Epigenetic Mechanisms of Crop Adaptation To Climate Change

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EPIBREEDING – NEW TOOL FOR TACKLING CHANGING ENVIRONMENT?

Velimir Mladenov[1], Borislav Banjac[1], Svetlana Vujić[1], Đorđe Krstić[1], Aleksandra Radanović[2], Sandra Cvejić[2], Siniša Jocić[2], Ana Marjanović Jeromela[2], Ankica Kondić Špika[2], Jegor Miladinović[2], Boško Dedić[2], Milan Jocković[2], Dragana Miladinović[2]

[1]University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia
[2]Institute of Field and Vegetable Crops, National Institute of Republic of Serbia, Novi Sad, Serbia

Creation of new crop varieties using classical breeding methods is a long-term process, sometimes not efficient enough to meet demands of changing environment and market demands. Recent studies showed that epigenetic mechanisms may play a role in enhancing crop resilience to stresses and therefore may be an important tool generate new, more environment-flexible varieties. The introduction of modern techniques, such as epibreeding, into crop breeding programs, could became a new way for more efficient incorporation of desired traits into commercial varieties. Climate change and its consequences placed breeder's focus on selection of crop genotypes tolerant to not only specific, but also combination of abiotic stresses. Since abiotic stress related traits are mostly quantitative, epigenomic selection could be a tool of choice in breeding programs in the future. In order to become useful tool in crop improvement, epibreeding studies should address not only the effect of epigenetic variation on abiotic stress related traits, but also stability and heritability of those variations. More work needs to be done in creation of new, reliable and efficient breeding methods that will enable breeders to move beyond mere correlation between epigenetic variation and the desired trait. One step towards this is identification and further validation of epi-molecular markers, as well as genome editing tools that will further enable either the use of natural epigenetic variation or targeted modifications of the epigenome in creation of environment-flexible phenotypes.

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Contact: Velimir Mladenov, University of Novi Sad, Faculty ofAagriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia. Email: velimir.mladenov@polj.edu.rs

EPIGENETIC VARIATION IN APPLE – ENVIRONMENTAL INDUCTION, STABILITY AND HERITABILITY

Janne Lempe, Henryk Flachowsky, Andreas Peil

Julius Kühn Institute (JKI) –Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Fruit Crops, Pillnitzer Platz 3a, D-01279 Dresden, Germany

Climate change and the loss of biodiversity pose big challenges on agricultural production and requires development of novel crop varieties and breeding strategies. We propose to investigate epigenetic variation as a novel resource for phenotypic variation and to test its potential for crop breeding. In particular, we will focus on environmental inducibility, stability and heritability of epigenetic variation in apple. To do so, we established collaborations with four partners - from Spain, Italy, Poland and Sweden – gathering five sites from different climatic regions within Europe. We will assess whether long-term environmentally induced epigenetic variation exists, that is specific to particular climatic conditions. Therefore, we will compare methylomes of apple trees of two different top cultivars that have been growing at these climatically distinct sites across Europe for more than five years. As apple cultivars are propagated vegetatively by grafting, we want to establish, whether epigenetic variation can be transmitted to offspring via clonal propagation. Growing grafted trees from these distinct sites at one common place will allow us to determine the stability of long-term methylome variation. To complement these results, we will distribute uniform plant material to the different sites to investigate short-term induced methylome variation. For all trees, we will assess traits related to flowering time and winter dormancy and test for association with methylome variation in particular candidate genes. Thus, we will be able to compare the spectrum of short-term and long-term environmentally induced epigenetic variation. Furthermore, we will investigate if alternate bearing is methylation sensitive. The anticipated results of our study on epigenetic variation – short-term, long-term, stability and heritability – will provide valuable knowledge on the potential of epigenetic variation in crop breeding.

DEVELOPMENT OF SYNBIO TOOLS FOR THE BIO-ENGINEERING OF RICE CHLOROPLAST PROCESSES AND PATHWAYS

Álvaro Eseverri[1,2], Can Baysal[3], Vicente Medina[3], Teresa Capell[3], Paul Christou[3,4], Luis M. Rubio[1,2] and Elena Caro[1,2*]

1Centre for Plant Biotechnology and Genomics, Universidad Politécnica de Madrid (UPM) - Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Pozuelo de Alarcón, Spain
2Departamento de Biotecnología-Biología Vegetal, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain
3Department of Plant Production and Forestry Science, University of Lleida-Agrotecnio Center, Lleida, Spain
4ICREA, Catalan Institute for Research and Advanced Studies, Barcelona, Spain

Plant synthetic biology is a valuable discipline in the fight against global warming, offering the possibility of the development of new ways to synthesize carbohydrates from atmospheric CO2, generating fuel and starting material for the chemical industry. In this context, the tools enabling chloroplast pathway engineering are especially useful for projects tackling climate change.
Nucleus-encoded plastid proteins are synthesized as precursors with N-terminal targeting signals called transit peptides (TPs), which mediate interactions with the translocon complexes at the outer (TOC) and inner (TIC) plastid membranes. These complexes exist in multiple isoforms in higher plants and show differential specificity and tissue abundance. While some show specificity for photosynthesis-related precursor proteins, others distinctly recognize nonphotosynthetic and housekeeping precursor proteins.
Here, we present our work on TPs from four Arabidopsis thaliana proteins to determine whether they were able to mediate import of a nuclear-encoded marker protein into plastids of different tissues of a dicot and a monocot species.
Our results show that Arabidopsis TPs mediate plastid import in rice callus, and in leaf and root tissues with very high efficiency, providing new biotechnological tools for crop improvement strategies based on recombinant protein accumulation in plastids by the expression of nuclear-encoded transgenes.

