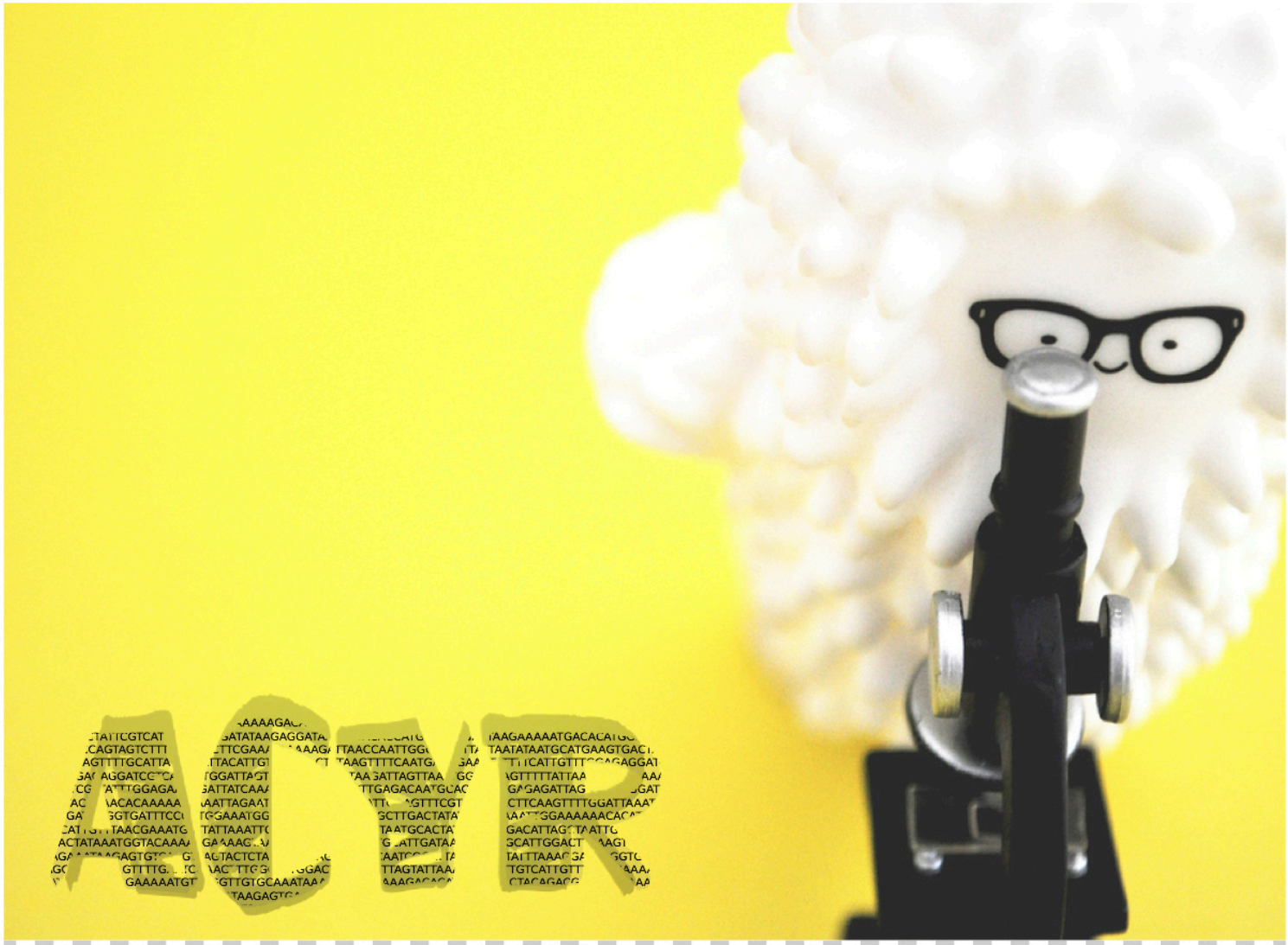

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June 2nd

Monday morning

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Opening talk

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June 3rd

Tuesday morning

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5M2	**Alexandra Contreras Jodar**	Signals of recent selection across the genome of Iberian compared with Landrace pig breeds.	*June 3rd*	*10.00 - 10.30*

Plant Development and Signal Transduction

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Plant Responses to Biotic and Abiotic Stress

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3T2	**Mireia Bundó Barberà**	The role of OsCPK4 in the defense signaling of rice plants.	*June 2nd*	*15.00 - 15.30*
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Plant Development and Signal Transduction

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P3	**Maria José Molina Contreras**	About the dual action mechanism and the importance of N-terminal region of ATHB4 in the Shade Avoidance Syndrome
P4	**Mariana Bustamante**	Genomic analyses of the CUC1 network

Plant and Animal Genomics

P5	**Irene Julca Chávez**	Identification and validation of genes responsible of agronomic traits in peach (Prunus persica L. Batsch)
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Plant Responses to Biotic and Abiotic Stress

P14	**Marina Puigvert Sánchez**	Negative retrocontrol of the hrp genes in the bacterial plant pathogen Ralstonia solanacearum

BRIARDO LLORENTE

Reviving the past to understand the present

The plastid proteome built up over evolutionary time both by proteins of endosymbiotic origin and proteins acquired via horizontal gene transfer (HGT)[1,2], yet much remains unknown about the forces shaping its composition. Here we describe and experimentally reconstruct the ancient HGT of a bacterial polyphenol oxidase (*PPO*) gene to the nuclear genome of an early common ancestor of all land plants, inferring how this bacterial enzyme evolved into a plastidial form driven by natural selection. Using *Arabidopsis thaliana*, which belongs to the only known plant lineage that lost all PPO-coding genes, we recapitulated the PPO bacteria-to-plants transition by expressing an ancestral-like version of PPO lacking subcellular localization signals and a modern version harboring a plastid transit peptide. Expression of the ancestral-like enzyme was associated with reduced growth rates and delayed bolting, suggesting that directional selection favored the fixation of the modern plastidial PPO. By recreating the likely evolutionary history followed by PPO circa 500 millions years ago, we have identified forces underlying plastid proteome evolution. Ultimately, the study emphasizes the usefulness of recreating parallel evolutionary scenarios *in vivo* to directly examine evolutionary processes.

ROSSANA HENRIQUES

New tools for old traits

SESSION 1M

1M1: Characterizing Flavonoid content of Strawberry fruit in a F.vesca NILs population

María Urrutia-Rosauro[1], **Amparo Monfort**[1]

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Strawberry is a general term to refer to several species from the *Fragaria* genus (*Rosaceae* family).The genus *Fragaria* includes species with different levels of ploidy, ranging from diploid (mainly wild or woodland strawberries, ex. *F.vesca*) to octoploid (mainly commercial varieties ex. F.x ananasa). The diploid strawberry *F.vesca* is the model species for the *Fragaria* genus due to the sequenced and relatively small genome (240 Mb) [1] and the high degree of synteny that it shares with the octoploid species [2].

Strawberries have a great economic importance in Spain as it is the 1st exporter and the 4th producer worldwide (data from FAO, http://www.fao.org/economic/ess/ess-trade/en/). Furthermore it's a highly appreciated product by consumers for its nutritional properties, especially for its antioxidant capacity, mainly due to its flavonoid content. That is why many efforts are addressed to develop new strawberry varities with enhanced quality traits.

