmosphere (CA) and conditioning. Besides we evaluated the effect of an ethylene inhibitor on CI development. Our results showed that early varieties did not present CI despite of storage conditions prone to mealiness occurrence. On the other hand, late harvested varieties consistently showed CI, and only in specific cultivars treatments of CA or conditioning helped to reduce the disorder severity.

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P 116

IN SILICO PROMOTER ANALYSIS AND HORMONAL REGULATION OF XYLOGLUCAN ENDOTRANSGLYCOSYLASE/HYDROLASE GENES FROM *FRAGARIA CHILOENSIS*

Mª C. Opazo[1], **R. Lizana**[1], R. Herrera[1], M. A. Moya-León[1]
mopazo@utalca.cl

[1]Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Talca, Chile.

The Chilean strawberry fruit (*Fragaria chiloensis* (L.) Mill.) has emerged as a new berry for its excellent organoleptic characteristics, however the fast softening of strawberries is a limiting step for their commercialization. During fruit ripening, significant modifications in cell wall structure take place associated with cell wall components whose solubility increases, polymer size decreases, and linkages between the polymers alter in parallel with decreasing fruit firmness. Several enzymes have been isolated and studied in relation to ripening of strawberry fruit. Xyloglucan endotransglycosylase/hydrolase (XTH) enzyme is associated to the cell wall and it is believed to alter the composition of xyloglucan chains, which are assembled with the cellulose microfibrils. In this work, the promoters of Fc-XTH1 and Fc-XTH2 genes, previously described in *F. chiloensis*, were obtained by Genome walker, cloned and in silico analyzed to reveal putative cis elements using PlantCARE. Both promoters showed several regulatory elements responding to hormonal regulators such as auxin, abscisic acid, giberellin and ethylene, indicating a probable regulatory role of these hormones. Therefore, treatments with auxin (NAA), gibberellins (GA3), 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 shows a significant variation in the expression of Fc-XTH1 and Fc-XTH2 after the hormonal treatments, giving an inhibitory role to auxin and ethylene, and a differential expression pattern of both isoforms by GA3 and ABA treatments, which is consistent with the regulatory elements described in the promoter sequences.

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P 117

ASSEMBLING THE CHILEAN NATIVE POTATOES BASE COLLECTION

Flor Rodríguez[1], Annette Fahrenkrog[1], Carolina Folch[1], Sandra Orena[1], Alfredo Kido[2], Alejandro Peña[2], Julio Kalazich[1], José Santos Rojas[1]
flor.rodriguez@inia.cl

[1]INIA *CRI* Remehue, Osorno, Chile

[2]SAG, Servicio Agrícola y Ganadero, Osorno, Chile

The Chilean native potato (*Solanum tuberosum* subspecies *tuberosum*) is the only native potato germplasm adapted to long days available worldwide, and breeders prefer this well-adapted germplasm with interesting properties as research material. However, only few Chilean native potatoes have been used in breeding programs worldwide, despite the fact that this germplasm is deposited in several germplasm banks around the world including Chile. This is partly due to the fact that these collections still need characterization for several important characters. Currently, there is a renewed worldwide interest in our native potatoes because its uniqueness, its richness in important characters, and because they are crossed easily with modern varieties.

INIA and UACH conserve a collection of Chilean native potato and recently UACH has registered more than 200 landraces in the official 'Registry of Described Varieties'. Today, INIA holds a collection of 332 entries, besides 204 that are held by SAG in its Evaluation Station in Osorno. These 536 accessions are being evaluated at INIA-Remehue using morphological descriptors and 6 nuclear microsatellites first to identify duplicates in our collection and to assemble our base collection free of duplicates. Second, to identify genotypes of our collection those match the already registered landraces. Finally, to homologize our collection with the aim to assess potential duplication in the collections maintained at INIA, UACH and SAG, and to assemble and fingerprint a single base collection of Chilean native potato.