RUBICSO AND GSTF GENES AT WHEAT CULTIVAR DAJTI ARE DIFFERENTLY EXPRESSED UNDER SALINE STRESS

Ariola Bacu
Department of Biotechnology, University of Tirana, Albania
E_mail:ariola.bacu@fshn.edu.al

Local wheat cultivar Dajti (Triticum aestivum L.) was created in Albania through conventional selection practices, and was evaluated as resistant toward environmental stresses. Trying to understand the molecular basis of this resistance, we controlled the expression of GST and Rubisco genes. Glutathione S-transferases (GSTs), play an important role among effective defense mechanisms against stress-induced oxidative damages. Meanwhile, photosynthetic rate is highly correlated with the amount of Rubiscos encoded by rubisco genes (rbcL/S), which are also regulated from rubisco activases.

In our study we controlled the transcription of GSTF1 gene and rubisco genes (rbcL/S) at leaves of wheat of local cultivar Dajti under saline conditions. For these, seeds of the local cultivar were kept at 4^{o}C for 24 h and were allowed to germinate in Petri dishes supplied with water only at 22^{o}C for 3 days; further supplied with Hoagland solution for 14 days, and finally were grown in Hoagland culture modified with different concentrations of NaCl (50, 100, and 200 mM). Total RNA was extracted from leaf material at 0–3–6–10–24–72 h following saline treatment, and specific RT-PCRs were developed to control the expression of GSTF1 and Rubisco genes (rbcL/S). Concentrations of amplicons were evaluated in agarose gels.

As a result, synthesis of new rubisco gene products compared to controls was evidenced; Since photosynthetic rate is highly correlated with the amount of Rubisco, the presence of new transcripts of rubisco genes could be interpreted as plant's response for the regulation of the amount of the enzyme in order to keep the pigment's amount in leaves as stable as possible in shortly applied saline stress conditions. The transcription of GSTF1 was increased immediately after saline treatment, reduced during the time of exposure, and did not depend on salt concentration. This might be connected to the specific osmoregulation and more specifically to the inhibition of chloride accumulation at the roots of this cultivar.

In conclusion, the expression mode of both gene categories differs under saline conditions, suggesting this way that epigenetic mechanisms related to their transcription are involved, as part of the defense system of the plants.

INFLUENCE OF ENVIRONMENTAL CONDITIONS ON EPIGENETIC DETERMINISM OF ARABIDOPSIS CLUBROOT RESISTANCE

Petitpas M.[1], Lemoine J. [1], Lariagon C. [1], Gravot A. [1], Manzanares-Dauleux M.J. [1], Jubault M.[1]

IGEPP, INRAE, INSTITUT AGRO-AGROCAMPUS OUEST, Université de Rennes, F-35000, Rennes, France.

The individual impact of biotic or abiotic stresses on plants has been widely studied, but few researches have been conducted on the effect of combined stresses. Epigenetic variation was previously found to be involved in Arabidopsis resistance to clubroot, a major Brassicaceae disease caused by the protist Plasmodiophora brassicae. Moreover, detection of some clubroot resistance QTLepi was found to be dependent of the temperature and/or soil hydric status (Liégard et al., 2019; Gravot et al., 2016). We aim to study the influence of environmental conditions on the epigenetic determinism of Arabidopsis resistance to clubroot. For this, we will evaluate the impact of either a punctual increase of temperature or punctual soil hydric status variation on the Arabidopsis response to P. brassicae using epigenetic recombinant inbred lines (epiRIL). EpiRIL were used in order to distinguish epigenetic and genetic variability as they are genetically identical and only differ from their epigenome (Johannes et al., 2009). Assessment of 123 epiRILs lines issued from the cross ddm1 (partially resistant to clubroot) x Col-0 (susceptible to clubroot) (Johannes et al., 2009) for response to clubroot will be carried out under classical growth conditions (22°C during the 16h of light and 19°C during the night) and under punctual abiotic stress conditions. Higher temperature (25°C/22°C) or different water availability conditions (flooding or mild drought) will be applied at the beginning of the secondary phase of infection (i.e. 7 days post inoculation) for 7 days. Clubroot responses will be assessed through several variables at 21 days post infection. Linkage analysis will be carried out enabling us to identify genomic regions harbouring methylation variations associated with clubroot resistance in the different growth conditions. QTLepi identified will be compared between all environmental conditions and with QTL and QTLepi previously detected.

THE EFFECT OF SEQUENTIAL ABIOTIC STRESSES ON PHYSIOLOGICAL AND MOLECULAR DEFENSE RESPONSES IN ARABIDOPSIS THALIANA

Soyanni Holness , Giovanna Serino and Paola Vittorioso

Sapienza University of Rome, Department of Biology and Biotechnology 'Charles Darwin', Piazzale Aldo Moro 5,00185, Rome
Contact email: soyanni.holness@uniroma1.it

Single abiotic stress treatments with drought or light can trigger a molecular response that can last from several days to weeks and can help plants to deal with reoccurring stresses. However, it is often not clear if this is due to acclimation or to epigenetic memory (priming), and the underlying mechanisms that help plants to cope with subsequent stresses. We are interested in investigating the potential priming effects in plants subjected to subsequent stresses. Here we selected two specific abiotic stresses: high light (HL) and drought (D). Arabidopsis plants were subjected to a short-term HL stress, to a long-term D stress or to the two stresses administered sequentially. The stress responses were assessed at the physiological and molecular level and compared. In fact, RD29A (D), ELIP1(HL) and ABA1(both) increased upon stress induction. Additionally, HL seems to mediate a priming response against D through RD29A, ABA1 and NCED3 gene expression. Our results provide an initial characterization of the molecular mechanisms through which one stress can influence the plant response to a subsequent stress.