Our group has previously developed a Near Isogenic Lines (NILs) population with a genetic background from *F.vesca* and homozygous overlapping introgressions from *F.bucharica.* This population comprises 42 lines and has been mapped using a set of 80 SSRs. It has shown a wide range of segregating characters including total polyphenol content, and has proved being useful for detecting several QTLs for agronomical quality traits.

We have recently focused our interest in further characterize the flavonoid composition of wild strawberry fruit and to find QTLs responsible for the observed differential accumulation of specific compounds among our population. In order to do so, we performed an untargeted LCMS experiment in two consecutive years followed by a QTL statistical analysis. As a result we were able to detect more than 900 unknown compounds, unambiguously identify 23 of them and map several QTLs for most of the compounds.

References:
1. Shulaev *et al*. (2011). Nat genetics , vol 43, num 2, Pages 109-116
2. Rousseau-Gueutin *et al* (2008). Genet, vol 179 . Pages 2045-2060

Acknowledgments: Project funding by AGL2010-21414. Fellowships: BES-2011-043633

1M2: Chromorespiration as a novel bioenergetic process identified in tomato fruit chromoplasts

Marta Renato[1,2], Albert Boronat[2], Joaquín Azcón-Bieto[1]

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Chromoplasts are plastids specialized in the synthesis and accumulation of carotenoids. During tomato (*Solanum lycopersicum*) fruit ripening chloroplasts differentiate into chromoplasts, losing their photosynthetic activity and synthesizing large amounts of carotenoids. Chromoplasts present a barely studied electron transport chain ending in O_2 uptake activity, known as chromorespiration. In this work, respiratory assays using chromoplasts isolated from tomato fruit were conducted. The obtained results showed that O_2 uptake activity of chromoplasts is stimulated by the electron donors NADH and NADPH and repressed by octyl gallate, an inhibitor of the plastidial terminal oxidase (PTOX). O_2 uptake also responds to the uncoupler carbonylcyanide m-chlorophenylhydrazone (CCCP), indicating that a membrane proton gradient develops inside the chromoplast. The ATP synthesis rate of isolated chromoplasts was evaluated. It was found that ATP synthesis is dependent on NAD(P)H, is inhibited by octyl gallate and CCCP, and is sensitive to 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), an inhibitor of the cytochrome b_6f complex. Cyt *f* was detected in chromoplasts samples by immunoblot analysis and was localized in inner chromoplastic structures by TEM. Taken together, these results strongly suggest that cyt b_6f is present in chromoplasts and participate in chromorespiration. To analyse the significance of chromoplast respiration in tomato fruit during ripening, the effect of octyl gallate on oxygen uptake and ATP content was studied in pericarp tissue samples of fruits harvested at different ripening stages. Our results suggest that chromorespiration increases during ripening, being responsible of about 26% of total oxygen consumption in ripe fruit, and represents a relevant contribution to ATP synthesis in red pericarp. Preliminary studies are being conducted to identify additional components involved in chromorespiration, like a type-II NAD(P)H dehydrogenase and the cytochrome c_6.

Acknowledgments: This work was supported by grants of the Spanish Ministerio de Ciencia e Innovación (BIO2009-09523 to A.B., including European Regional Development Funds), the Spanish Consolider-Ingenio 2010 Program (CSD2007-00036 Centre for Research in Agrigenomics) and the Generalitat de Catalunya (2009SGR0026). M.R. is recipient of a predoctoral fellowship from the Spanish Ministerio de Educación, Cultura y Deporte.

SESSION 2M

2M1: Genetic dissection of fruit ripening in the melon climacteric line SC3-5-1

P. Rios-Rodriguez[1]*, J. Vegas[1], W. Sanseverino[1] and J. Garcia-Mas[1]

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Fruit ripening is a complex metabolic and physiological process that is highly regulated and has a great influence in the organoleptic quality and economical value of the fruit. Ethylene is the plant hormone that regulates ripening and, depending on its expression pattern, fruits can be classified as climacteric and non climacteric. The existence of both climacteric and non-climacteric genotypes and the advances in the development of genetic and genomic tools make melon a suitable model for fruit ripening studies. In previous studies, two QTLs for climacteric fruit ripening, *ETHQB3.5* and *ETHQV6.3*, were detected in the climacteric near isogenic line SC3-5-1 containing two introgressions of the accession PI 161375 in the genetic background of 'Piel de Sapo' (PS), the two being non-climacteric varieties. Both QTLs are capable of inducing climacteric ripening alone, but when together there is a stronger phenotype due to an epistatic interaction (Vegas et al. 2013). In this work, a new segregating population containing only *ETHQV6.3* has been developed aiming for the fine mapping and positional cloning of the QTL and, complementarily, an RNA-seq of the fruit has been performed between the unripe and ripe stages of melon lines PS and SC3-5-1 in order to understand the transcriptomic changes associated with climacteric fruit ripening.

Interaction between QTLs induces an advance in ethylene biosynthesis during melon fruit ripening - Vegas, J., Garcia-Mas, J., and Monforte, A. (2013) Theor Appl Genet, 126(6): 1531-44

Acknowledgments: Project funded by the MEC project AGL2012-40130-C02-01

2M2: Analysis of transposon-related structural variation in melon

Cristina Vives, Josep Casacuberta

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Massive sequencing of crop varieties allows correlating genetic and phenotypic variation. Recently, melon genome (DHL92, a double haploid line) has been sequenced and transposons have been annotated using different bioinformatic approaches.
Moreover, seven melon varieties, including several cultivated varieties, have been resequenced.
The purpose of this study is to analyze the impact of transposition on gene and genome evolution between these seven varieties. To this end, we identified polymorphisms due to the presence or absence of transposable element at a given loci. The analysis of transposon polymorphic insertions can help to redefine and characterize better some transposon families, allows to identify the transposon families recently active in the melon genome and provides new information on genetic polymorphisms that can be linked to traits selected during melon domestication and breeding. All these new information will shed light on the impact of transposons in the evolution of plant crops.

2M3: Movement protein of cucumber mosaic virus determines systemic infection in the melon accession PI161375

Cèlia Guiu-Aragonés*[1], Juan A. Diaz-Pendón[2], Eduardo Peña[3], Manfred Heinlein[3] and Ana Montserrat Martín-Hernández[1]

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Resistance to CMV in the exotic melon accession PI161375 shows a complex mixture of qualitative and quantitative resistance, depending on the strain. Previously, we had reported the presence of a recessive gene (*cmv1*) in the linkage group XII conferring total resistance to CMV strains of subgroup II, but not to strains of subgroup I. Strains of subgroup II are probably not able to interact with *cmv1* to complete their infectious cycle, those of subgroup I can interact.
Using infectious clones of strains LS (subgroup II) and FNY (subgroup I) we have made combinations between RNAs of both strains showing that the determinant of the virulence is located in RNA3. Chimaeras between FNY and LS showed that the determinant of the virulence is in the N-terminal 208 amino acids of movement protein (MP). We are currently trying to identify the specific amino acid changes that confer virulence to overcome the resistance provided by *cmv1*.
We have also analysed the mechanisms of the resistance provided by *cmv1*. We report that the strain LS is able to replicate and to move cell to cell in the inoculated leaf of the resistant line. However, the virus is not able to invade the sieve elements of the resistant line. Our current work is focused on identifying the cell type of the phloem tissue where the virus invasion is interrupted.
Altogether, our results demonstrate that the resistance determined by *cmv1* involves interruption of the virus entry into the vascular system and therefore, inability to develop a systemic infection.

