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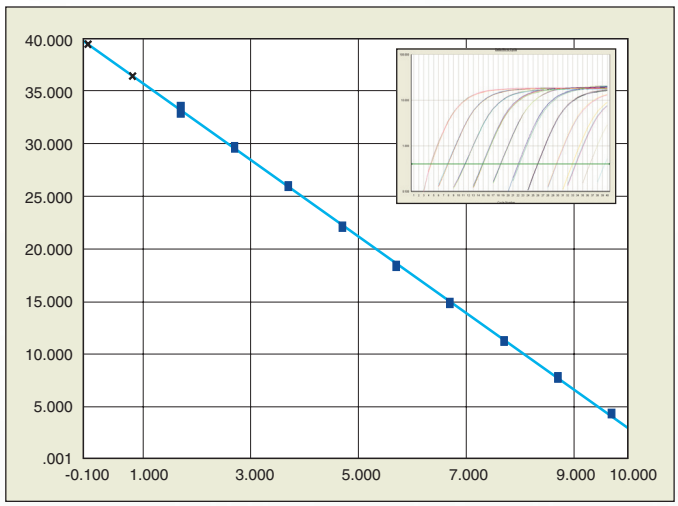

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Opening Talk

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PROGRAM

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TALKS ABSTRACTS

Afternoon Session 1A

1A1: Genomic analyses of the CUC1 network

Mariana Bustamante[1] , **Jian Jin** [1] , **José Luis Riechmann** [1,2]

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The global aim of our group is the characterization and understanding of gene regulatory networks underlying plant development, using the Arabidopsis flower as our model and experimental system. CUC1, together with the related factor CUC2, plays a pivotal role in the separation of organs and also between 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. 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 (microarrays), the same approach that led our group to the thorough description of the AP1 network in (Kaufmann et al., 2010). We have analyzed ChIP-Seq data obtained from a CUC1 overexpressing line. We are correlating these results with gene expression profiling obtained from the microarray analyses of the same line and from an alternative overexpressing line. With this information, we will identify putative direct target genes of CUC1 and aim to further understand the downstream events that take place after CUC1 activation. Kaufmann et al. (2010). Orchestration of floral initiation by APETALA1. Science (New York, N.Y.), 328(5974), 85–89. doi:10.1126/science.1185244 Project funded by BFU2011-22734, MICINN

1A2: Identification and validation of CNVs in Swine

Manuel Revilla [1,2], Anna Puig-Oliveras [1,2], Daniel Crespo-Piazuelo [1,2], Anna I. Fernández[3] , Maria Ballester[4] , Josep Mª Folch [1,2]

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An experimental Iberian x Landrace population (IBMAP) has been widely studied to identify structural variants associated with economically relevant traits, mainly for pork quality and pig growth. Landrace is a commercial lean breed with an efficient growth meanwhile Iberian is a fat rustic breed characterized for its meat quality. In the present study, a genomic analysis of porcine copy number variants (CNVs) based on next generation sequencing data (NGS) was carried out with the aim of identifying CNVs segregating in the IBMAP population and studying their association with growth and meat quality traits. The genomes of seven founders of the IBMAP population (two Iberian boards and five Landrace sows) were obtained by using next generation sequencing. All reads were mapped to the reference genome (Sscrofa10.2) having a mean coverage of 13.3x. CNVs were detected using deepsequencing data in Control-FREEC [1]. A total of 1423 CNVs including duplications and deletions were detected; these ranged from 264 (Iberian) to 121 (Landrace), with an average of 203 CNVs per sample. To define the size of each copy number variable regions (CNVRs) in the genome, the overlapping region between CNVs was used. The analysis led to the detection of 482 CNV regions (CNVRs) containing 326 genes. Remarkably, 28 of the CNVRs identified in our research (57%) have been also reported in Ramayo-Caldas et al. (2010) study [2]. Some of the identified CNVRs contained relevant functional genes (e.g., KIT and CLCA4), which were validated in a larger number of animals by quantitative PCR. Furthermore, correlation studies were performed for RQ values and indices of fatty acid metabolism and growth traits. Suggestive correlations were observed for CLCA4 gene expression and octadecenoic acid (C18:1(n-7)) (r=0.24), pH24 (r=-0.21), ham weight (r=- 0.24) and live weight at 125 (r=-0.21) and 155 days (r=-0.24). The genetic validation allowed us to test the segregation of the CNVRs identified from NGS data. These CNVRs may help in the study of economically important traits in the pig industry. References: [1] Control-FREEC: a tool for assessing copy number and allelic content using next-generation sequencing data – Boeva et al. (2012). Bioinformatics, 28:423-5. [2] Copy number variation in the porcine genome inferred from a 60k SNP BeadChip – RamayoCaldas et al. (2010). BMC Genomics, 11:593. Acknowledgments: Project funding by MICINN AGL2008-04818-C03/GAN and MINECO AGL2011-29821-C02 and the Innovation Programme Consolider-Ingenio 2010 (CSD2007- 00036).

1A3: 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, as well as new elements of the transcriptional regulatory network. 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.