Keywords: Abiotic stress, drought, high light, priming, sequential

SHEDDING LIGHT INTO THE MODULATION OF VITIS VINIFERA L. DEFENSE: THE ROLE OF EPIGENETIC-RELATED TRANSCRIPTS AGAINST P. VITICOLA OUTBREAKS INDUCED BY CLIMATE-CHANGES

Azevedo V.*, Daddiego L.**, Cardone M.F.***, Perrella G.**, Sousa L.****, Malhó R.*, Figueiredo A.*, Bergamini C.***, Marsico A.D.***, Alagna F.**

*Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016, Lisboa (Portugal); **ENEA, Centro Ricerche Trisaia, S.S. 106 Ionica Km 419+500, Rotondella, MT (Italy); ***CREA, Centro di ricerca Viticoltura ed Enologia (CREA-VE), via Casamassima 148, 70010, Turi, BA (Italy) ****DEIO and CEAUL, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016, Lisboa (Portugal)

Plants plasticity against biotic stresses rely on the interconnection between immunity and defense. Epigenome modifications, including DNA methylation, histone modifications, chromatin remodelling and RNA modifications, influence the adaptive gene expression response. Up to date little is known on the epigenetic control of plant-pathogen interactions, particularly between grapevine and Plasmopara viticola. Downy mildew, caused by P. viticola is one of the most important diseases worldwide. In a climate change scenario, viticulture is facing new emerging challenges and more frequent re-occurrences of diseases such as downy mildew. Thus, a deeper knowledge on this pathosystem is crucial to define new disease management strategies. Our study aims at identifying the main epigenetic associated genes that are differentially modulated after P. viticola inoculation. Thus, a transcriptome analysis was performed on two new grapevine genotypes (N20/020 and N23/018) showing high and low susceptibility responses when inoculated with P. viticola, respectively. A microarray assay was carried out at 6 and 24 hours after inoculation (hpi) followed by statistical and gene ontology analysis. A total of 3664 differentially expressed genes were obtained with FDR cut-off <0,20. Of these, 662 were defence-related transcripts and 106 were associated to epigenetic mechanisms. In particular, the N23/018 appeared to have significantly modulated genes related to DNA methylation and chromatin modifications. This last mechanism is also part of the defense-related dataset differentially expressed in presence of the pathogen. Moreover, this genotype displays a quicker (6hpi) and more robust response since genes encoding disease resistance proteins are upregulated. Meanwhile, N20/020 presented significant modulation of genes encoding histone modification and chromatin remodelers. Furthermore, this genotype displays a later defense response (24hpi) with significant overexpression of protease inhibitor proteins. Overall, our results reveal that chromatin modifications could play a role in defense and particularly that the epigenetic machinery might have a function during P. viticola infection.

DNA METHYLATION ANALYSIS OF FLAX (LINUM USITATISSIMUM L.) GROWN UNDER THE CHRONIC RADIATION EXPOSURE IN CHERNOBYL

Veronika Lancíková, Martin Jopčík, Jana Libantová, Andrea Hricová

Institute of Plant Genetics and Biotechnology, Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Nitra, Slovakia

Plants are an excellent system when investigating the adaptation mechanisms to the radioactive contamination in environment. Response to the long-term radiation exposure can be manifested by genetic or epigenetic change. Changes in DNA methylation pattern are commonly associated with response to stress conditions. In the 2007, two experimental plots were established in the Chernobyl area, radioactive and nonradioactive (remediated). Cultural plants, soybean, flax, sunflower, wheat were cultivated on the plots. In this study, DNA methylation pattern had been investigated in flax grown under the chronic radiation exposure in Chernobyl for multiple generations. The study of flaxseed oil composition showed the increased content of alpha-linolenic acid. Fatty acid desaturase 3 genes encode the conversion of linoleic into α-linolenic acid. Gene-specific DNA methylation study of the FAD3A gene isoform had been performed using the combination of bisulphite sequencing and methylation-sensitive high resolution melting analysis. Demethylation in the coding region of the targeted gene had been identified. Genome-wide methylation-sensitive AFLP analysis showed the comparable level of DNA methylation in the seed samples collected from radioactive and nonradioactive plots.
Shortly after the Chernobyl accident, native plants and trees in the area responded to the acute irradiation by genome hypermethylation. However, decades later, flora developed the efficient adaptation mechanisms and survived in the changed environment. Unlike native flora, flax was intentionally sown on the experimental plots. The results suggested the ability of flax to cope with the persisting environmental stress.

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EXPLOITING EPIGENETIC VARIATION WITHIN IFVCNS OIL CROP COLLECTIOS – SETTING THE STAGE

Dragana Miladinovič1, Aleksandra Radanović1, Sandra Cvejić1, Siniša Jocić1, Ana Marjanović Jeromela1, Ankica Kondić Špika1, Jegor Miladinović1, Boško Dedić1, Milan Jocković1, Velimir Mladenov2

1Institute of Field and Vegetable Crops, National Institute of Republic of Serbia, Novi Sad, Serbia, 2University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia

Oil crops breeding at Institute of Field and Vegetable Crops (IFVCNS) has a successful 50-year long tradition that resulted in substantial collections of genetic resources of sunflower, rapeseed, pumpkins and 24 minor oil crops. IFVCNS sunflower collection is one of the largest collections of annual and perennial sunflower species, interspecific hybrids, sunflower varieties, lines and hybrids worldwide (http://www.nsseme.com/about/inc/oilcrops/wild.php). This collection has been actively used in breeding as a source of disease resistance and abiotic stress resistance genes. The species of the genus Helianthus have very broad spectrum of life forms and occupy diverse habitats. Long-term adaptation to different habitats and environmental conditions could lead to appearance of epigenetic variants that create the basis for survival of the Helianthus species in a wide variety of habitats. Within the framework of ongoing projects, we have started setting the stage for exploitation of epigenetic variations within the IFVCNS oil crops collections, with emphasis on sunflower, and identification of genotypes with stable and heritable epigenetic variants. Those would be further used for creation of EpiRILs complemented with molecular analyses for identification of epiQTLs correlated to abiotic and biotic stresses. The final aim of this new approach to exploitation of IFVCNS oil crop collections to identify genotypes with desirable traits that could be used for creation of highly productive resilient oil crop varieties, as well as to create an ideotypes specific for certain agro-ecological conditions.