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P 118

NATIVE CHILEAN POTATOES AS A SOURCE OF BIO-HYDROGEN PRODUCTION

Sergio Diez-de-Medina[1], Uwe Conrath[2], Herman Silva[1]
hesilva@uchile.cl

[1]Laboratorio de Genómica Funcional y Bioinformática, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile

[2]Plant Physiology Department, RWTH Aachen University, 52056 Aachen, Germany

Potato (*Solanum tuberosum*) is one of the most important crops in the world. Chile has been proposed as one of the possible origins of this specie, showing an important biodiversity of ancestral potato species. Based on a transgenic strategy, in the Conrath lab a potato line mutant for an ADP/ATP transporter was obtained (Aatp1). This line has higher free sugar content than its genetic background showing 1.43% of glucose, 0.58% of fructose and 0.34% of sucrose, and has been proposed to be used as biomass for bio-hydrogen production. In order to look for the bio-fuel potential within chilean germoplasm we compared free sugar and starch content in native and improved varieties, and we used potato extracts as substrate in Rhodobacterium culture reactors to produce bio-hydrogen. We screened seven native potato varieties and over 20 improved varieties. Our findings show that native chilean varieties have low levels of free sugars, with 0.38% of glucose, 0.1 % of fructose and 0.63% sucrose in the varieties with higher free sugar yield. Despite this, the amount of glucose that can be extracted from starch in native varieties (up to 22% of its fresh weight) is considerably higher than the transgenic line (8.4%). The extracts used for photo-fermentation to produce bio-hydrogen demonstrate that the best native line yields 8.4 fold of hydrogen than the transgenic line. Our projections estimate that with an hectare of the best native chilean variety could yield in optimized fermentator conditions enough bio-hydrogen to move a commercial available hydrogen car for approximately 10,000 kilometers.

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P 119

DIVERSITY IN CAPTURE AND USE SOLAR RADIATION OF NATIVE POTATOES

Carolina Lizana [1]
carolina.lizana@uach.cl

[1]Instituto de Producción y Sanidad Vegetal, Universidad Austral de Chile, Valdivia, Chile

Resources efficiency use by crops is a priority to increase crop production. Native potato genotypes have wide variability in architecture that could change its radiation use efficiency and provide useful traits for plant breeding. The objective of the study was to evaluate the efficiency of radiation capture of native potato germplasm and its association with productivity. We used eight native potato genotypes (239UA1388, NG133, NG6, NG5, NG85, 275CON756, 38CON918, 453CON901) interesting for high content of phenolic compounds in the skin and flesh. We determined the radiation interception, leaf area index, leaf size, leaf angle, yield and its components, dry matter percentage of tubers, specific gravity and percentage of starch. The results showed a wide variation in the structure of the canopy. Leaf angles varied between 53 and 85 ° (from vertical) and the average leaf size between 6.8 and 23 cm2. Leaf area index was not significantly associated with the efficiency of radiation, but the leaf angle ($r2 = 0.32$, $P < 0.05$) and the average size of leaves ($r2 = 0.37$, $P < 0.05$) were positively associated with this trait. Genotypes with higher efficiency in radiation use had a higher specific weight of tubers (1.09) and higher percentage of starch (22%). One of the genotypes with higher EUR and better yield performance has the highest concentration of phenolic compounds making it promising for use in potato breeding.

P 120

MOLECULAR VALIDATION OF SOPHORA TOROMIRO SPECIMENS AND IN VITRO PROPAGATION

Jessica Devia[2], Carolina Serrano[1], Jaime Espejo[3], Patricio Arce-Johnson[1]
jidevia@gmail.com

[1]Departamento de Genética Molecular yMicrobiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile

[2]Programa Doctorado Ciencias Silvoagropecuarias y Veterinarias, Facultad de Ciencias Agronómicas, Universidad de Chile

[3]Empresa Forestal Mininco

Sophora toromiro, is a forestry species, originally described as endemic to Rapa Nui or Eastern Islands, now extinct in the wild. Some specimens remain in botanical gardens in Chile and others have been maintained and propagated in European greenhouses. While several copies of toromiro trees are present in different places, it is unknown if all of these are indeed within the same species, therefore to identify and propagate genuine individuals is a very important work in the preservation of this species. This work involve the analysis of more than 20 specimens of toromiro found in private collections, an specimen from a Chilean National Park and also a sample from the original Sophora toromiro tree preserved in the Museum of Natural History of Santiago, as a control for genetic validation of the species, making a difference with others previous studies. DNA samples were obtained from leaves of the different specimens for molecular analysis using inter-SSR molecular markers. This assay allows differentiate those Sophora toromiro plants free of genetic hybridization. Subsequently, to confirm this information with accuracy, we used capillary electrophoresis with the specimens that form the basis of our ex situ germplasm bank. The specimens validated by molecular techniques, are being propagated in vitro using seeds and shoots collected from adult trees. We established a disinfection protocol for cuttings and another for seeds, obtaining as a result a 77,3 % of cutting sprouted and 100% germination, respectively. The shoots obtained are being maintained in multiplication stage and different concentrations of auxin were tested until a suitable rooting. The micropropagation results are very promising since toromiro is a recalcitrant species and currently part of this collection is successfully maintained under in vitro conditions.