Acknowledgments: Project funding by FP7-PEOPLE-2013-ITN. Fellowships: Marie Curie – ITN

2M1: QTL analysis of the slow melting flesh character in peach

Octávio Serra [1] , Jordi Giné[2] , Iban Eduardo[1] , Joan Bonany[3] , Gemma Echeverría[2] , Christian Larrigaudière[2] and Pere Arús[1]

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Peach (Prunus persica (L.) Batsch) is a diploid tree species (2n=16) native from China and cultivated worldwide with a great economic importance. Peach fruits produced for freshmarket have a very short shelf life and cannot be stored at low temperatures for long periods, unlike other fruits like apple or pear. With the peach industry demanding new varieties with an enhanced postharvest behaviour, a better understanding of peach fruit ripening and texture is needed. Peach texture can be classified in different flesh types, namely melting flesh (MF), non-melting flesh (NMF) and non-softening flesh (NSF) depending on the alleles present in the F-M locus located in chromosome 4 and controlled by two endopolygalacturonase genes separated by 34 kb. Using markers based on these sequences marker-assisted selection is possible for these flesh types, allowing breeders to anticipate the fruit texture of trees at an early stage. However there are some varieties presenting a different flesh consistency that cannot be distinguished by these markers, the slow-melting flesh (SMF), a trait highly desired due to the slower firmness loss of the fruit after harvest than MF. In this work we studied the firmness loss and the texture profile analysis of fruits in two segregant nectarine populations: 'Bigtop' x 'Armking' (BtxAk) and 'Bigtop' x 'Nectaross' (BtxNr). 'Bigtop' is a cultivar that presents the SMF phenotype. Fruit firmness loss was used for quantitative trait loci (QTL) mapping for two seasons (2013 and 2014). Results showed that a major QTL in chromosome 5 of 'Bigtop' can explain up to 30% of the firmness loss trait. However, the same trait maps in chromosome 4 for the other parental lines (MF), co-localizing with the maturity date (MD) locus, which indicates a possible pleiotropic effect of MD and the SMF trait.

2M2: High efficiency transposition of the tobacco retrotransposon Tnt1 in Physcomitrella patens

Cristina Vives [1] , Beatriz Contreras[1] , Florence Charlot[2] , Fabien Nogué[2] and Josep Casacuberta[1]

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Transposons have become an useful tool to generate mutant collections in animals and plants due to the capacity to insert copies into the genome. In plants, some retrotransposons have been shown to integrate preferentially near genes making them particularly interesting for mutagenesis. Among them, tobacco retrotransposon Tnt1 has been used to generate mutants in different plants species. The moss Physcomitrella patens is a model for studying different biological plant processes. Homologous recombination is an efficient process in P.patens which makes it highly suitable for reverse genetics. Due to the low integration efficiency of non-homologous sequences, it's very difficult to develop mutant collections. The purpose of this study is to analyze the capacity of the tobacco retrotransposon Tnt1 to transpose in P.patens and to develop Tnt1-derived vectors for high efficiency transposition that could be used to generate insertion mutant collections in a bryophyte specie.

2M3: Inhibition of the cell death regulator METACASPASE 1 by the protease inhibitor SERPIN1 in plants

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Our research aims at filling a major gap of knowledge by providing a detailed picture of the mechanisms controlling and executing pathogen-triggered programmed cell death (PCD) in plant cells. We used an orthogonal approach to study deathosome dynamics during the hypersensitive response (HR) cell death, a plant-specific localized immune response to pathogen recognition. In this context, we propose that the core members of the decision-making deathosomes are a limited set of proteins, most of which have a key role in HR cell death regulation. These include two type I metacaspases, which we have recently shown to antagonistically regulate this process, suggesting an ancient link between cell death control and innate immunity in plants and animals.
We have been able to identify native AtMC1-containing supramolecular complexes using gel filtration chromatography. In order to study their composition we have developed a novel technique to immunoisolate native protein complexes in planta. In a set of pilot experiments using this method, we have identified components of the AtMC1 interactome, which dynamically respond to PCD triggers and may be part of a regulatory network. Among the AtMC1-interactors we have identified several cell death and disease resistance regulators and putative metacaspase substrates, including a type II metacaspase.

In this work, we describe the identification and functional characterization of a novel Atmc1 interactor in basal conditions: the protease inhibitor Serpin1. Serpin1 interacts with AtMC1 in non-death inducing conditions. In addition, Serpin1 blocks auto-processing from AtMC1. Finally, Serpin1 inhibits cell death caused by AtMC1 putative active form by direct intraction via the catalytic domain in planta. Together, this data further supports the existance of an evolutionarily conserved cell death network. We are currently exploring whether pathogens exploit this cell death inhibition system to overcome plant defenses, as it is the case in animals.

3M1: Steryl glycoside metabolism in tomato: a novel target for improving fruit quality and stress tolerance in tomato

Nobahar Panahi [1] , Montserrat Arró [1,2] , Nidia Castillo [1] , Laura Gutierrez[1] , Alejandro Lara[1] , Albert Boronat [1,3], Albert Ferrer [1,2] and Teresa Altabella [1,4]

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Tomato (Solanum lycopersicum) is one of the most widely grown vegetables in the world and represents a very important source of nutrients in the human diet. Fruit ripening is a complex process, which involves a series of biochemical and metabolic changes that provide the optimal characteristics for consumption. However, the softening and increased susceptibility to microbial infections during the late stages of ripening result in a rapid deterioration of the fruit, affecting significantly postharvest handling and marketing. Another aspect related with tomato growing is its susceptibility to different plagues. Therefore, the development of pest resistant varieties is a major challenge in sustainable agriculture. Although the biochemical and metabolic processes related to fruit quality have been extensively studied in tomato, other less-characterized metabolic processes may also play important roles not only during fruit development and ripening but also in plant development and response to different types of stress. Sterol metabolism would be one of these processes. In plant tissues, sterols are found in free form (FS) and conjugated as esters (SE), glycosides (SG) and acylglycosides (ASG). Tomato, along with other species of the Solanaceae family, shows an atypical profile of conjugated sterols, accumulating particularly high amounts of SGs and ASGs (Duperon et al., 1984). However, the role of SG and ASG is currently unknown. SGs are synthesized from FS and UDP-sugar (glucose) in a reaction mediated by UDP-Glc:sterol glucosyltransferase (SGT). We have identified four tomato (S. lycopersicum cv. MicroTom) genes coding for putative SGTs, and the cDNAs corresponding to three of them (SlSGT1, SlSGT2, SlSGT3) have been cloned. These genes are differentially expressed in different tomato organs and fruit ripening stages, and encode cytosolic proteins whose functional identity has been demonstrated by complementation of the A. thaliana null mutant ugt80B1 (De Bolt et al. 2009). The possible involvement of the SlSGTs in plant response to stress will be discussed. These data will set the basis for further studies aimed at understanding the role of SG in tomato growth and development, fruit ripening and their response to biotic and abiotic stress. References: De Bolt et al. (2009). Plant Phys, 151: 78-87 Duperon et al. (1984). Phytochemistry, 23: 743-746