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INTEGRATED TRANSCRIPTOME, SMALLRNA PROFILE AND METHYLOME OF TOMATO-GEMINIVIRUS INTERACTION

Beatriz Romero-Rodríguez1, Marko Petek2, Maja Kriznik2, Chen Jiao3, Miguel A. Botella1, Zhangjun Fei3, Kristina Gruden2, Eduardo R. Bejarano1, Araceli G. Castillo1.

1 Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-CSIC (IHSM-UMA-CSIC), Campus Teatinos, 29010 Málaga, Spain. 2 Department of Biotechnology and Systems Biology, National Institute of Biology, Vecna pot 111, 1000 Ljubljana, Slovenia, 3 Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, New York, USA
Corresponding author: Araceli G. Castillo (ara@uma.es)

Geminiviruses constitute the largest family of plant-infecting viruses with small, single-stranded DNA genomes that replicate through double-stranded DNA intermediates. Because of their limited coding capacity, geminiviruses use plant nuclear machinery to amplify their genomes, which are packaged into nucleosomes forming chromatin as multiple circular minichromosomes. Thus, viral minichromosomes must encounter the nuclear pathways that regulate host gene expression and chromatin states. DNA methylation and post-transcriptional gene silencing play critical roles in controlling infection of geminiviruses and this pathogen can counteract these host defense mechanisms and promote its infectivity. Tomato Yellow Leaf Curl Virus (TYLCV) belongs to the Begomovirus genus and is transmitted
by the whitefly Bemisia tabaci. With only six viral proteins, this geminivirus must create a proper environment for viral replication, transcription and propagation. Behind the apparent simplicity of geminiviruses lies a complex network of molecular interactions with their host and their natural vector, which induces a wide variety of transcriptional, post-transcriptional and chromatin changes in the host. To better understand this virus-host interaction at a genetic and epigenetic level we have analysed the transcriptome, sRNA profile and methylome of tomato plants (Moneymaker) infected with the geminivirus, TYLCV. Total RNA and DNA was extracted from tomato–infected plants (three biological replicates) and analysed at 2, 7, 14 and 21-day post-infection (dpi). Analysis of the changes in host transcription during the infection and its correlation with changes in sRNA profiles and DNA methylation will be presented and examined.

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A PRELIMINARY ANALYSIS OF DNA METHYLATION IN QUERCUS ILEX BY USING THE METHYLATION SENSITIVE AMPLIFICATION POLYMORPHISM (MSAP) TECHNIQUE

Mónica Labella-Ortega1, Mónica Escandón Martínez1, Ana López2, Jesús V. Jorrín-Novo1, María-Dolores Rey1

1Department Biochemistry and Molecular Biology, Universidad de Córdoba, Córdoba, Spain, 2 Department of Plant Molecular Genetics, Spanish National Centre for Biotechnology, Madrid, Spain.

Corresponding author: b52resam@uco.es

DNA cytosine methylation is an epigenetic mark determining gene expression and regulating plant growth, development, and responses to stresses, among other biological processes. Its study in orphan forest tree species like Holm oak is very limited. Holm oak is the most representative and emblematic species of the Mediterranean forest and the dehesa/montado agrosilvopastoral ecosystem, with relevance from an environmental and economic point of view. In this work, a preliminary analysis of DNA methylation using the MSAP technique has been performed. It has been carried out with two different individuals in leaves from seedlings and adult trees, and in seed embryos, in order to detect permanent and transitory markers along the developmental stages. DNA was extracted (CTAB), quantified (qubit), digested with EcoRI and MspI/HpaII (methylation sensitive) and subjected to PCR with specific primers (Rico et al., 2014). The PCR products were analysed by capillary electrophoresis. In total, 290 loci were resolved, being 187 methylated or hemimethylated, and 103 unmethylated. Out of 187, only 8 were detected in all the samples, revealing the dynamic character of the DNA methylation. The DNA methylation pattern was different between individuals, organs (leaves and embryo) and developmental stage (adult and seedling leaves). Higher DNA methylation was observed in a polyembryonic individual, being probably related to this phenotype. DNA from leaves was more methylated in seedlings than adult trees. The methylation pattern included permanent marks (present in both organs, leaves and embryos, and developmental stages) as well as transient ones (present in only one organ or developmental stage). Sequencing of DNA is now in progress, in order to identify specific methylated genes.

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STUDY OF THE ROLE OF SMALL RNAS IN DORMANCY CYCLE OF APPLE TREE

Julio Garighan1, Etienne Dvorak1, Bruno Huettel2, Priscila Grynberg3, Julie Leclercq1 Evelyne Costes1, Fernando Andrés1

1 UMR AGAP Institut, Univ Montpellier, CIRAD, INRAE, Institut Agro, F-34398 Montpellier
2 Max Planck Institute for Plant Breeding Research, Genome Centre, Cologne-Germany
3 Bioinformatica Lab, Embrapa Recursos Genéticos e Biotecnologia – Cenargen, Brasilia-Brazil

Winter dormancy is an adaptative mechanism that temperate and boreal trees have developed to protect their meristems against low temperatures. In apple trees (Malus domestica), cold temperatures induce bud dormancy at the end of summer/beginning of the fall. Apple buds stay dormant during winter until they are exposed to a period of cold known as a chilling requirement. Once the chilling requirement is satisfied, dormancy is released, and buds can resume growth (budbreak) and initiate flowering in response to warm temperatures in spring. In the annual model plant species Arabidopsis thaliana, the thermo-regulation of flowering-time is strongly controlled by the epigenetic and post-transcriptional modification of floral-regulators genes mediated by small RNAs. Small RNAs modulate temperature responses also in woody plant species, such as grapevine, poplar, and pear. However, how small RNAs are involved in genetic networks of temperature-mediated to regulate dormancy and budbreak in fruit tree species remains unclear.