Acknowledgments: The work was funded by grant AGL2009-12698-C02-01/AGR from Spanish Ministerio de Ciencia e Innovación, and grant Consolider CDS2007-00036 from the Spanish Ministerio de Educación y Ciencia. CG-A was supported by grant BES-2010-030274 from Ministerio de Ciencia e Innovación

3T1: A new gene product present in the genome of Sweet potato feathery mottle virus (SPFMV)

Ares Mingot[1], Adrian Valli[2], Lluïsa Vilaplana[1], Juan José López-Moya[1]

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Sweet potato feathery mottle virus (SPFMV) is a member of the potyvirus genus that infects sweet potato (*Ipomoea batatas* (L.) Lam., family *Convolvulaceae*), the 6th most important food crop in the world. SPFMV causes grave symptoms and significant yield losses in this crop when it is present in mixed viral infections together with the unrelated crinivirus Sweet potato chlorotic stunt virus (SPCSV).
Potyviruses are RNA viruses with a common genomic organization. Recently, a novel ORF has been identified “in silico” in the genome of SPFMV and other viruses in the same lineage of potyviruses infecting sweet potatoes. The new ORF, tentatively called PISPO, can be found embedded within the P1 sequence region, and it could result in the production of a new gene product P1N+PISPO through frameshift translation starting in a conserved GGAAAAA domain.
The presence of this new gene product raises several questions about its role. The RNA silencing suppressor (RSS) activity in potyvirids is often found in P1 or HCPro. Interestingly, the RSS has not been described yet in the case of SPFMV, and the modes of action of other RSSs in sweet potato viruses are different to the ones previously reported in related viruses infecting other plant hosts.
To gain insights into the peculiar relationships between sweet potatoes and the viruses infecting them, we have initiated work with new SPFMV isolates found on commercial sweet potato samples. We have characterized these new isolates by sequencing different regions of the virus genome, setting up a detection method, and started to determine their host-range on different plant species. Analysis of the 5' region showed the presence of the expected 232-residues long PISPO sequence downstream of the conserved frameshift signature. Also, we have cloned different gene products (P1, P1N+PISPO and HCPro) in order to identify the corresponding viral RSS and try to establish their functions during the virus cycle.

Acknowledgments: Project funded by grant AGL2010-14949. AMM is recipient of a FPI fellowship BES-2011-045699.

3T2: The role of OsCPK4 in the defense signaling of rice plants

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Calcium-dependent protein kinases (CDPKs or CPKs) are protein kinases that directly bind calcium before phophorylating substrates involved in stress signaling pathways and other cellular processes. Ca^{2+} and phosphorylation are the main second messengers in the signaling network in eucaryots, and CDPKs integrate both signals in a single polypeptide chain. Thus, CDPKs play an important role in plant signaling. CDPKs constitute a large multigene family, for which a functional diversification has been proposed. Several CDPKs have been implicated in stress signaling in rice plants. Rice is the second more important cultivated crop worldwide and blast disease, caused by *Magnaporthe oryzae*, is the most devastating fungal diseases affecting rice production. Rice plants are able to defend themselves against most of the pathogens, and only a few successful pathogens are able to overcome the plants´ defenses. Therefore, there is the need to understand how rice plants sense the pathogen attack and characterize their defense response to develop strategies to improve rice blast disease resistance. We previously found that the expression of *OsCPK4* is induced after fungal elicitor treatment in rice plants, suggesting that this specific isoform might have a role in signaling defense responses. Our aim is to elucidate the function of OsCPK4 in the signaling cascade triggered by *M.oryzae* infection in rice plants. With this objective, gain-of-function plants by overexpressing constitutively the *OsCPK4* gene were generated and tested for resistance-susceptibility against *M.oryzae* infection. These plants were found to be more resistant than control plants to the infection, presumably due to a priming state of the defense response. The *OsCPK4* overexpression seems to promote also the accumulation of salicylic acid and the callose depositions, both typical defense plant responses. These results demonstrate the functional role of this CDPK in the rice defense and the potential as a target to improve disease resistance of rice crop.

3T3: Understanding drought response in maize: characterization of a novel transcription factor related to ABA signaling

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Abscisic acid (ABA) is the major phytohormone that mediates drought responses in land plants. ABA triggers several signaling transduction pathways that lead to an altered gene expression with effects on stress response and acquisition of tolerance. Protein kinases and phosphatases are of dominant importance in ABA signal transduction and among these proteins, a subgroup of the SnRK2s, including OST1, has been shown to be a major hub of this pathway[1]. When ABA levels increase, SnRK2 becomes active affecting other proteins and cellular processes, such as the activation of ABF transcription factors by phosphorylation altering gene expression.
However, a full description of the ABA-induced transcriptional regulation may also include the identification of alternative SnRK2 dependent transcriptional regulators. *Zea mays* SnRK2.8 (ZmOST1) has been identified as the functional homolog of arabidopsis OST1[2]. To isolate new transcriptional targets affected by SnRK2 activity in maize, we started this work performing a yeast two hybrid assay using ZmOST1 as a bait against a library of drought stressed leaves. This technique yielded, between others, a Zn-finger type transcription factor as a potential target. This protein is homologous to an arabidopsis regulator of proton responsive gene expression, AtSTOP1. Here, we corroborate the *in vivo* interaction between these two proteins, and we show ZmSTOP1 to be a phosphorylation substrate of ZmOST1. To assess ZmSTOP1 biological function, we are currently characterizing its implication in stomatal conductance and its function in ABA and in other abiotic and biotic stress responses.

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4T1: How chromatin modulates circadian clock function in *Arabidopsis thaliana*? A search for novel candidates and mechanisms

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Most living organisms exhibit a rhythmic pattern of activity that synchronizes every day with the 24-hour day-night cycle. The molecular machinery responsible for generating these biological or circadian rhythms is known as circadian clock. In plants, the molecular clockwork consists of a set of circadian regulated genes that are expressed at different phases during the day and night. These set of genes regulate each other's via complex transcriptional regulatory networks. Increasing evidence also highlights the importance of epigenetic marks modulating circadian gene expression at the core of the clock. However, we are still far from a complete understanding of the components and mechanisms linking chromatin remodeling with the circadian clock. In this context, my PhD project will focus on characterizing chromatin remodeling factors and their involvement in the regulation of circadian gene expression. Plants mis-expressing selected candidates (displaying an oscillatory pattern in their expression) will be used to examine altered circadian rhythmicity. Combining results of ChIP-Seq and RNA-Seq approaches will also provide a global view of the epigenetic and transcriptional networks underlying the function of these chromatin-related components and their connection with the clock. Protein immunoprecipitation followed by mass spectrometry analysis will also provide insightful information about how protein complexes assemble to generate higher-order structures essential for clock function.

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4T2: Regulation of plant stem cell quiescence by a novel Brassinosteroid signaling module

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The quiescent center (QC) maintains the activity of the surrounding stem cells within the root stem cell niche, yet specific molecular players sustaining the low rate of QC cell division remain poorly unknown. Here, we identified a R2R3-MYB transcription factor, *BRAVO (BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTRE),* acting as cell-specific repressor of QC divisions in the primary root of Arabidopsis. Ectopic BRAVO expression restricts overall root growth and ceases root regeneration upon damage of the stem cells, demonstrating the role of BRAVO in counteracting Brassinosteroid (BR)- mediated cell division in the QC cells. BR-regulated transcription factor BES1 (BRI1-EMS SUPRESSOR 1) directly repress and physically interacts with BRAVO *in vivo*, creating a bistable switch that modulates QC divisions at the root stem cell niche. Together, our results define a mechanism for BR-mediated regulation of stem cell quiescence in plants.