Acknowledgements: This work was financed by the Ministerio de Economia y Competitividad (AGL2013-13522-R). N.C. and A. L. are supported by predoctoral grants from CONACYT (México)

3M2: Cocos Island feral pigs and what happens when an admixed population stay isolated for 200 years

Bianco Erica [1,2*], Henry W. Soto[3] , Lourdes Vargas[4] , Miguel Pérez-Enciso[1,2,5]

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Cocos Island is a small Pacific island 500 km away from the Costa Rican west coast. In 1793, the English whaler Rattler left on shore a boar and a sow: no further pig introgressions were recorded. This makes Cocos population a good natural experiment to study the genetic of short term isolation in mammals. Our goal was to understand the origin of this population and to evaluate the effects of 200 years of isolation in a mammal genome. We analyzed four data sets: mitochondrial D-loop sequence; 60k SNP chip genotype data and twelve microsatellites of 12 individuals and the whole genome sequence of a male and a female. Principal component analysis (PCA) and Fst showed that Cocos population formed an isolated cluster. The two closest breeds in the PCA were two commercial breeds: Large White and Landrace. Using D-loops sequence, SSCX and SSCY haplotypes, we found that Cocos are hybrids between European and Asian pigs, as actual commercial breeds. Microsatellite data were compatible with the foundation event of N=2 individuals: only one marker had 4 alleles. Even if this population was isolated for 200 years and was founded by only two individuals, genomewide variability (Watterson's theta θ) was similar to commercial breed individuals: θ=1.9 for both Cocos. Using simulation, we showed that such a strong foundation event was still compatible with this level of variability. Cocos pig population admixed genome will help us to model the dynamics of feralization. Moreover, it is possible that this population carries alleles that were counter selected in actual commercial breeds since the introgression of Asian germplasm. Acknowledgments: this project was fund by MINECO AGL2010-14822 and AGL2013-41834- R grants. EB is recipient of FPI fellowship BES-2011043530.

3M3: Characterization of melon fruit ripening in the climacteric line SC3-5-1

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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 nonclimacteric 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). The development of a new segregating population containing only ETHQV6.3 allowed the identification of a candidate gene via the fine mapping and positional cloning of the QTL. Complementarily, a transcriptomic study of the fruit along ripening is being performed using PS, SC3-5-1 and two lines containing ETHQB3.5 and ETHQV6.3 separately. The validation and characterization of the candidate gene, together with the transcriptome profile of the lines, will help our understanding of the climacteric fruit ripening process in melon. 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

3M4: Unraveling the complexity of drought response: ZmSTOP1 transcription factor identified as OST1 substrate affects root growth and stomatal regulation in response to ABA

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Water deficit has become a very important threat to agricultural yield worldwide. The identification of new players in drought stress response among crop plants is vital to understand their adaptation to increasing environmental challenges. Abscisic acid (ABA) is a plant hormone known to mediate drought adaptative responses such as seed dormancy, stomatal closure and root growth arrest. In the case of stomatal regulation, ABA drives the activation of OST1 kinase, which phosphorylates different substrates, for example NADPH oxidases, ionic channels and transcription factors, which will finally lead to stomatal closure. Although stomatal closure in response to ABA is essential to avoid desiccation, and thus, the death of the plant, a certain level of stomatal conductivity must be maintained to permit gas exchange and transpiration, essential to drive water and solutes uptake through the roots. We identified and characterized a new maize C2H2 zinc-finger transcription factor that presents homology to the Arabidopsis Sensitive to Proton Rhizotoxicity (STOP)1, which is critical for aluminum and proton tolerance in acidic soils[1]. Due to the importance of protons in stomatal movement, we checked STOP1 implication in drought responses, determining that ZmSTOP1 is involved both in stomatal regulation and root growth. Here we show that ZmSTOP1 promotes a differential response to ABA in roots and shoots. Root growth is over-inhibited whereas stomata present insensitivity to the ABA signal. Through microarray analyses we determine that the genes affected by ZmSTOP1 are classified mainly in signaling, regulation of transcription and stress. Moreover, we establish that this transcription factor is a substrate of OST1 kinase. At the present moment we are studying the implications of OST1 phosphorylation on ZmSTOP1.

1.Iuchi et al. (2007). Zinc finger protein STOP1 is critical for proton tolerance in Arabidopsis and coregulates a key gene in aluminum tolerance. Proc Natl Acad Sci U S A.;104(23): p.9900-5.