To study the role of small RNAs on the control of apple dormancy, we performed a small RNA sequencing on bud samples during a time-course experiment. Using the method "TraPR" (Trans-kingdom, rapid, affordable Purification of RISCs), 373 different potential micro RNAs (miRNAs) were identified, including 105 known apple miRNAs. A total of 39 of them were differentially expressed during the dormancy cycle, suggesting their role in this process. The possible targets were identified in silico and classified according to their potential molecular function. Remarkably, targets of the identified miRNAs were related to growth control and hormonal signaling, cell wall modification, global transcriptional and post-transcriptional regulation, among other significant pathways. In conclusion, we identified several apple miRNAs and their targets potentially involved in genetic networks controlling dormancy and budbreak in fruit tree species.

INFECTIVE ENDOGENOUS VIRAL ELEMENTS ARE TIGHTLY CONTROLLED BY RNA SILENCING PATHWAYS IN BANANA

Pierre Olivier Duroy1,2*, Jonathan Seguin3, Sébastien Ravel1,2, Rajendran Rajeswaran 3*, Nathalie Laboureau1,2, Frederic Salmon4,5, Jean-Marie Delos4,5, Mikhail Pooggin6, Marie Line, Iskra-Caruana1,2 and Matthieu Chabannes7,8

1 CIRAD, UMR PHIM, 34398 Montpellier, France. 2 PHIM Plant Health Institute, Univ Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France. 3 Botanical Institute, Department of Plant Physiology, Zürich-Basel Plant Science Center, University of Basel, Basel, Switzerland. 4 CIRAD, UMR AGAP Institut, F-97130 Capesterre-Belle-Eau, Guadeloupe, France. 5 UMRAGAP Institut, Univ Montpellier, CIRAD, INRAE, Institut Agro, F-34398 Montpellier, France. 6 PHIM Plant Health Institute, Univ Montpellier, INRAE, CIRAD, Institut Agro, IRD, Montpellier, France 7 CIRAD, UMR AGAP Institut, F-34398 Montpellier, France.
8 UMR AGAP Institut, Univ Montpellier, CIRAD, INRAE, Institut Agro, F-34398 Montpellier, France.

Corresponding author: Matthieu Chabannes, Phone: +33 4 67 61 58 38; e-mail: matthieu.chabannes@cirad.fr

To protect genome integrity against invasive nucleic acids, most eukaryotic organisms have evolved gene silencing mechanisms, often involving small interfering RNAs (siRNAs). Endogenous banana streak virus (eBSV) integrants derived from three distinct species, present in Musa balbisiana (B) but not Musa acuminata (A) banana genomes are able to reconstitute functional episomal viruses in interspecific triploid AAB banana hybrids, causing banana streak disease. In contrast, the seedy diploid (BB) parent line, which harbours identical eBSV loci, does not develop disease. Illumina sequencing revealed that eBSV loci produce low-abundance transcripts covering most of the viral sequence and generate predominantly 24-nt siRNAs, in contrast to predominantly 21–22 nt viral siRNAs derived from the respective episomal viruses in M. acuminata or AAB hybrid plants. Remarkably, siRNA production is restricted to duplicated and inverted viral sequences present in eBSV loci. Using bisulfite sequencing we found that both siRNA-producing and non-producing sequences in the eBSV loci are methylated at 100 % symmetric CG and CHG sites and up to 50% in non-symmetric CHH sites unlike episomal virus DNA previously found to be largely unmethylated. Our data suggest that eBSVs are controlled at the epigenetic level in the BB diploids. This regulation prevents both their awakening and systemic infection of the plant, but probably also serves to confer the resistant phenotype observed in BB plants against mealybug-transmitted viral particles. These findings are thus of relevance to other plant resources hosting integrated viruses.

PARENTAL DNA METHYLATION STATES PREDICT METHYLOME REMODELING AND PHENOTYPIC HETEROSIS IN ARABIDOPSIS

Ioanna Kakoulidou1, Rhonda C. Meyer3, Robert Piecyk1*, Thomas Altmann3, Frank Johannes1,2

1 Department of Molecular Life Sciences, Technical University of Munich, Freising, Germany
2 Institute for Advanced Study (IAS), Technical University of Munich, Garching, Germany
3Department of Molecular Genetics, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben,Germany
* presenting author robert.piecyk@tum.de

There is growing evidence that, in addition to genetic factors, epigenetic factors also contribute to heterosis. We previously used near-isogenic but epigenetically divergent parents to create so-called epigenetic F1 hybrids (epiHybrids) in Arabidopsis thaliana. Preliminary phenotypic analysis in our lab revealed that specific differentially methylated regions (DMRs) in parental genomes are associated with heterosis in the epiHybrids. We hypothesized that these DMR-heterosis associations are mediated by extensive methylome and transcriptome remodeling. To test this hypothesis systematically, we generated a panel of 500 epiHybrid families by crossing a male sterile plant to 500 different ddm1-derived epigenetic recombinant inbred lines (epiRILs). Whole genome methylation sequencing and high-throughput phenotyping was performed for 169 parents-hybrid trios. Genome-wide analysis revealed that hybrid methylomes undergo extensive remodeling in each trio, with non-additive methylation levels clustering mainly around pericentromeric regions. Using stable DMRs as physical markers in a QTL mapping analysis, we found that parental DMRs in these non-coding regions predicted remodeling patterns not only locally (i.e. in cis) but also in trans at regulatory regions of thousands of genes. Further analysis showed that these same DMRs were also associated with heterosis in many morphological and developmental traits. Our results thus establish a model by which parental methylation differences in heterochromatin-rich pericentromeric regions act as major re-organizers of genome-wide methylation patterns in hybrids. This methylome remodeling induces downstream non-additive phenotypic effects in the form of heterosis. That these effects are achieved in isogenic plant material indicates that epigenetic variation is a major molecular determinant of this classical phenomenon.