SESSION 5M

5M1: A novel mechanism to control SUMOylation by SUMO E1-activating enzyme.

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Small-Ubiquitin-related MOdifier, SUMO, is a post-translational modifier belonging to the Ubiquitin family. SUMOylation consists on a covalent and reversible SUMO attachment to a lysine residue in the substrate. In plants, SUMO conjugation controls different biological processes: ABA signalling, development, biotic and abiotic stress responses. However the molecular mechanisms controlling SUMO conjugation *in vivo* are far from being understood.
Arabidopsis thaliana have eight putative SUMO isoforms; although only four were found to be expressed: *At*SUMO1, 2, 3 and 5 (Kurepa et al., 2003). We analysed the biochemical properties of these paralogues and we found that the surfaces involved in these interactions are well conserved in *At*SUMO1/2 isoforms, whereas *At*SUMO3 shows a lower degree of conservation, and *At*SUMO5 is the most divergent isoform. We have observed that *At*SUMO3 is less efficiently conjugated than *At*SUMO1/2, and *At*SUMO5 shows the lowest conjugation level *in vitro*. Our results provide evidence for the preferential conjugation of *At*SUMO1/2 compared with *At*SUMO3/5 and that is based on SUMO paralog selection by the SUMO activating enzyme (E1).
The E1 is the first control point in the regulatory pathway; is a heterodimer consisting of a large subunit, SAE2, and a small subunit, SAE1. We focused the study on the E1 diversification; SUMO conjugation rate is dependent on the SAE1 isoform contained in the E1 holoenzyme. In order to explore SUMO conjugation *in vivo atsae1a* mutant plants were characterized; these plants displays SUMOylation defects upon abiotic stress, consistent with a SUMOylation defective phenotype. Interestingly, we have identified SAE2 as a putative SUMO substrate, deeper knowledge will allow us to better understanding regulation of SUMO post-translational modifications in plants.
Overall, these results point to a regulatory role of the SUMO E1-activating enzyme during SUMO conjugation and provide a novel mechanism to control SUMOylation in vivo by diversification of the E1 small subunit.

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5M2: Signals of recent selection across the genome of Iberian compared with Landrace pig breeds.

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Modern breeding practices lead to the formation of the currently European commercial breeds with different morphologic and productive characteristics. Meanwhile, still remain traditional breeds as the Iberian pig that has not been improved because it has remained isolated for centuries. While Iberian breed exhibits an extreme trend to obesity and appetite and a great capacity to accumulate intramuscular and subcutaneous fat, Landrace is an international breed developed and improved by selection. It is one of the most productive breeds due to their low feed conversion rate, good daily gain and high lean meat content.
Here we describe the use of whole genome sequencing of two opposite breeds for growth, fatness and meat quality to identify regions with selective sweeps and candidate mutations that may have had an important role in the phenotypic differentiation of these breeds. Thus, we report, after quality control, a total of 4,555,419 autosomal and shared SNPs between breeds to calculate and contrast the decay of a single site between Landrace and Iberian breeds by Extended Haplotype Homozigosity (EHHS) as described TANG *et al.* (2007) in humans. Therefore, a total of 129 EHHS intervals were identified displaying significant over-representation of Gene Ontology Biological process terms like muscular tissue development, digestive system, retinol metabolism and fatty acid oxidation, immunology and reproduction among others. Moreover, these intervals overlapped with many QTL related to fatty acid composition, intramuscular and subcutaneous fat content.
On the other hand, fixed SNPs in Iberian breed and at low frequency in Landrace were annotated and functionally analyzed. The main overrepresented pathways were metabolism of lipids and lipoproteins for non-synonymous coding, upstream and 5'UTR and ribosome and spliceosome for 3'UTR SNPs and candidate genes were annotated to functional validation. These results will help in the identification of genomic regions and genes relevant for the application of better selection strategies in the pig industry.

Tang, K., K. R. Thornton and M. Stoneking, 2007 A new approach for using genome scans to detect recent positive selection in the human genome. PLoS Biol 5: e171.

5M3: MicroRNAs in rice innate immunity

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MicroRNAs (miRNAs) are small non-coding RNAs that regulate eukaryotic gene expression. They negatively regulate gene expression by triggering mRNA degradation or translational repression of the cognate mRNA. Increasing evidence also support that microRNAs (miRNAs) play a fundamental role in the plant response to pathogen infection. Most of these studies have been approached in bacterial resistance (Navarro et al. 2006). To identify miRNAs potentially involved in rice immunity, we performed deep sequencing of small RNA populations from rice tissues treated, or not, with elicitors prepared from the fungal pathogen *Magnaporthe oryzae.* This fungus is the causal agent of the rice blast disease, one of the most devastating diseases of rice worldwide. Based on its economic and scientific relevance, rice blast is considered to be among the 10 most important fungus-caused diseases in plants (Dean R. et al 2012).The rice/*M. oryzae* pathosystem is also a model in the study of plant-microbe interactions (the genome sequence of the two partners is available). High-throughput sequencing of small RNA libraries prepared from elicitor-treated rice tissues revealed dynamic alterations in the accumulation of a diverse set of known microRNAs, These studies also allowed us to identify novel miRNAs from rice (Campo S. et al. 2013). Global transcriptome analysis and degradome sequencing is being used to identify targets of pathogen-regulated rice miRNAs. Finally, the identification of rice mutants affected in small RNA biogenesis is also pursued in our group. The discovery of novel pathogen-regulated miRNAs and their mode of functioning will improve our understanding on the adaptation of rice plants to pathogen infection.

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6M1: *Ralstonia solanacearum* AWR5 effector acts as an inhibitor of the TOR signalling pathway

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The present work focuses on the characterization of a multigenic family of 5 type III effectors called AWR from the plant pathogen *Ralstonia solanacearum*. These effectors are involved in bacterial infection as previous experiments *in planta* showed that their deletion renders the bacterium less virulent on tomato (Solé et al., 2012). Our previous work showed that AWR5 protein causes growth inhibition phenotypes on budding yeast *S. cerevisiae*. Expression of T3SS effectors in *Saccharomyces cerevisiae* oversteps the limitations of their study in plants, as yeast lacks resistance (R) proteins that can trigger ETI responses.
In order to characterize AWR5 function, we performed a gene expression profiling using heterologous expression of AWR5 in the yeast *Saccharomyces cerevisiae*. Expression of AWR5 in yeast has showed a gene expression profile reminiscent of TOR complex inhibition: down-regulation of genes involved in ribosome biogenesis or rRNA processing and up-regulation of genes involved in the metabolism of nitrogen. Data will be presented on the molecular mechanism(s) underpinning these AWR5-dependent changes in gene expression in *S. cerevisiae*. We are currently investigating the AWR5 effect on TOR pathway on non-host *Nicotiana benthamiana,* where it causes hypersensitive response-like cell death.
This research project will unravel the mechanism of action of a type III effector which has a dramatic impact on plant physiology.

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The *awr* gene family encodes a novel class of *Ralstonia solanacearum* type III effectors displaying virulence and avirulence activities.
Solé M, Popa C, Mith O, Sohn KH, Jones JD, Deslandes L, Valls M..
Mol.Plant.Microbe Interact. 2012, 25 (7), 941-953.