4A1: Improve strawberry fruit quality using a NIL collection

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Wild strawberries (Fragaria vesca spp.) are close diploid relatives of the octoploid cultivated strawberries (Fragaria x ananassa spp.). The availability of a high quality sequenced genome and annotation for F. vesca [1], the synteny studies between diploid strawberry, octoploid strawberry and other species from the Rosaceae family [2], its short inter-generational period and its reduced plant-size has positioned it as a model species among the the Fragaria and Rosaceae research community. Strawberry is an economically important crop, traditionally appreciated for its aroma. However, breeding programs in the past decades addressed more efforts to improve its agronomical performance rather than its organoleptic quality resulting in a set of varieties producing appealing big brilliant red fruits but lacking in flavor and aroma. In a recent work, we developed and phenotyped for agronomical traits a near isogenic line (NIL) collection in F. vesca with homozygous exotic introgressions from F. bucharica [3]. Several stable quantitative trait loci (QTL) were mapped successfully for traits such as fruit shape, flowering habit, runner production, flower color, number of petals or sugar content. Taking in account this background, our objective was to map stable QTL for metabolites (mQTL) implicated in organoleptic fruit quality, mainly volatile compounds responsible for its aroma and also mQTL related with its nutritional properties, mainly (poly)-phenolic compounds. A in depth metabolic analysis of the F. vesca NIL collection was performed using LC-MS and GC-MS techniques. 100 volatile and 24 (poly)-phenol compounds were unambiguously identified and 178 stable mQTL were mapped in total, including mQTL for 'key'-compounds in the strawberry aroma, using a whole genome genotyping SNPs array IStraw90® [4]. Finally, a set of four NIL with outstanding nutritional or aromatic characteristics were selected for a differential expression analysis by mRNA sequencing in order to detect possible candidate genes responsible for the observed phenotypes.

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4A2: Comparative proteomics for safety assessment of genetically modified maize

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The necessity of studies on the impact of genetically modified plants in the EU is being questioned. The project GRACE (GMO Risk Assessment and Communication of Evidence) is going to reflect on this debate by re-evaluating the methodologies for assessing the potential risks of GM maize. Nowadays the only GM approved for cultivation in EU is the insect resistant MON810 maize (Bt-maize) and 90% of this maize is grown in Spain. 2-D comparative proteomics is a proposed method for assessing the potential risks of the MON810 maize. A total of 11 maize samples (two MON810 lines, two respective parentals, two naturally fungi contaminated and five other conventional verities) were analyzed in 2D gels. All varieties passed with proteome correlation values between varieties among the 70% resolving more than 1400 spots/gel. 121 differential spots were identified between all varieties when compared all together. The analysis of global differences clearly separated two varieties infected by fungi from all other varieties. All uninfected varieties were placed altogether in the PCA map, in a “cloud” with minimal differences between them. When comparing separately the proteomes of GM versus isogenic maize varieties we observed a major separation of varieties according to the seed producer. Finally, a MALDI/TOF analysis of all relevant spots found will be performed; including the most relevant spots from PCA analysis. In conclusion, when analyzing the GM maize proteomes we can’t see any differences associated to the expression and/or accumulation of the transgenic protein. Our results suggest that the genetic background has a major effect on differentiating the maize varieties than the transgen insertion itself.

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P1: Unravelling the climacteric versus non-climateric ripening in melon (Cucumis melo L.) using a recombinant inbred line (RIL) population

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Ripening is the most important physiological change in fruit, involving significative transformations as sugar and carotenoid accumulation, aroma production, chlorophyll degradation, abscission… The classical model species to study ripening is tomato, however, melon is a very interesting species to elucidate the genetic basis of ripening, because varieties with climacteric and non-climacteric ripening are found and many genetic and genomic resources are avaliable. Ripening in climacteric fruits is characterized by an autocatalytic synthesis of ethylene, producing a peak of ethylene and an increase of respiration. In non-climacteric fruits, the characteristic peaks of ethylene and respiration are not present. In order to elucidate the molecular basis of ripening in melon, a population of recombinant inbred lines (RIL) between two commercial varieties, the climacteric Vedrántais and the non-climacteric Piel de Sapo melon type was developed, which contains approximately 90 F8-F7 lines. A genotyping-by-sequencing (GBS) approach will be performed to obtain a genetic map for QTL analysis. The population will be phenotyped for fruit quality: flesh colour, skin colour, fruit shape, size and weight, sugar and organic acid content. Trying to establish an alternative method to measure ethylene in planta, we will use an electrochemical sensor to determine the ethylene production in developing fruits. The information about which of them are producing ethylene and the quantity of the hormone will be used on one hand, to perform a QTL analysis and, on the other hand, to see whether any specific characteristic of climacteric ripening can be related not only qualitatively with the presence or absence of ethylene but also quantitatively. The results will clarify some aspects about the relationship between ethylene and the different changes observed during fruit ripening, and to identify QTLs responsible of fruit quality in melon.

P2: Unveiling the role of DXS-Interacting (DXI) proteins in the regulation of plastidial isoprenoid biosynthesis

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A complex network of biosynthetic pathways in different subcellular compartments supports plant life. In particular, plastids provide a number of unique metabolic pathways among eukaryotes, including the methylerythritol 4-phosphate (MEP) pathway for the production of isoprenoid precursors. MEP-derived isoprenoids include compounds essential for photosynthesis and growth regulation. The first and main rate-limiting reaction of the MEP pathway is catalyzed by deoxyxylulose 5-phosphate synthase (DXS). Post-translational regulation of DXS is central to regulate the levels and activity of this enzyme, but the specific mechanisms and players involved remain little known. To gain insights into this type of regulation, we identified a number of DXS-interacting (DXI) proteins by yeast two-hybrid and co-immunoprecipitation approaches. To test the relevance of these potential interactions for DXS activity, we first aimed to analyze whether T-DNA insertion lines with reduced levels of DXI proteins showed changes in the resistance to clomazone (CLM), an inhibitor of DXS activity that produces a bleached phenotype. From the analysis of 22 T-DNA insertion lines, we found changes in the resistance to CLM in mutants for three genes encoding proteins involved in photosynthesis: FBA1, SBPASE and FNR1. These proteins have been fused to tags and transiently expressed in plant cells to confirm both their plastidial localization and their interaction with DXS in vivo. Next, DXS activity and plastid isoprenoid levels will be measured in DXI mutant backgrounds. The data generated will allow to better understanding the relevance of protein-protein interaction to modulate DXS activity and the flux of the MEP pathway.