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P 121

IDENTIFICATION AND BIONFORMATIC ANALYSIS OF XYLOGLUCAN ENDOTRANSGLYCOSYLASE/HYDROLASE (XTH) GENES FROM FRAGARIA VESCA

M.C. Opazo[1], H. Brumer[2], R. Herrera[1], M. A. Moya-León[1]
amoya@utalca.cl
[1]Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Casilla 747, Talca, Chile.
[2]Division of Glycoscience Royal Institute of Technology (KTH) Stockholm, Sweden.

XTH proteins have been detected in all plant cell types and diverse studies have described the expression of the associated gene during cell growth and differentiation. It is known that XTH genes belong to a multigenic family, and with the aim to identify XTH genes in F. vesca, the database located at http://www.strawberrygenome.org was used. A tblastn search was performed using the protein sequences of Arabidopsis thaliana XTHs, selecting one gene sequence from each group (I, II, IIIA and IIIB). The search allows the identification of 26 genes encoding for XTHs. The name for each gene was given according to the A. thaliana XTHs available nomenclature. Analysis of the number of introns and exons was made for each gene. A phylogenic analysis of the different XTHs identified was performed. The results obtained allowed the classification of F. vesca XTHs genes: 9 genes belong to group I, 12 genes to group II and 5 genes to group III (2 to group IIIA and 3 to group IIIB). Interestingly, in the wild strawberry two genes from group IIIA were identified, indicating that they could present only one catalytic activity: xyloglucan hydrolase. Based on the crystal structure of an XTH (PDB id: 1UN1) an alignment analysis was performed to identify structural elements shared between selected F. vesca amino acid sequences and XTH protein. The analysis revealed the existence of shared structural elements such as the catalytic site, shown as DEIDFEFLG, which is highly conserved. Also the characteristic pattern of b-sheets and the a-helices at the N-terminal were present. This work is the initial step for the complete analysis of the Fragaria vesca XTH family.

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P 122

IDENTIFICATION OF DROUGHT TOLERANCE GENES IN QUINOA USING A TRANSCRIPTOME ANALYSIS APPROACH

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

andreamoralesa@gmail.com

[1]Centro Estudios Avanzados en Zonas Áridas, CEAZA. [2]Laboratorio de Genómica Funcional y Bioinformática, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile.

Chenopodium quinoa is an important grain crop from the Andean region of South America. Quinoa has gained international attention for its high nutritional value; the protein quality and quantity in quinoa seed is often superior to more common cereal grains. Therefore it is considered well balanced for human and animal nutrition, similar to milk protein. Moreover, quinoa is characterized by its ability to tolerate several abiotic stresses.

Field determinations had shown that quinoa is tolerant to drought. We have assessed drought tolerance in a Chilean ecotype, R49, in order to find quinoa´s genes related to this abiotic stress. We performed a strategy with two different approaches: the first one will allow unravelling stress tolerance genes without any prior knowledge of the genome based on the expression of drought tolerance quinoa cDNA libraries in the plant *Arabidopsis thaliana*. A cDNA drought tolerance quinoa library was constructed and used to transform Arabidopsis through *Agrobacterium*-mediated gene transfer. We are analyzing 350 different transgenic Arabidopsis lines using Poliethylenglicol 8000 to reduce the water potential into the medium and we have some candidate lines with tolerance to drought.

For the second approach, we are using a massive sequencing technology, ILLUMINA, to obtain information on the quinoa transcriptome. We sequenced adult quinoa transcriptome in two different conditions, drought and control conditions. We have 103 x 10^6 paired end reads, that assembled into 150,952 contigs, and 18,124 contigs over 1 kb. We performed a digital expression analysis with these contigs and we have identified 737 differential expressed genes with >4 fold of change (p-value < 0.01). We found 527 genes overexpressed by drought conditions, where 237 contigs exhibited homology to known genes, 33 contigs with homology to unknown or hypothetical proteins and 257 contigs without homology to public databases.

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