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6M2: RVE8/LCL5 and the LIFs Regulates Responses to Biotic Stress in a Circadian Fashion

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The circadian clock is the molecular mechanism responsible for generating 24-hour rhythms in biological processes. Circadian clocks are ubiquitously present in nature, from bacteria to flies and humans. In plants, many crucial players of the circadian network have been already described. However, little is known about how these circadian components connect the clock with plant physiology and metabolism. In our studies, we have analysed the role of the clock component *RVE8/LCL5* (*REVEILLE8/ LONG HYPOCOTIL CIRCADIAN CLOCK ASSOCIATED LIKE5*) modulating plant responses to biotic stresses. *RVE8/LCL5* is a MYB transcription factor that positively regulates the central clock component *TOC1 (TIMMING OF CAB EXPRESSION1*) by direct binding to its promoter and facilitating histone acetylation. Our preliminary studies have shown that plants mis-expressing *RVE8/LCL5* displayed altered responses to bacteria and fungus infection. Yeast-two hybrid analysis has also shown that RVE8/LCL5 interacts with four proteins belonging to the same family (LIFs, LCL5 Interacting Factor). Analysis of *lif* mutant plants showed altered responses to pathogen infection, suggesting that RVE8/LCL5 and LIFs modulate the circadian timing of plants responses to pathogen attack. Future studies involving changes in chromatin remodelling and the molecular mechanisms underlying RVE8/LCL5 and LIFs function will provide further insights into how RVE8/LCL5 and LIFs connect the circadian clock with plant responses to biotic stress.

6M3: The role of miRNAs in rice immunity

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Rice (*Oryza sativa*) is one of the most important crops in the world with very high economical and social relevance. It is the staple food for more than half of the human population. Rice diseases caused by pathogens are responsible of important yield loss. The most devastating disease worldwide is rice blast caused by the fungus *Magnaporthe oryzae*. Plants, including rice, are well capable of defending themselves against most pathogens through innate immunity, although successful pathogens overcome the plant defenses. Emerging evidences support the notion that microRNAs (miRNAs) have a regulatory role of the plant responses that counteract pathogen infection. Our objective is to find some rice miRNAs functionally involved in the defense response against *M. oryzae* infection. For this purpose, we selected 10 known rice miRNAs based on their differential expression under *M. oryzae* elicitor treatment in a small-RNAseq analysis. The bioinformatics target prediction of the selected miRNAs identified putative target genes with roles in disease resistance, defense signaling and other defense-related responses. By degradome analysis, we validated 8 target genes for 7 of the selected miRNAs by finding the corresponding cleaved transcripts, most of them with putative functions in defense response. In addition, the "*in silico*" analysis of promoter sequences showed that most of the selected miRNAs and their putative targets contain promoter motifs related to defense response. The 10 selected miRNAs are generated from 7 different miRNA precursors. We isolated and cloned these precursors in plant transformation vectors. Transient expression assays using the obtained plant transformation vectors showed that precursors were efficiently processed to the corresponding mature miRNAs in *Nicotiana benthamiana*. Nowadays, we are generating transgenic rice plants for the overexpression of each of these 7 miRNA precursors. These plants will be useful for the functional characterization of the selected miRNAs in the rice defense response against *M. oryzae*. Our studies will provide us with important insights on the molecular mechanisms mediated by miRNAs in rice immunity that eventually will help to develop strategies to obtain resistant plants to blast disease.

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7T1: *In vivo* regulation of SUMO conjugation

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Posttranslational modification with Small Ubiquitin-related Modifier (SUMO) is an essential regulatory mechanism of protein function in eukaryotes. In plants, genetic studies have established a role for SUMOylation in plant development and environmental stress responses. However, the molecular mechanism through which SUMO regulates those biological processes is poorly understood.
SUMO is synthesized as a precursor that is processed by the specific ULP proteases. As a first step in SUMO conjugation, the mature SUMO is activated by the heterodimeric E1 activating enzyme in an ATP-dependent reaction. Next, SUMO is transferred to the E2 conjugating enzyme, which is competent for transferring SUMO to a lysine in the target substrate, although this reaction is facilitated by E3 ligases.
In previous studies, our group has focused in the study of the regulatory role of the heterodimeric E1 (SAE2/SAE1) activating enzyme. We showed that the SUMO E1 contributes to SUMO paralog discrimination, providing a novel mechanism to favor conjugation of the essential AtSUMO1/2 paralogs. In addition, we have established that evolutionary diversification of the E1 small subunit, SAE1, contributes to regulation of SUMO conjugation rate, suggesting that in vivo dynamics of SUMO conjugation could be mediated by the E1 activating enzyme.
Recent results pointing to the existence of novel posttranslational modifications that could modulate SUMO activation in vivo will be presented.

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7T2: A cellular dynamics approach to understand Brassinosteroid contribution to root growth in *Arabidopsis thaliana*

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Organ growth is the result of a fine coordination between cell division and cell expansion in multiple organisms. In plants, the primary root of Arabidopsis exhibits spatially separated zones for cell division (meristem zone, located at the root tip), and cell elongation (elongation zone, located immediately above the meristem). Plant growth regulators Brassinosteroids (BRs) control root growth by controlling different cellular activities, i.e. the normal progression of the cell cycle in the meristem, cell elongation and differentation[1,2]. Despite these findings, phenotypic analysis has so far failed to establish a hierarchy for these developmental effects. Through high-resolution Confocal Laser Scanning Microscopy (CSLM) imaging we quantify multiple cellular traits in wild type and BR mutant Arabidopsis roots. Aided by mathematical modeling and automated analysis of the data we characterize how BRs drive changes in these traits and consequently modify root growth. Our study provides a new methodology to quantify morphological and dynamical cellular root growth traits and reveals that BRs have a complex role in coupling meristematic and elongation root zones.

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P1: Role of RAV1 and RAV1-like genes in floral induction

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Floral induction is probably the most important process in plant development since perpetuation of plant species depends on that. In *Arabidopsis thaliana,* floral induction is controlled by several genetic pathways that respond to either environmental and/or endogenous stimuli. *TEMPRANILLO (TEM)* genes belong to the RAV transcription factor family, a six member family that includes *RAV1, RAV1-Like , RAV2 (TEM2), RAV2-Like (TEM1)* , *RAV3* and *RAV3-Like*. It has been previously described how *TEM1* and *TEM2* control flowering time in *Arabidopsis thaliana* (Castillejo and Pelaz, 2008; Osnato et al., 2011). However, it is still unknown if other *RAV* genes are involved in controlling floral induction. Consequently, we have studied the *RAV1* and *RAV1L* contribution during the control of floral induction as well as their possible levels of redundancy with *TEM1* and *TEM2*. For that purpose, we have studied the loss-of-function *rav1* and *rav1-like* single mutants and in combination with *tem1-1* and *tem2-2.* In addition, we generated and analyzed the over-expression of *RAV1* and *RAV1L* generatig *35S::RAV1* and *35S::RAV1L* transgenic lines. Moreover, as *RAV1* gene has been described to act in response to cold temperatures (Fowler and Thomashow,2002) we studied its role in low ambient temperatures. Phenotypic analyses showed that *RAV1* seems to be involved in delaying floral induction only at low temperatures (16ºC) under both LD and SD conditions. We have also performed spatial/temporal analysis of the expression pattern of *RAV1* and *RAV1-like* by generating p*RAV1::GUS* and *pRAV1L::GUS* reporter lines. Similarly to *pTEM1::GUS* and *pTEM2::GUS* plants, *pRAV1::GUS* and *pRAV1L::GUS* expression was observed mainly in the vascular tissue of rosette leaves as well as slightly in sepals, petals and stamens. However, *pRAV1-GUS* expression, unlike *TEM1* and *TEM2*, was also detected in the inflorescence ramifications, suggesting a possible exclusively role of *RAV1* in branching.

