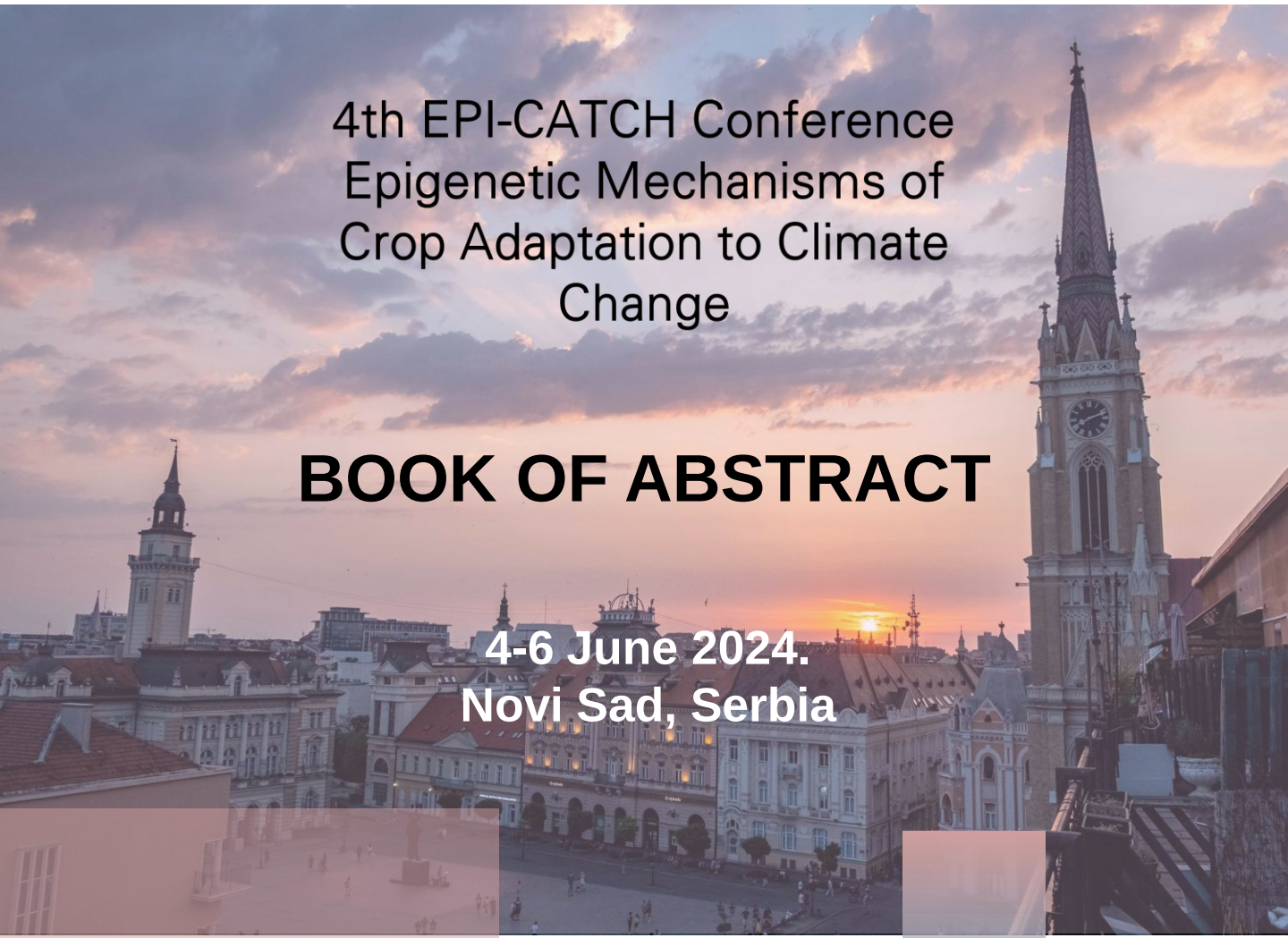
epicatch

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY



4th EPI-CATCH Conference Epigenetic Mechanisms of Crop Adaptation to Climate Change