P3: 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.

P4: Mixed infections of plant viruses in crops: effects on dissemination and resistance

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Mixed infections of plants by two or more pathogenic viruses are quite common in nature. Contrarily to single infections, the presence of more than one virus may lead to complex diseases and synergistic reactions in plants. However, in many cases, mixed infections might remain unnoticed, and that could reduce the effectiveness of control measures selectively implemented to combat viruses individually. Indeed, epidemiological surveys are revealing complex situations with frequent mixed infections that could compromise the efficiency of virus control measures. The present work aims to address problems caused by mixed infections, exploring pathosystems of different host plants, including Cucurbitaceae (melon) and Solanaceae (tomato), different potential vectors (aphids and whiteflies), and virus combinations of criniviruses with ipomovirus/potyvirus in melon, or with begomoviruses in tomato. The choice of these experimental systems is based mainly on the current situation in our crop production conditions, and in the scientific and technological interest of the combinations of viruses. The main objectives are: 1) to establish mixed infections in the different pathosystems, and characterise their combined effects; 2) to evaluate the potential effects of mixed infections in transmissibility by vectors, and to compare their different vital parameters; and 3) to analyse the interference of mixed infections with the resistance mechanism in host plants, focusing on the action of viral suppressors of gene silencing. Results will serve to anticipate pro-active measures favouring virus control. For instance, procedures to generate RNAi responses in insect vectors to interfere with virus transmission. Also, characterization of viral counter-defences against plant responses, in particular based on the combined activities of gene silencing suppressors during multiple infections. All these approaches will generate new knowledge on the dynamics of mixed infections, and could derive into recommendations for disease control that help to maintain productivity of highvalue horticultural crops.

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P5: Identification through RNA-sequencing of splicing variants differentially expressed in pigs with divergent lipid phenotypes

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We have used RNA-Seq to examine the gluteus medius (GM) muscle transcriptome of commercial Duroc pigs with divergent phenotypes (HIGH and LOW) for 13 fatness traits. The whole transcriptome of 52 GM samples from HIGH (n=26) and LOW (n=26) pigs were sequenced with a HiSeq2000 platform. An average of 70-million sequence reads were obtained from each sample and mapped to the pig reference genome. In all samples, 80% of the reads were categorized as mapped. Differential expression analysis was performed at the gene and transcript (splicing variants) levels by comparing the mRNA profiles of the LOW and HIGH groups. We detected 87 genes and 39 splicing variants differentially expressed between pigs showing divergent fatness phenotypes (p1.5). Noteworthy, 8 splicing variants happened to be novel (unannotated in the last pig reference genome available (Sus scrofa 10.2.79)). In order to detect genes acting as key regulators of metabolic pathways, we carried out a pathway analysis with Ingenuity IPA taking into consideration the differentially expressed splicing variants. This approach evidenced that most of differentially expressed splicing isoforms belong to the glycolysis and gluconeogenesis, oxidative phosphorylation and PPAR pathways. Interestingly, we identified several splicing variants corresponding to genes associated with lipid metabolism. For instance, stearoyl-CoA desaturase (SCD) and TIMP1 genes encode splicing variants that appear to be differentially expressed (p2). On the other hand, specific segregation of SNPs was identified between HIGH (n=2,955) and LOW (n=1,467) groups. A next step will be to identify to map the unannotated splicing variants to specific loci. Acknowledgments: Project AGL2010-22208-C02-01 funding by Ministry of Economy and Competitiveness. The authors thank Batallé Selection and IRTA- Monells staff in obtaining animal material and phenotypic data.

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P6: Understanding DRACULA2, a putative dynamic nucleoporine linking the nuclear pore and shade signaling

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Plants are exposed to many unfavorable conditions in nature. One of them is living in an environment of high plant density where presence of nearby plants results in a reduction of the red (R) to far red (FR) ratio (R:FR) percieved. This is caused by a specific enrichment of the FR light reflected from the surface of neighboring leaves. Phytochrome photoreceptors (mainly phytochrome B) perceive this as a signal and triggers a set of responses aimed to adapt plant's growth and development, known as Shade Avoidance Syndrome (SAS). This results in an increased hypocotyl and stem elongation and early flowering. In the laboratory, plant proximity conditions can be simulated by supplementing white light with various amounts of FR light. To identify new regulatory components of the SAS a genetic screen was performed in Arabidopsis thaliana based on a transgenic luciferase line responsive to shade. As a result, dracula 2 (dra2) mutant seedlings displayed longer hypocotyls in white light but an attenuated hypocotyl response in simulated shade. DRA2 encodes the plant nucleoporine NUP98a, constituent of the nuclear pore complex (NPC) in plants. Although the NPC is well characterized in yeast and animals, less is known in plants. We have found that in addition to dra2, other mutants deficient in various NUPs have an impaired hypocotyl response to simulated shade indicating that the NPC has a transport-dependent role in SAS regulation. Unlike other NUP mutants, dra2 seedlings exhibit attenuated expression of several shade marker genes suggesting that DRA2 participates in a transport-independent mechanism that associates active genes of the chromatin with the NPC. With this in mind, we want to address the transport-independent role of DRA2 and its potential dynamic properties. We are obtaining and characterizing transgenic plants of Arabidopsis overexpressing a diversity of functional and tagged versions of DRA2. The latest results will be presented.