miRNA-MEDIATED PHOSPHATE STARVATION REGULATORY MECHANISMS RELATED TO EMBRYOGENIC COMPETENCE MAINTENANCE IN TAMARILLO

Daniela Cordeiro, Ana Pedrosa, Jorge Canhoto, Sandra Correia

University of Coimbra, Centre for Functional Ecology, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

Somatic embryogenesis (SE) is a process by which somatic plant cells express totipotency, developing into embryos and later into plants. It is a valuable tool for the rapid and large-scale micropropagation of economically important species and an efficient system for genetically modified plant production and for studies on plant embryogenesis and cellular developmental plasticity.
In tamarillo (Solanum betaceum Cav.) SE, embryogenic (EC) and non-embryogenic callus (NEC) can be induced from the same explant. These calli lines can be kept in culture for several years. However, long-term (LT) subcultures cause loss of embryogenic potential and EC become unable to develop viable somatic embryos (LTC).
Previously obtained data, generated from tamarillo SE-induced cell lines, showed that specific miRNAs are differentially expressed among EC, NEC and LTC. Interestingly, two phosphate (Pi)-starvation-induced miRNAs, miR399 and miR827, were found to be up-regulated in callus with lower embryogenic capacity. Since Pi-starvation responses are mediated by sugar metabolism and subcultures are carried out with high sucrose levels, we hypothesise that the long exposure to high sucrose rates could be related to the loss of embryogenic competence.
To validate the smallRNA-Seq results, qPCR was used. miRNAs and target genes expression were normalized with miR166a, since this revealed to be the best reference for tamarillo calli analysis. Furthermore, Pi-quantification was performed in the different callus types.
miR399 and miR827 showed up-regulated expression in LTC and NEC compared with EC and, concomitantly, their targets, involved in cellular Pi-transport responses (PHOSPHATE2 and PHOSPHATE TRANSPORTER 5), were down-regulated in LTC. It was also found that LTC and NEC have Pi contents three times lower than EC, which explain the miRNAs up-regulation.
More assays are being obtained on the functional validation of both miRNAs. However, the results so far obtained, point out to an interaction between Pi levels, sugar metabolism and embryogenic competence.

TRANSGENERATIONAL EFFECT OF WATER STRESS ON SUNFLOWER SEED GERMINABILITY

Baptiste Vancostenoble1, Nicolas Blanchet2, Nicolas B. Langlade2, Christophe Bailly1

1 Sorbonne Université, IBPS, CNRS, UMR 7622 Biologie du Développement, Paris, France
2 LIPM, INRAe, CNRS, Toulouse, France.

Tolerance of crop plant to water stress is a major issue of climate change since adverse environmental conditions are expected to tragically reduce crop yields in a nearly future. Rapid and homogeneous seed germination is a major component of final crop yield but it can be dramatically impaired by the environmental condition prevailing at the time of sowing. In addition, the maternal environment conditions during seed development on the mother plant have been also demonstrated to have a marked effect on subsequent seed germinability. In sunflower, a moderate water stress applied to the mother plant after flowering enables a better germination under water stress of the seeds produced in these conditions, thus suggesting a maternal effect or a transgenerational effect on the progeny. To determine whether an epigenetic component could be associated with the induction of water stress tolerance by the maternal environment, we have investigated the effect of water stress applied on mother plants on the methylome of the resulting seeds at the time of shedding and during their germination in sub-optimal conditions. DNA methylation was determined after bisulfite treatment and HT Sequencing. The number and the methylation level of differentially methylated regions (DMRs) were not markedly modified during seed germination, either in optimal and no-optimal conditions, suggesting that modifications of DNA methylation occurred during seed development. Indeed, water stress on the mother plants modified pattern of DMRs of seed DNA. DMRs were found in the gene body, in the promoter or in the post coding sequence region of a set of differentially express genes identified by RNA seq. Comparison of DNA methylation with changes in gene expression induced by the seed maternal environment will be presented in order to draw a comprehensive model of transmission and inheritance of stress tolerance at the germination stage.

EPIGENETIC MODULATORS ENHANCE SOMATIC EMBRYO PRODUCTION FOR REGENERATION AND BREEDING OF CORK OAK

Elena Carneros, Esteban M. Díaz-Luzza, Yolanda Pérez-Pérez, Ivett Bárány I, Pilar S. Testillano

Pollen Biotechnology of Crop Plants, CIB Margarita Salas-CSIC, Madrid, Spain
Corresponding author: Pilar S. Testillano (testillano@cib.csic.es)