BOOK OF ABSTRACT

4-6 June 2024.
Novi Sad, Serbia

PLANT BREEDING IN VIEW OF EPIGENETIC MECHANISMS AND TECHNOLOGICAL ADVANCEMENTS

РЕПУБЛИКА СРБИЈА
АУТОНОМНА ПОКРАЈИНА ВОЈВОДИНА
ПОКРАЈИНСКИ СЕКРЕТАРИЈАТ ЗА ПОЉОПРИВРЕДУ, ВОДОПРИВРЕДУ И ШУМАРСТВО

РЕПУБЛИКА СРБИЈА
АУТОНОМНА ПОКРАЈИНА ВОЈВОДИНА
ПОКРАЈИНСКИ СЕКРЕТАРИЈАТ ЗА ВИСОКО ОБРАЗОВАЊЕ И НАУЧНОИСТРАЖИВАЧКУ ДЕЛАТНОСТ

Funded by the European Union

BOOK OF ABSTRACT

SCIENTIFIC COMMITTEE

Federico Martinelli – University of Florence, Italy
Velimir Mladenov – University of Novi Sad, Serbia
Stéphane Maury – University of Orléans, France
Sotirios Fragkostefanakis – Goethe University Frankfurt, Germany
Michal Lieberman-Lazarovich – Volcani Center, Israel
Eirini Kaiserli – University of Glasgow, United Kingdom
Valya Vassileva – IPPG, Bulgarian Academy of Sciences, Bulgaria
Pilar Testilliano – CIB Margarita Salas – CSIC, Spain

LOCAL ORGANISING COMMITTEE

Branko Ćupina, Full Professor
Velimir Mladenov, Associate Professor
Đorđe Krstić, Full Professor
Borislav Banjac, Associate Professor
Svetlana Vujić, Associate Professor
Ksenija Mačkić, Associate Professor
Jovana Šućur Elez, Associate Professor
Bojana Blagojević, Assistant Professor
Rada Šućur, Teaching Assistant
Teodora Feher, Junior Teaching Assistant

TUESDAY, JUNE 4TH

RNA-BASED EPIGENETIC REGULATION & CHROMATIN DYNAMICS AND HISTONECODE IN PLANT STRESS RESPONSE

KEYNOTE SPEAKERS

Exploring the chromatin-based regulation of enhancer promoter contact and its impact on gene expression in tomato

Moussa Benhamed[1]

[1]University Paris Saclay, France, Paris

The complex and dynamic three-dimensional organization of chromatin within the nucleus makes understanding the control of gene expression challenging, but also opens up possible ways to epigenetically modulate gene expression. Because plants are sessile, they evolved sophisticated ways to rapidly modulate gene expression in response to environmental stress, that are thought to be coordinated by changes in chromatin conformation to mediate specific cellular and physiological responses. However, to what extent and how stress induces dynamic changes in chromatin reorganization remains poorly understood. Here, we comprehensively investigated genome-wide chromatin changes associated with transcriptional reprogramming response to heat stress in tomato. Our data show that heat stress induces rapid changes in chromatin architecture, leading to the transient formation of promoter-enhancer contacts, likely driving the expression of heat-stress responsive genes. Furthermore, we demonstrate that chromatin spatial reorganization requires HSFA1a, a transcription factor (TF) essential for heat stress tolerance in tomato. In the light of our findings, we propose that TFs play a key role in controlling dynamic transcriptional responses through 3D reconfiguration of promoter-enhancer contacts.

Keywords: chromatin, epigenetics, heat stress, transcription factors, histone

An evolutionary tale of PWOs and PRC2 proteins

Ahamed Khan[1], Abdoallah Sharaf[1], Saqlain Haider[2], Alžbeta Kusová[3], Petra Procházková Schrumpfová[3], Iva Mozgová[1], Sara Farrona[2]

[1]Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic
[2]School of Biological and Chemical Sciences, College of Science and Engineering, National University of Ireland, Galway, Ireland
[3]Laboratory of Functional Genomics and Proteomics, National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic

Evolution may occur through gradual changes over time or by sudden novel events. We are interested in understanding the evolutionary history of PWWP-DOMAIN INTERACTOR OF POLYCOMBS (PWO) proteins and their molecular functions across the green lineage. Our phylogenetic analyses demonstrate that PWOs suddenly emerged in the Lycophytes, contrasting with more ancestral plants, such as moss, that lack PWOs. Structural analyses of PWOs across plant species, from ancestral to modern ones, show the conservation of an N-terminal PWWP domain and a short C-terminal motif. Through a synthetic biology approach using Selaginella moellendorffii (Sm) PWOs and Arabidopsis thaliana PWO1 respectively as ancestral and modern protein models, our goal is to understand how PWOs may have contributed to the evolution of chromatin dynamics and developmental traits. Here, I will show data of the conservation of PWOs' interactions to components of POLYCOMB REPRESSIVE COMPLEX 2 (PRC2), indicating the functional significance of PWOs in epigenome regulation during land plant evolution.

Keywords: PWOs, PRC2, epigenetics, evolution

ORAL AND POSTER PRESENTATION

Heat stress transcription factors of B class act as molecular switches for the activation and attenuation of stress response to optimize thermotolerance

Ayat Bakery[1], Natalia Sapia[1], Kerstin Zehl[1], Christos Bazakos[2,3], Moussa Benhamed[4], Sotirios Fragkostefanakis[1]

[1]Goethe University Frankfurt, Germany, Frankfurt am Main
[2]ELGO DEMETER, Greece, Thessaloniki
[3]Max Planck Institute for Plant Breeding Research, Germany, Koeln
[4]University Paris Saclay, France, Paris

Plants experience heat stress (HS) at temperatures where growth and development are negatively affected. Survival from HS is dependent on the upregulation of hundreds of genes with protective functions for protein and cellular homeostasis such as heat shock proteins (HSPs). During recovery from stress, the response mechanisms are turned off to allow the re-establishment of homeostasis. However, some of these mechanisms are maintained in a standby mode, allowing the plants to quickly respond in case of a new HS incident. All these processes are controlled at the level of transcription, mainly by members of the family of HS transcription factors (HSFs). In tomato, HsfA1a is the master regulator of HS response and therefore essential for thermotolerance. Class B HSFs act as both as co-activators and co-repressors, therefore serving as ON/OFF molecular switches in a condition and target gene-dependent manner. Our current model on the bivalent function of HsfB factors and their relation to histone modifiers will be discussed.

Keywords: heat stress, transcription factors, histone, thermotolerance

The Role of Epigenetics in clinical and Environmental fungi

Letterio Giuffrè[1]

[1]University of Messina, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, Italy, Messina

The term “epigenetic” refers to cellular activity modification mediated by several factors that affect the expression of target genes without any DNA sequence changes. In recent years, a growing body of research has demonstrated that epigenetics modification plays a dynamic role between the genome and the environment, in both clinical and environmental fungal species. In clinical fungi, epigenetic modifications are crucial in shaping virulence-determining traits, drug resistance, and host-pathogen interactions, while, in environmental fungi, epigenetics changes seem to be involved in stress-response and adaptive mechanisms. Moreover, unusual epigenetic modifications appear to be involved in several other biological process in fungi, including sexual reproduction and origin of new species. This oral presentation will provide an overview of the current knowledge surrounding the impact of epigenetic mechanisms on the biology, pathogenicity, evolution, and adaptation of fungi in clinical and environmental settings.

Keywords: epigenetic, fungi, bioinformatics

Epitranscriptomics: an overview on RNA modifications in prokaryotes and eukaryotes

Maria Rosa Felice[1]

[1]Department of Chemical Biological Pharmaceutical and Environmental Sciences University of Messina, Messina, Italy

Epitranscriptomics is a relatively new and emerging area of post-transcriptional gene regulation research involving many RNA modifications that affect its functionality, stability, folding, splicing and translation. During this talk, I will present and analyze the most common modifications that occur post-transcriptionally on either prokaryote and eukaryote messenger RNAs (mRNAs), especially those produced by plants. I will also discuss the key enzymes already known to be involved in the dynamism of this process and the potential effects that RNA modifications have on normal plant physiology. In conclusion, I will briefly describe the experimental techniques mainly used to discover and/or confirm new RNA modifications, including their limitations and strengths, with a particular look towards modern nucleic acid sequencing methods.