Fellowships: FI-DGR 2015

P7: Brassinosteroids at the stem cell niche: molecular dissection of the bravo pathway

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Brassinosteroid hormones (BRs) are essential regulators of plant growth and development. In Arabidopsis, BR signaling has been widely investigated by genetic and biochemical approaches that led to the identification of central BR signaling components ubiquitously expressed in the plant, from the BRI1 receptor at the plasma membrane to downstream acting BR-regulated BES1 and BZR1 transcription factors in the nuclei. Our laboratory has pioneered the identification of BR signaling components with spatiotemporal resolution in Arabidopsis. In the primary root, BRs control cell cycle progression in the meristem, as well as the transition of these cells to differentiation1 . In the root stem cell niche, BRs promote the renewal of the quiescent centre (QC) cells and the differentiation of distal stem cells, suggesting that counteracting BR signaling is a mechanism to preserve the low rates of cell division in the QC. Using cell-specific transcriptomics we have identified BRAVO as the first QC-specific repressor, preserving the root quiescence2 . BRAVO encodes a MYB transcription factor that is recruited by the BR-activated form of the transcription factor BES1, creating a bistable switch that modulates QC divisions at the root stem cell niche. Our findings unveil the existence of a QC cell-specific pathway to control stem cell division and renewal, which is necessary for plant development and adaptation to environmental stresses. Currently, we aim to identify the native BRAVO protein complex in the stem cell niche by using in vivo immunoprecipitation. We will present our current understanding of BRAVO pathway and its functional relevance for stem cell development in plants. 1 González-García M-P, Vilarrasa-Blasi J, Zhiponova M, Divol F, Mora-García S, Russinova E, Caño-Delgado AI (2011). Brassinosteroids control meristem size by promoting cell cycle progression in Arabidopsis roots. Development ,138: 849-859. 2 Vilarrasa-Blasi J, González-García MP, Frigola D, Fàbregas N, Alexiou KG, López-Bigas N, Rivas S, Jauneau A, Lohmann JU, Benfey PN, Ibañes M, Caño-Delgado AI (2014). Regulation of plant stem cell quiescence by a brassinosteroid signaling module. Dev Cell, 30(1): 36-47.

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P8: TEMPRANILLO regulates the age-dependent developmental pathway at different levels

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Arabidopsis plant development can be divided in three distinct phases: Juvenile phase, adult phase and reproductive phase. These developmental phases are controlled by different genetic pathways that respond to diverse environmental and endogenous stimuli including photoperiod, temperature, hormones and age. The age-dependent pathway controls the transition from the juvenile to the adult vegetative phase. The expression level of the microRNA156 (miR156) is high during early stages of plant development, while decreases in adulthood. Most SPL (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE) genes are silenced by miR156, and become active as miR156 decays in the adult phase when they in turn activate miR172. TEMPRANILLO (TEM) genes act as repressors of flowering. The similarity in the temporal expression pattern of miR156 and TEMs suggested a putative role of TEMs in the juvenile-toadult transition. We found that as miR156, TEM genes control this developmental transition. However, TEMs affect to a greater extent the length of the adult phase, whereas miR156 shows a major role in controlling the juvenile phase. Phenotypic and expression studies indicate that TEMs regulate these developmental transitions by regulating miR156 levels and by directly acting on miR156 downstream genes. TEMs are able to repress SPLs (SPL9, SPL3) and miR172 expression by binding in vivo to their regulatory regions. Therefore, TEMs act in a miR156-dependent and -independent manner in the age-dependent pathway.

P9: Auxin methylation is required for differential growth in Arabidopsis thaliana

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The IAMT1 gene encodes an indoleacetic-acid (IAA) methyltransferase that inactivates auxin in Arabidopsis thaliana [1]. The analysis of the iamt1-1 mutant revealed that several auxinrelated processes of the seedling aerial development were affected in the absence of a functional IAMT1. Dark-grown iamt1-1 seedlings had longer hypocotyls compared to wild type plants, and apical hook opening was faster. In addition to a faster growth rate and opening of the apical hook, iamt1-1 mutants had additional defects in differential growth, specifically in gravitropism. Monitoring gravitropic response showed that the mutants were largely impaired in gravitropic reorientation. Since IAA content in iamt1-1 seedlings was not different from that of wild type seedlings, we studied if the mutants could have local auxin changes. The introduction of different auxin reporters [2] into the iamt1-1 showed that during hook development and gravistimulation auxin activity was stronger and had a wider domain than in wild-type plants. This indicated that IAA methylation affects the accumulation of auxin, or its downstream signalling, rendering the hypocotyls unable to form a proper auxin gradient upon reorientation. Since expressing IAMT1 specifically in the endodermis was sufficient to rescue iamt1-1 agravitropic phenotype, and aerial gravitropism is regulated by the endodermis-specific expression of the auxin carrier PIN3, we checked if polar auxin transport (PAT) was affected in the mutant. The application of NPA, a PAT inhibitor, partially alleviated agravitropism, while transcript levels of different PIN genes were highly upregulated in the mutant. The pPIN3::GPF-PIN3 reporter showed that differential positioning of the protein was abolished in the mutant, impairing differential IAA transport during gravitropic responses or apical hook opening, preventing differential growth. Overall, we show that IAA methylation is an integral component of the generation of lateral auxin gradients that lead to differential growth, although the mechanism linking IAA methylation and PINs transcriptional regulation remains unclear. [1] Qin et al. (2005). Plant cell 17, 2693-2704. [2] Brunoud et al. (2011). Nature 482, 103-106.