P2: Role of the PIF3-regulated type 2C phosphatase MIDA9 in regulating seedling deetiolation

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The phytochorme (phy)-interacting basic helix-loop-helix transcription factors (PIFs) sustain the etiolated state of dark-germinated seedlings by actively repressing deeetiolation in darkness. This activity is rapidly reversed upon light exposure by phy-induced proteolytic degradation of the PIFs. Through an strategy based on microarray analysis coupled with functional profiling, we identified four PIF3-regulated genes misexpressed in the dark (MIDAs) that are novel regulators of seedling deetiolation. We are currently studying the cellular and molecular functions of one of the identified genes, MIDA9, a type 2C phosphatase involved in hook development. First, through morphologic and cellular analyses of mutant and transgenic lines, we are defining the spatial and temporal actions of MIDA9 during the different phases of hook development (i.e. formation, maintenance and opening). Second, through a yeast two hybrid screen, we are searching for putative MIDA9-interacting proteins that might be targets of the MIDA9 phosphatase activity. We will discuss how these strategies are contributing to determine the function of MIDA9 during the seedling deetiolation developmental transition.

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P3: About the dual action mechanism and the importance of N-terminal region of ATHB4 in the Shade Avoidance Syndrome

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Plants perceive the neighboring vegetation as a reduction in the ratio of red to far-red light, that produce a set of responses known as the **S**hade **A**voidance **S**yndrome (SAS). These responses are involved in different developmental changes aimed to outgrow the neighboring plants (characterized by enhanced elongation, reduced leaf expansion, decreased branching and ultimately early flowering). ATHB4 is an *Arabidopsis thaliana* member of the HD-ZIP II family of transcription factors whose expression is rapidly upregulated after plant proximity perception. Members of this family are described as transcriptional repressors. ATHB4, as others *PHYTOCHROME RAPIDLY REGULATED* (*PAR*) genes, has a role in the SAS regulation (Sorin et al., 2009) as well as controlling leaf polarity (Bou-Torrent et al., 2012). After structure-function analyses, we found that the Nterminal region, of unknown function, was fundamental for ATHB4 activity. In addition, we have hypothesized that ATHB4 has a dual molecular mechanism of action in the regulation of different physiological traits: it acts as a transcriptional cofactor in the SAS regulation, whereas it acts as a transcription factor when controlling leaf polarity. To further investigate these possibilities, we generated transgenic plants overproducing ATHB4 fused to the potent transcriptional activator VP16 protein (35S:ATHB4-VP16). In these plants we analyzed three different traits: the hypocotyl response to simulated shade, molecular analysis of marker genes of shade perception, and the phenotype of rosette's leaves. To address the putative role of the N-terminal region of ATHB4, we next analyzed the ability of the N-terminal region to interact with other proteins. The latest results will be presented.

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P4: Genomic analyses of the *CUC1* network

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The global aim of our group is the characterization and understanding of gene regulatory networks underlying plant development, using Arabidopsis flower development as our model and experimental system.
Our goal is to characterize the *CUP-SHAPED COTYLEDON1* (*CUC1*) gene regulatory network and identify its direct target genes, through the analysis of correlated transcription factor genome-wide binding (ChIP-Seq) and transcriptomic data, the same approach that led to the thorough description of the AP1 network by our group in (Kaufmann et al., 2010).
CUC1 (together with the related factor CUC2) plays a pivotal role in the separation of organs and of organs and meristems: it is expressed in organ boundaries, and acts as a growth antagonist. Previous work showed that *CUC1* is regulated by miR164. Like in the case of *AP1*, much has been learned about how their expression is regulated, but so far the genes that they regulate are largely unknown.
We are currently analyzing the first ChIP-Seq data obtained for a CUC1 transgenic line: pCUC1:CUC1m-GFP pAP1:AP1-GR *ap1 cal*. This data will be correlated with gene expression profiling data obtained from the microarray analyses of pCUC1:CUC1m-GFP pAP1:AP1-GR *ap1 cal* (CUC1m levels are elevated due to a silent mutation in CUC1 that makes it insensitive to the miR164 downregulation) and of 35S:AP1-GR eep *ap1 cal* (CUC1 elevated because eep is a miR164 mutant). With this data, we will identify putative direct target genes of CUC1 and characterize the *CUC1* gene regulatory network.

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P5: Identification and validation of genes responsible of agronomic traits in peach (*Prunus persica* L. Batsch)

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Peach (*Prunus persica* L. Batsch) fruit shape (flat/round) is a mendelian character controlled by a single gene (S/s) located at the end of chromosome 6. The flat phenotype is produced by S/s heterozygous genotype; while the round phenotype is produced by s/s homozygous genotype; the homozygous genotype S/S do not produce fruits (called aborting phenotype). Previous association genetics studies done in our lab found a candidate gene for this trait containing an 8bp INDEL exclusive for the flat phenotype. This polymorphism was validated in a panel of 300 varieties and in a natural reversion from the flat to the round shape. Currently, we are cloning the flat allele through the Long Range PCR and sequencing techniques, but we face with the difficulty that this gene is highly repetitive in the genome of peach.

On the other hand, the validation of gene function in peach is not feasible so far through the transgenic approach. Hence, we are developing a technique to validate the function of genes in peach using a potyvirus (PPV, plum pox virus) as an expression vector of the candidate genes in infected peach plants. For this purpose, we are modifing PPV-based expression vectors to incorporate the visually traceable reporter gene Ros1 from *Antirrhinum majus*, and the first constructs are being tested for infectivity in different host plants, including *Nicotiana benthamiana*, *N. tabacum* and *P. persica*.

P6: An improved version of cultivated strawberry linkage map using the IStraw90 Axiom® Array for QTL analysis.

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Strawberries are economically important fruits around the world. Fragaria x ananassa, the cultivated strawberry, is an allo-octoploid specie (2n=8x=56), their ploidy level difficult the application of marker assisted breeding. F. vesca has been considered as a diploid model organism. Berries, including strawberries, are known for their rich nutritional profile. Understanding the genetic bases controlling the production of phenolic and volatile compounds is extremely important for the selection of new varieties of cultivated strawberry with greater consumer acceptation.
The genetic map of a F2 population (CamarosaxDover) posses 192 loci distributed along the 28 expected linkage groups, representing each homeologous group with a high coverage (>70%) when compared to F. vesca genome. LC-MS analysis for polyphenol metabolites in full ripen fruits allowed the quantification of 22 metabolites and mapping 146 metabolic QTLs. The IStraw90TM (International Strawberry 90K Axiom®) Affymetrix array developed by Rosbreed based on diploid and octoploide sequences was hybridized with 120 F2 individuals and parentals. The analysis of the results using GenotypeConsole and SNPolisher softwares shows a high quality of hybridization, with a >97% probe call rate for all individuals. For the 93937 valid SNPs: 75% were classified as MonoHighResolution, 9356 as NoMinorHomozygous, and 4939 as PolyHighResolution segregating in our population. The SNPs position in F.vesca and the SSR map allow to construct 4x7 homeologous groups with an increased resolution and higher coverage. Distribution of SNPs in homeologous groups will help the assembly of contigs and scaffolds of the genome draft sequence covering 154x of one population individual.