Somatic embryogenesis is a widely used biotechnological tool for large-scale mass propagation of selected material, genetic transformation and breeding, with many advantages in the case of forest tree improvement. However, its application is limited in woody species due to low efficiency and recalcitrance. Cell reprogramming, totipotency acquisition and somatic embryogenesis initiation involve changes in the developmental program of the cell, which affect global genome organization. We have reported, in rapeseed and barley, that microspore embryogenesis initiation is associated with DNA hypomethylation (Solís et al. 2012, 2015). However little is known in trees about DNA methylation dynamics during somatic embryogenesis (Rodríguez-Sanz et al. 2014, Corredoira et al. 2017). In this work we analyzed the changes in global DNA methylation levels and nuclear distribution
of methylated DNA as well as gene expression profiles of several DNA methyl transferases during somatic embryogenesis in Quercus suber L. (cork oak), by biochemical, molecular and immunocytochemical approaches. Furthermore, effects of a small bioactive molecule, the DNA demethylating agent 5'-azacytidine (AzaC), on somatic embryogenesis were analyzed. Results showed low levels of global DNA methylation at early stages of somatic embryogenesis, in proembryogenic masses, followed by a progressive increase in DNA methylation at later stages, during somatic embryo differentiation. AzaC treatment reduced global DNA methylation of proembryogenic masses and promoted the proliferation of somatic embryogenesis cultures, favoring somatic embryogenesis initiation. At advanced stages, AzaC prevented embryo differentiation, an effect that reverted by eliminating the drug from culture medium. AzaC treatment increased the expression of the early embryogenesis marker gene QsSERK1-LIKE, while the late embryogenesis gene QsLEA-LIKE was repressed. Elimination of AzaC from treated cultures led to the formation of higher proportion of embryos compared with control cultures. These findings provide new insights into the epigenetic regulation underlying somatic embryogenesis in cork oak, a forest species of high economic and ecologic value. Moreover, they provide a promising avenue for pharmacological intervention by using small molecule epigenetic modulators, to improve somatic embryogenesis yields for forestry breeding and propagation programs.

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02

DNA-methylation profiles of primed and unprimed clonal *Petunia hybrida* plants after several generations of hydric stress

Rétif Félix, Tisserand Myriam, Brouard Nathalie, Porcher Alexis, Gaillard Sylvain, Bucher Etienne*, Landès Claudine, Huché-Thélier Lydie, Verdier Jérôme, Leduc Nathalie

Univ Angers, Institut Agro, INRAE, *IRHS*, *SFR* QUASAV, F-49000 Angers, France
*current address: Agroscope, Nyon, Switzerland

Crop adaptation to stress-inducing environment is a key issue in the general context of climatic change but also to face the increasing demand for adapted plants to urban adverse growth conditions (building shade, irregular watering or restricted water usage, heat, poor soils). Genetic selection of new varieties through crossing of parental lines is the classical way to improve plants, but is a long process. New methods for crop adaptation are therefore studied, among them priming. Priming is the memorization of a physiological state following exposure to a stimulus (*e.g*. a stress) that allows a plant to respond quicker or more efficiently to next stimuli. Priming can be associated to epigenetics marks, such as DNA methylation changes induced by the stimulus. Priming has been studied only in few plant species and yet, little is known about the duration, intensity required for a stress to induce efficient priming, or on the length of plant memory of such priming state. Using *Petunia hybrida*, we currently address these questions through the study of successive vegetative clones exposed to water restriction. Developmental, physiological and molecular responses of primed and unprimed plants to water stress are compared as well as their DNA methylation profiles after whole genome bisulfite sequencing. First results concerning differentially methylated regions between these two batches of plants will be presented.

This research was conducted in the framework of the regional programme "Objectif Végétal, Research, Education and Innovation in Pays de la Loire", supported by the French Region Pays de la Loire, Angers Loire Métropole and the European Regional Development Fund." This project contributes to the scientific program of UMT STRATège (ASTREDHOR-INRAE - Université d'Angers, Institut Agro Agrocampus Ouest, Beaucouzé, France).

THE NEXT-GENERATION SEQUENCING TOOL WAS USED TO COMPARE TWO REFERENCE APPLE GENOMES

Andrea GULYÁS[1], Norbert HIDVÉGI[1], Judit DOBRÁNSZKI[1]

1 Centre for Agricultural Genomics and Biotechnology, Faculty of the Agricultural and Food Science and Environmental Management, University of Debrecen, Nyíregyháza P.O. Box 12, H-4400,

Researchers are now concerned with a number of issues in molecular genetics, including how plants respond to environmental changes and how they affect genetic stock. We examined how different sequencing methods yield different results, e.g., fitting the genomic details of an apple into two different genome reference genomes. We needed to see whether there was a distinction between these methods and, if so, which approach could be shown to be superior. As a result, we compared first-generation (Sanger) to another hybrid reference sample. Sanger sequencing identified 30,294 genes, while hybrid sequencing identified 45,116 genes. To assess where the methylated cytosines are found in the CpG, CHG, and CHH contexts, our sequencing findings were plotted on both reference genomes. The CHH background has the largest difference between the two sequencing groups, with 63,011 contexts for Sanger sequencing and 15,547 contexts for hybrid sequencing. We will learn what roles our sequenced genes play in biological processes, what molecular functions they serve, and what cellular components they make up, with Omicsbox. Except in hybrid sequencing, where the cellular variable organization genes are different in biological systems, the two blasts are almost identical. For both sequencing outcomes, Omicsbox contrasted the three contexts. As previously stated, there was a disparity in cellular organization between CpG and CHG sequencing and Sanger sequencing in the case of CpG and CHG. A biogenesis mechanism has already arisen and been added to biological processes in the form of CHH. Except for the cellular elements, there was no discernible distinction between the two sequencing methods. We sequenced the apple varieties to the reference genomes after adapting them to the reference genomes and found no significant differences in the sequencing methods. Sanger has 30,294 genes and the hybrid genome has 45,116 genes annotated, there could be minor variations.