P10: Identification and validation of a candidate gene for the flat shape of peach fruits

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The flat shape of peaches is caused by a dominant allele of the gene S mapped in chromosome 6. Although this allele works as dominant, flat peaches are only observed in heterozygous genotypes (Ss) while fruits with the SS genotype abort. An association analysis has identify a 10 kb deletion, affecting the promoter and the first coding region of a LRR-kinase, that co-segregates with the flat phenotype. A three-primers PCR experiment has been designed to identify the deletion with a simple PCR analysis. These primers have been used to validate the association in 300 varieties consisting of flat, round and aborting peaches. In the meantime, we are studying and validating the function of the LRR-kinase candidate gene in a round peach generated from a natural somatic mutation of a flat peach variety. Faint amplification of flat allele is observed in mutated round peach while a legible amplification is observed in flat one suggesting the presence of chimeric DNA caused by the mutation of only one of the three meristematic bud layers. PCR amplification of DNA extracted from the mutated flesh confirmed this hypothesis as well as the role of the candidate gene in the phenotype. Future studies will be oriented to clone the mutated allele and to investigate the role of the gene in determining fruit shape.

P11: The Arabidopsis Synaptotagmin1 Is Enriched in Endoplasmic Reticulum-Plasma Membrane Contact Sites and Confers Cellular Resistance to Mechanical Stresses.

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Eukaryotic endoplasmic reticulum (ER)-plasma membrane (PM) contact sites are evolutionarily conserved microdomains that have important roles in specialized metabolic functions such as ER-PM communication, lipid homeostasis, and Ca^{2+} influx. Despite recent advances in knowledge about ER-PM contact site components and functions in yeast (*Saccharomyces cerevisiae*) and mammals, relatively little is known about the functional significance of these structures in plants. In this report, we characterize the Arabidopsis (*Arabidopsis thaliana*) phospholipid binding Synaptotagmin1 (SYT1) as a plant ortholog of the mammal extended synaptotagmins and yeast tricalbins families of ER-PM anchors. We propose that SYT1 functions at ER-PM contact sites because it displays a dual ER-PM localization, it is enriched in microtubule-depleted regions at the cell cortex, and it colocalizes with VAP27, a known ER-PM marker. Furthermore, biochemical and physiological analyses indicate that SYT1 might function as an electrostatic phospholipid anchor conferring mechanical stability in plant cells. Together, the subcellular localization and functional characterization of SYT1 highlights a putative role of plant ER-PM contact site components in the cellular adaptation to environmental stresses.words.

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P12: Metabolism of conjugated sterols in tomato

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Phytosterols are integral components of plant membranes that modulate membrane fluidity and permeability. Recent studies have shown that sterols play also an essential role not only in plant growth and development but also in their responses to different types of stress. Plant sterols are found in free form and conjugated as esters (SE), glycosides (SG) and acylglycosides (ASG). Tomato, one of the most widely grown vegetable crops worldwide and a very important source of nutrients in the human diet, along with other species of the Solanaceae family, shows an atypical content and profile of conjugated sterols. However, the biological and evolutionary significance of this peculiar sterol metabolism is currently unknown and the knowledge about the enzymes involved in the synthesis of conjugated sterols is still very limited. These include phospholipid sterol acyltransferase and Acil-CoA sterol acyltransferases, both involved in SE biosynthesis, steryl glucosyl transferases involved in the biosynthesis of SG and steryl glucoside acyltransferases, involved in the biosynthesis of ASG. We have identified, cloned and characterized the tomato enzymes involved in the biosynthesis of conjugated sterols which will set the basis for further studies aimed at understanding the role of these compounds in tomato growth and development, fruit ripening and their response to biotic and abiotic stress.

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Development of the Maxwell® 16 LEV Plant DNA Kit, and its application to plant leaf tissues

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Poster# P0083

1. Introduction

Increasingly, microgram amounts of purified DNA from plant tissues are required in order to perform complex molecular studies with plant populations.

These studies require laborious approaches to genomic DNA extraction, such as manual organic extraction methods.

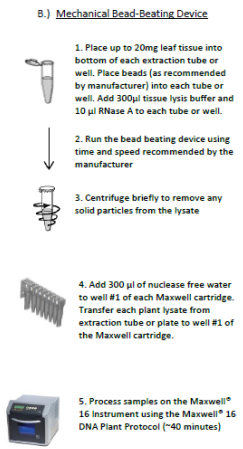
Automated DNA isolation relieves the burden of sample processing using laborious and inefficient manual purification methods.

DNA isolation methods designed around a paramagnetic particle, and associated chemistries have successfully been automated on bench top particle handling instruments, such as the Maxwell® 16 instrument.

Promega has recently designed and developed an automatable DNA purification chemistry to address the problem of automated DNA purification from crude plant lysates.

This chemistry features a novel paramagnetic particle with a high binding capacity, which allows automated DNA isolation from plant tissue samples, and then elution in small volumes (50 µl).

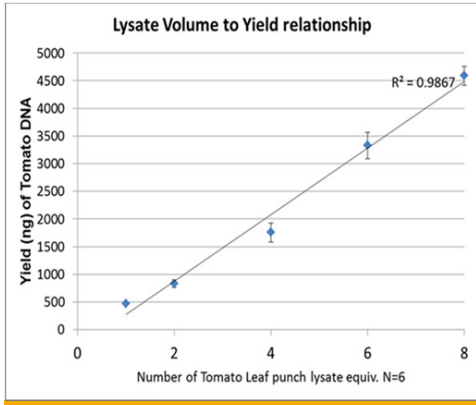
2. Overview of sample extraction and isolation



All buffers to support extraction, pre-dispensed and sealed cartridges, elution buffers, and the plastics required to perform isolation chemistry are all provided within the kit (commercially available as Maxwell® 16 LEV Plant DNA Kit, Promega Catalog #AS1420)

3. Efficiently recovers up to microgram quantities of DNA from plant lysate

The chemistry at the heart of the Maxwell® 16 LEV Plant DNA Kit consists of paramagnetic cellulose particles, which features an extremely high bind capacity for nucleic acids, allowing for the isolation high quality DNA from plant tissue samples.