P7: New insight into the SSC8 genetic determination of fatty acid composition in pigs

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Fat content and fatty acid (FA) composition in swine are important factors of meat quality. A QTL for FA composition in backfat was previously detected on porcine chromosome 8 (SSC8) in an Iberian x Landrace cross (IBMAP). Afterwards, a genome-wide association study for muscle FA composition detected the same genomic region in a backcross population. A polymorphism (*ELOVL6:c.-533C<T*) in the promoter region of *ELOVL6*, a strong positional candidate gene for this QTL, was associated with the percentage of palmitic and palmitoleic acids in muscle and adipose tissues. Here, a combination of single-marker association and haplotype-based approach was used to analyze backfat FA composition in 470 animals of the IBMAP population genotyped with 144 SNPs distributed along SSC8.
Two trait-associated SNP regions were identified at 93 Mb and 119 Mb on SSC8. The strongest statistical signals of both regions were observed for palmitoleic acid (C16:1(n-7)) content and C18:0/C16:0 and C18:1(n-7)/C16:1(n-7) elongation ratios. *MAML3* and *SETD7* were studied as positional candidate genes in the 93-Mb region and two novel microsatellites in *MAML3* and nine SNPs in *SETD7* were identified. However, no significant association for the *MAML3* microsatellite genotypes was detected and the *SETD7:c.700G>T* SNP, although statistically significant, was not the strongest signal in this region. In addition, the expression of *MAML3* and *SETD7* in liver and adipose tissue varied among animals, but no association was detected with the polymorphisms of these genes. In the 119-Mb region, the *ELOVL6:c.- 533C>T* polymorphism showed a strong association with percentage of palmitic and palmitoleic FA and elongation ratios in backfat.
Our results suggest that the polymorphisms studied in *MAML3* and *SETD7* are not the causal mutations for the QTL in the 93-Mb region. However, the results for *ELOVL6* support the hypothesis of a pleiotropic effect on backfat and intramuscular FA composition of the *ELOVL6:c.-533C>T* polymorphism.

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P8: GMO Risk Assessment on animal and human health in the EU

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The research on the impact of genetically modified plants in the EU is nowadays set by the execution of animal feeding trials for testing food and feed based on OECD[1] and EFSA[2] guidelines. Nonetheless the necessity for and the relevance of these test procedures for the safety assessment of whole GM food and feed is being questioned[3]. Within the EU funded project GRACE (GMO Risk Assessment and Communication of Evidence) is going to reflect on this debate by re-evaluating the feeding trials on animal studies and exploring other options such as cell cultures. Firstly, three 90-day feeding trials with two different MON810 maize varieties and a GM potato will be carried out. The importance of chronic studies will be explored by extending the feeding trials of up to one year. Secondly, alternative analytical methods such as proteomics and NGS transcriptomics as well as bioinformatics tools will be set on plant material and animal tissues. The aim of the project is to conclude and/or advice on the necessity of the subchronic to chronic toxicity studies with GM plants fed to rats as part of the risk assessment established in international guidelines. And also to explore current alternative approaches for the characterization of GM plant material, alternative "omics" technologies and bioinformatics tools for testing new variables into animal tissues and cell cultures. Finally, the current animal and human health risk assessment procedures for GMO food and feed will be re-evaluated and adapt.

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P9: Identifying genetic variation at porcine lipid genes through whole-genome sequencing

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In order to investigate the genetic basis of lipid metabolism in pigs, a half-sibs population of Duroc pigs was phenotyped for several fatness traits (i.e. serum lipids, intramuscular fat content and composition, backfat thickness). Also, the genomes of the five boars that founded it (50% of the variation of the Lipgen population comes from these five sires) were sequenced at 30-fold coverage. The independent comparison of these genomic sequences with the pig reference genome allowed us to identify 13,328,140 variants including 10,002,757 SNPs and 2,867,142 indels. As a first step, we aimed to evaluate the amount of non-synonymous variation at 760 loci that have a well-established role in lipid metabolism. Filtering of the data revealed a total of 59,535 variants within these candidate genes. Among them, 328 variants (0.5%) corresponded to missense polymorphisms. Besides, we have found a putative nonsense polymorphism at the 7-dehydrocholesterol reductase gene, which plays a key role in cholesterol synthesis. We are now planning to perform a gene centric association analysis with 192 SNPs, selected on the basis of their predicted functional effects, in order to determine the effect of non-synonymous variation on lipid phenotypes.

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P10: Endometrial gene expression profile from pregnant sows with extreme phenotypes for reproductive capacity

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Prolificacy is one of the most important traits in swine industry because of its positive impact on productivity. Reproductive success can be influenced by multiple factors including ovulation rate, uterine capacity and foetal survival rates. The availability of a pig genome sequence and development of high-throughput techniques such as RNAseq have increased the discovery of genes and non-coding regulators involved in relevant biological processes. However, mechanisms involved in pig litter size variation remain unknown. The aim of this study was to identify key differences in gene expression associated to swine reproductive efficiency.
We performed a whole transcriptome analysis in 12 sows at day 30-32 of its gestation, resulting from an Iberian x Meishan F_2 population. Individuals were classified according to its estimated breeding value (EBV) as High (EBV>0) and Low (EBV<0) prolificacy. Uterine endometrium was collected and RNA sequenced to profile the mRNAome and miRNAome using an Ion 318™ Chip (Ion Torrent PGM) for each library and sample (High n=6; Low n=6). Computational analysis of mRNA libraries evinced 66 differentially expressed (DE) genes. Subsequent Gene Ontology analysis pointed out 5 candidate genes that were further validated by RT-qPCR on the whole extreme F_2 population (High n=16; Low n=20): *PTHLH* (Mammary gland development - $p<0.05$*; H/L ratio=3.69), *MMP8* (Embryonic development - $p<0.05$*; H/L ratio=4.41), *SCNN1G* (Response to hypoxia - $p<0.05$*; H/L ratio=3.42), *PTGS2* (Placental Development - $p<0.05$*; H/L ratio=3.50) and *HPGD* (Pregnancy - $p=0.118$; H/L ratio=1.69). Same approach was used on miRNA libraries identifying 14 DE miRNAs. RT-qPCR analysis revealed no significant differences on their expression levels, nevertheless, we identified a robust negative correlation between these miRNA and validated target genes expression levels. These correlations were statistically significant for miR-133a ($p<0.01$) and miR-92a ($p<0.01$).
The identified and validated differentially expressed RNAs provide a list of powerful candidate genes that contribute to a better understanding of the genetic architecture of prolificacy-related traits.

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P11: Manipulating plastidial protein quality control components to improve carotenoid contents in tomato.

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All photosynthetic organisms produce carotenoids, a group of isoprenoid metabolites with industrial and nutritional relevance. In plants, they are synthesized in all plastid types, where they play different functions and accumulate in different doses [1]. The plastids present in many yellow to red flowers and fruits, termed chromoplasts, are actually specialized in accumulating large amounts of carotenoids. For example, the accumulation of the carotenoids beta-carotene (provitamin A) and lycopene (a potent protector against prostate cancer) in tomato (*Solanum lycopersicum*) fruit during ripening results in the transformation of chloroplasts into chromoplasts and a concomitant change in the fruit color from green to red [2]. Plant carotenoid precursors are generated by the methylerythritol 4-phosphate (MEP) pathway. It has been shown that increased levels of any of the first two enzymes of this pathway, deoxyxylulose 5-phosphate (DXP) synthase (DXS) and DXP reductoisomerase (DXR), result in higher carotenoid production in several plastid types [1]. However, overexpression strategies have only resulted in moderate increases in enzyme levels, suggesting that post-transcriptional control of DXS and DXR protein accumulation could be an important limiting factor for a successful biotechnological overproduction of carotenoids and other MEP-derived products in crops.