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SYNTHETIC PROMOTER-DRIVEN PADEF IMPARTS RESISTANCE AGAINST PHYTOPATHOGENS IN THE TRANSGENIC PLANT TO INTERCELLULAR COMMUNICATION AND PLASMODESMATA IN PLANT DEVELOPMENT AND DISEASE

Lini Sethi

Institute of Life Sciences (ILS), Bhubaneswar

Phytophatogens cause damage to crops globally, influencing the economy and agriculture extensively. Extensive use of chemicals for the treatment leads to the expansion of several resistant strains of phytopathogens that have been reported. The development of a plant system expressing antimicrobial peptides could be an alternative to overcome this issue. We analysed the full-length synthetic transcript (Flt) promoter of the Blueberry red ringspot virus (BRRV) and synthesized two chimeric promoters (MBR3 and FBR3). Transcriptional activities of these chimeric promoters were found equivalent to that of the CaMV35S2 promoter. We have raised transgenic plants expressing antimicrobial peptide PaDef fused with an apoplastic signalling sequence (aTP) to drive the encoded transgene under these chimeric promoters in the apoplast. This plant-derived Padef ensures protection from a broad range of bacteria, where the antifungal property against two phytopathogenic fungi, namely *Alternaria alternata* and *Phoma exigua* var. *exigua* was studied using in vitro agar-based killing assays. Antibacterial properties of plant made PaDef was also evaluated against two Gram- negative bacteria i.e., *E. coli*, and *P. aeruginosa* and two Gram-positive bacteria i.e., *S. aureus*, and *R. fascians*, by OD_{600} assays. In vitro agar-based, well diffusion antifungal assays have shown that the recombinant PaDef expressed peptide driven by MBR3, FBR3, and $CaMV35S^2$ promoters indicate a distinct equal zone of inhibition against *A. alternata*. This suggests that the newly developed chimeric promoter can also effectively convey defense against plant pathogenic fungus. Alongside, the present study using recombinant PaDef showed a broad range of antibacterial properties by inhibiting the growth of Gram-positive and Gram-negative bacteria, including *E. coli*, *S. aureus*, *P. aeruginosa*, especially against phytopathogenic bacteria *Rhodococcus fascians*. This study provides probable chimeric caulimoviral promoters with the distinct cis-arrangement that could be useful in propelling transgenic technologies for plant-based research.

DNA METHYLATION ASSOCIATED GENES HELP TO TELL THE EVOLUTIONARY HISTORY OF CASTANEA GENUS

Sofia Alves1, ngelo Braga2, Carolina Soares2, Manuela Ribeiro Costa3, Leonor Morais-Cecílio1

1Linking Landscape, Environment, Agriculture and Food, Institute of Agronomy, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal
2Institute of Agronomy, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal
3Biosystems and Integrative Sciences Institute, Plant Functional Biology Centre, University of Minho, 4710-057 Braga, Portugal

Castanea is a genus belonging to the Fagaceae family, with high economic and ecological importance, due to the value of its fruits and wood. Epigenetic regulation is a process of gene silencing and activation important for plant mechanisms like bud dormancy and flower development. Epigenetic regulators such as DNA methyltranferases (DNAMTases) are proteins related to the maintenance and de novo DNA methylation, while demethylases (DDME) are associated with active DNA demethylation. With the intent to characterize these proteins in different species of Fagaceae, we searched for orthologs of 7 DNAMTases (MET1, CMT1, CMT2, CMT3, DRM2, DRM3 and DNMT2) and 3 DDMEs (DME, ROS1 and DML2) in different species of the genus Castanea (C. sativa from Europe, C. dentata from North America, C. crenata from Japan, and C. henryi from China), in Quercus (Q. robur) and in Fagus (F. sylvatica e F. crenata). Free access RNA-seq information was used to assemble de novo transcriptomes of those species. To establish a phylogenetic history of these genes we used the amino acid sequences of all expressed DNA methylases and demethylases together with the respective sequences from A. thaliana, Q. suber, Q. lobata and C. mollissima, to create phylogenetic trees constructed with Maximum Likelihood as the optimization criteria.
The DDMEs tree shows individual gene clusters according to the expected phylogenetic relationships, however the DNAMTases family tree gave some incompatible results. In order to understand this result, a phylogenetic tree was constructed with the concatenated aligned sequences. This final tree reflected the phylogenetic relations of the studied genus, supporting the evolutionary history of the Castanea genus in the Fagaceae family.

Key words: Fagaceae, Epigenetic regulators, Phylogenetic analysis, DNA methyltransferases, DNA demethylases, Castanea
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MOLECULAR PROPERTIES OF EPIMUTATION HOTSPOTS

Rashmi R. Hazarika1,2, Michele Serra1, Zhilin Zhang1 , Yinwen Zhang3 , Robert J. Schmitz2,3, Frank Johannes1,2

1Technical University of Munich, Department of Molecular Life Sciences, Freising, Germany
2TUM Institute for Advanced Study, Garching, Germany
3The University of Georgia, Department of Genetics, Athens, USA

Mistakes in the maintenance of CG methylation (mCG) are a major source of spontaneous epimutations in plants. Once acquired, these stochastic changes can be transmitted mitotically and meiotically, and thus accumulate in plant genomes over time. Multigenerational surveys show that the rate of spontaneous epimutations varies substantially across the genome, with some loci being particularly prone to maintenance errors. These “epimutation hotspots” contribute substantially to methylation diversity patterns in natural populations; yet their molecular basis remains poorly understood. Using Arabidopsis as a model, we show that epimutation hotspots are indexed by a specific set of chromatin states (CS) that map to sub-regions of gene body methylation (gbM) genes. Although these regions comprise only ~9.4% of all CGs in the genome, they account for ~50% of all epimutation events per unit time. Molecular profiling of these regions revealed that they contain unique sequence features, harbor steady-state intermediate methylation levels, and act as putative targets of DNA demethylases. We demonstrate, experimentally, that even subtle shifts in steady-state methylation in these hotspot regions are sufficient to significantly alter local epimutation intensities. Our study lays the groundwork for exploring the molecular mechanisms and evolutionary consequences of epimutation hotspots in plants.