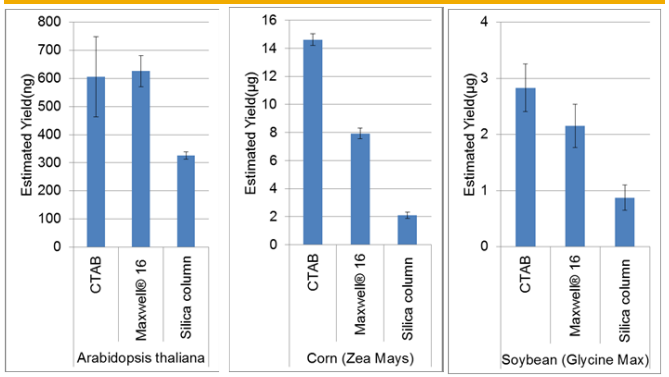


Increasing amounts of a tomato lysate pool, representing extract from between 1 and 8 punches (5 mm diameter) of tomato leaf material were loaded into the cartridge, N=6 per replicate point.

Resulting DNA isolates were quantified using qPCR to estimate DNA yield.

The linearity of response between lysate input and yield indicates no reduction in isolation efficiency into the microgram range of DNA yield from plant leaf lysates.

4. Competitive sample extraction trials across three extraction approaches



Equal volumes of plant leaf tissue (by mass in the case of A. thaliana, or 5 x 5 mm diameter leaf punches in the case of Corn or Soybean) were extracted and isolated with three separate protocols: a traditional CTAB Organic extraction process(CTAB), the new Maxwell®16 Plant DNA Kit(Maxwell® 16), and a silica-based purification column (silica column) provided by another commercial vendor. Resulting eluates were quantified for yield using a species-specific qPCR method against a commercially available source of DNA used as a control. Each yield estimate is presented ±1 Standard Error of the Mean (SEM)

5. Success at extraction of additional species at Promega and by Department of Botany, University of Wisconsin–Madison

Tested at Promega	Tissue	Tested by Early Adopter	Tissue	Tested by Early Adopter	Tissue
Arabidopsis thaliana	**Leaf**	*Ageratina altissima*	Leaf	*Hylodesmum glutinososum*	Leaf
Zea mays	**Leaf**	*Allaria petiolata*	Leaf	*Impatiens pallida*	Leaf
Glycine max	**Leaf**	*Amphicarpea bracteata*	Leaf	*Maianthmum canadense*	Leaf
Fragaria x ananassa	Leaf	*Arisaema triphyllum*	Leaf	*Maianthmum recemosum*	Leaf
Solanum tuberosum	Leaf	*Carex Albursina*	Leaf	*Mitella diphylla*	Leaf
Solanum lycopersicum	Leaf	*Caulophyllum thalloctroides*	Leaf	*Osmorhyza claytonii*	Leaf
	Root	*Circaea Lutetiana*	Leaf	*Phryma leptostachya*	Leaf
	Stem	*Clintonia borealis*	Leaf	*Pilea pumila*	Leaf
Nicontinium tabacum	Leaf	*Eupatorum rugosum*	Leaf	*Rhamnus carthatica*	Leaf
Spinacia oleracea	Leaf	*Eurybia macrophylla*	Leaf	*Ribes missouriense*	Leaf
Triticum aestivum	Leaf	*Gateopsis tetrahit*	Leaf	*Sanguinaria canadensis*	Leaf
Oryza sativa	Leaf	*Geranium maculatum*	Leaf	*Streptopus lanceolatus (var. longipes)*	Leaf
		Hydrophyllum virginianum	Leaf	*Trillium grandiflorum*	Leaf

Over the course of the development of the Maxwell® 16 LEV Plant Kit, a panel of varied species have been extracted and isolated with the system.

Extractions were performed by Promega Corporation and the University of Wisconsin. In all cases, milligram amounts of tissue were extracted and isolated with the Maxwell® 16 LEV Plant Kit.

Resulting eluates were quantified with qPCR or absorbance methods.

For every species and tissue type listed in this table, indicated quantifiable DNA with qPCR-based methods, spectrophotometric methods, or both approaches to quantitation (*Applications in Plant Sciences* 2014 2 (10): 1400048; http://www.bioone.org/loi/apps © 2014 Moeller et al. Published by the Botanical Society of America)

6. Conclusions and Product information

- ❖ Cellulose-based particles provides higher binding capacity than traditional silica-based DNA purification methods.
- ❖ Quickly purify up to 16 plant tissue samples simultaneously without organic solvents
- ❖ Improve workflow efficiencies and minimize re-isolation of samples, cutting costs and increasing reliability of results
- ❖ Automated method for genomic DNA isolation from plant materials
- ❖ Isolation method is compatible with a broad panel of species.
- ❖ Underlying chemistry isolates amplifiable DNA efficiently into the microgram range from plant tissue lysates.

Product Information:

- ❖ Instrument used is the Maxwell® 16 instrument (Promega Catalog #AS2000), configured for Low Elution Volume (LEV) mode.
- ❖ The kit is available commercially as the Maxwell® 16 LEV Plant DNA Kit (Promega Catalog # AS1420)
- For further technical information about these chemistries, contact Promega

NOTES