Recent results in our laboratory have unveiled a key role for several protein quality control (PQC) components in the post-transcriptional accumulation of active DXS and DXR enzymes in *Arabidopsis thaliana* chloroplasts. PQC mechanisms are essential to deal with damaged (misfolded) proteins either by stabilization, refolding, or degradation. In the case of DXS, the plastidial J-protein J20 recognizes misfolded (inactive) forms of the enzyme and delivers them to the Hsp70 chaperone for either refolding (i.e. reactivation) or degradation by the stromal Clp protease complex [3, 4]. In the case of DXR, refolding and Clp-dependent degradation are mediated by a different PQC pathway involving the chaperone Cpn60 complex. Although chromoplasts have similar chaperone and protease systems, their role in PQC of MEP pathway enzymes remains completely unknown. In this work we have explored the potential of manipulating those PQC components in tomato fruit for increasing carotenoid contents. A VIGS-based screening has shown that a reduction of tomato fruit homologues of Hsp70 and Cpn60 chaperones leads to an increase in fruit carotenoid levels. On the other hand, down-regulation of Clp protease activity produces a general delay in ripening, suggesting a novel role for this plastidial protease complex in the transition of chloroplasts to chromoplasts during tomato fruit ripening. Our data suggest that PQC systems are also important for regulating the flux to carotenoids in chromoplasts, but not exactly in the same way as that originally observed in chloroplasts. Additional data from the analysis of amiRNA transgenic lines will also be presented.

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P12: A central role for HY5 in the crossroads of retrograde and shade signaling pathways

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HY5 is a bZIP transcription factor with an important role in the regulation of light signaling [1]. In particular, it accumulates in the light to repress hypocotyl elongation and promote photosynthetic development during deetiolation [2, 3]. Additionally, HY5 participates in other signaling pathways, including the response to hormones such as strigolactones [4] and ABA [5] and retrograde signaling [6]. All these signaling pathways need to be integrated when plants compete for light as an energy resource. When sunlight is filtered through or reflected from plant photosynthetic (green) tissues, the resulting light becomes richer in the far-red (FR) spectrum. This FR-enriched light acts as a signal of plant proximity that unfolds the shade avoidance syndrome (SAS), a set of adaptive responses that typically result in enhanced elongation growth and decreased accumulation of photosynthetic pigments. The role of HY5 in this important process, however, remains to be characterized.
Our work using *Arabidopsis thaliana* mutants and overexpression lines shows that HY5 acts as a negative regulator of hypocotyl elongation in response to simulated shade. This role is particularly evident when photosynthetic functions are challenged. In wild type plants, the inhibition of photosynthesis with herbicides like norflurazon (NFZ, which blocks carotenoid synthesis) or phosphinothricin (PPT, an inhibitor of glutamine synthetase) results in an increase in HY5-encoding transcripts and a concomitant attenuation of the elongation response to FR-enriched light (i.e. simulated shade). Also in agreement with HY5 having a role in preventing the shade-triggered elongation growth, *hy5* mutants elongate in response to this light signal even in the presence of NFZ concentrations that completely block the elongation response in the wild-type. However, inhibition of photosynthesis with PPT blocks hypocotyl elongation in response to FR-enriched light in both wild-type and *hy5* mutants. To confirm these results, we performed similar experiments in the presence of CPTA (another inhibitor of the carotenoid pathway) and lincomycin (which inhibits protein synthesis in plastids). Both inhibitors prevented chloroplast development and shade-induced hypocotyl elongation in wild type plants, whereas only the CPTA treatment allowed the *hy5* mutant to elongate. These results suggest that a carotenoid-related retrograde signal is required for the HY5-mediated repression of elongation growth after simulated shade.
In summary, our results indicate that the disruption of photosynthetic activity in chloroplasts has a strong impact in light-regulated developmental responses (such as hypocotyl elongation in response to simulated shade). Furthermore, we show that HY5 is a central component of the mechanism that integrates both light and retrograde signals, a mechanism that is appears to be specifically modulated by carotenoid-related metabolites.

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P13: Cloning and characterization of tomato PSAT involved in the biosynthesis of steryl esters

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Phytosterols are key structural components of cell membranes that modulate its fluidity and permeability, and the activity of a number of membrane-related proteins. Sterol acylation is an essential process for maintaining sterol homeostasis in cells that is catalyzed by both phospholipid:sterol acyltransferases (PSAT) and AcylCoA:sterol acyltransferases (ASAT). In plants, accumulation of steryl esters (SE) has been described during maturation and senescence or when plant cell cultures reach stationary phase. It has been suggested that PSAT is involved in the recycling of both the phospholipid fatty acids and the free sterols released from cell membranes as senescence progresses (1).
We have identified a single tomato (*Solanum lycopersicum* cv. Micro-Tom) gene coding for PSAT (*SlPSAT)*. A cDNA corresponding to this gene has been cloned, sequenced and found to encode a protein of 630 amino acid residues that is 77% identical to the previously reported Arabidopsis PSAT1, which consists of 633 amino acids (2). The functional identity of the enzyme was demonstrated by ectopic expression of a *CaMV35S::SlPSAT* gene in an *A. thaliana psat* null mutant (1). When Arabidopsis *psat* mutant plants were exogenously supplied with mevalonate, the precursor of sterols, in contrast to wild-type plants, they cannot transform the excess of free sterols into SE and the first become highly toxic. Overexpression of *SlPSAT* restored normal growth to the Arabidopsis *psat* mutant in these conditions. Expression of *SlPSAT* also reversed the premature senescence observed in excised *psat* rossette leaves and restored SE levels to wild-type range in the retransformed *psat* mutant. Expression analysis of *SlPSAT* by qRT-PCR showed that the gene is predominantly expressed in the leaves. Finally, expression of *SlPSAT* fusions with YFP in agroinfiltrated *N. benthamiana* leaves revealed that the protein localizes to spherical structures that are distributed throughout the cytoplasm and might represent SE storage vesicles.

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P14: Negative retrocontrol of the *hrp* genes in the bacterial plant pathogen *Ralstonia solanacearum*

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Ralstonia solanacearum can cause the bacterial wilting disease in plants through the Type III Secretion System (T3SS), through which it injects effector proteins inside the plant cell to change its normal physiology subverting defence responses. The T3SS is encoded by the *hrp* (HR and pathogenicity) genes. Expression of the T3SS is controlled by a regulatory cascade that involves several regulators. The inputs that positively activate the cascade have been well described and include environmental signals as well as plant cell contact. In contrast, no negative regulation of the system has been reported to date. In this work we describe a negative feedback regulation exerted on the master T3SS transcriptional regulator HrpB when bacteria grow in co-culture with *Arabidopsis thaliana* cells. We study if the responsible for this negative feedback regulation is HrpB or HrcC, a T3SS structural protein whose gene is expressed in a transcription unit together with *hrpB*. We will also provide insight of which level of the regulatory cascade is the HrpB\HrcC negative control acting in. We present data on transcriptional levels over time measured in both minimal media and co-culture conditions using a wide array of luminescent reporter strains created in the present study. The new negative regulatory pathway we describe may help to understand how expression of the T3SS system and its related effectors is turned off during the infection process after their rapid initial activation.

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