Keywords: epitranscriptomics, RNA, plant, sequencing

Effects of RNAi-mediated crown gall tumor suppression in transgrafted walnut trees

Federico Martinelli[1,2], Sriema L. Walawage[1], Ramona Abbattista[1], Houston Saxe[1], Paulo A. Zaini[1], Hossein Gouran[1], My Phu[1]; Monica Britton[1], Brad Hanson[1], Charles A. Leslie[1], Gregory T. Browne[3,4], Daniel Kluepfel[3,4], Matthew A. Escobar[1,5], Abhaya M. Dandekar[1]

[1] Department of Plant Sciences, University of California, Davis, CA, USA
[2] Department of Biology University of Florence, Sesto Fiorentino, Italy
[3] Department of Plant Pathology, University of California, Davis, CA, USA
[4] Crops Pathology and Genetics Research Unit, United States Department of Agriculture, Agricultural Research Service, Davis, CA, USA
[5] California State University San Marcos, CA, USA

Transgrafting is defined as the combination of non-transgenic (NT) scion with transgenic rootstock (or vice versa). In this work, we have obtained walnut transgrafted trees composed of NT Chandler scions transgrafted in transgenic Paradox rootstocks expressing dsRNA generated from self-complementary tryptophan monooxygenase (iaaM) and isopentenyl transferase (ipt) gene fragments. The two specific aims of this work were: 1) to determine if dsRNA are able to transit across the graft union, and 2) to analyze possible effects on the bark proteome and metabolome as well as possible changes in the expression of horticultural and disease-related traits. Small RNA sequencing showed the absence of any significant movement of dsRNA from rootstock to the scion through the graft union. Expressed dsRNA mapped mostly on Ti plasmid of Agrobacterium. Proteomic analysis confirmed no transit of transgenic proteins (KanR and GUS) into the scion. No effects of RNAi-mediated transgrafting were observed on horticultural traits as well as susceptibility to key pathogens (Phytophtora spp. and Xanthomonas arboricola pv. jugalandis) and weed population control. Although 28.6% of identified proteins in scion barks of transgrafted trees were significantly affected in comparison to wild-type grafted trees, these changes reflected small changes at the metabolome level. In the J1-1A transgrafted line, an enhancement of maltose, galactinol, and raffinose and an altered carbon-nitrogen balance was observed. These results confirmed that the transgenic RNAi-mediated rootstock strategy is effective in reducing safety concerns due to the absence of dsRNA migration through the graft union and the reduced metabolomic effects in scion tissues.

Keywords: walnut, RNA-interference, biotic stress

Light and warm temperature signal integration at the chromatin level

Eirini Kaiserli[1]

[1]University of Glasgow, Glasgow, United Kingdom

Plants are highly plastic organisms and therefore provide an ideal system to study how environmental stimuli shape their morphology and growth. Light and temperature fluctuations are interconnected and act as informational signals to modulate critical developmental transitions and architecture during the life-cycle of flowering plants balancing growth and adaptation. Light and thermal signalling components localise in highly dynamic sub-nuclear hubs to control chromatin accessibility and changes in gene expression. The talk will focus on the role of light signal integrators in modulating thermomorphogenesis and thermotolerance at the transcriptional and chromatin level.

Keywords: light, warm temperature, chromatin, transcription, nuclear bodies, Arabidopsis

eIF3M2 maintain pollen tube integrity during heat stress via HSP70 up regulation

Zahra Kahrizi[1,2], Christos Michailidis[1], Karel Raabe[1,2], David Honys[1], Said Hafidh[1]

[1]Laboratory of Pollen Biology, Institute of Experimental Botany of the Czech Academy of Sciences, Czech Republic
[2]Department of Experimental Plant Biology, Faculty of Science, Charles University, Czech Republic

Translational control is crucial for pollen tube growth, which in turn ensures proper fertilization and seed generation. Heat stress (HS) causes a global halt to translation, which protects proteins from misfolding and promotes the synthesis of the proteome that cells need to survive. In this study, we demonstrate that under normal conditions, the Arabidopsis double mutant of the translation initiation factor subunits eif3m1 and eif3m2 has a higher rate of seed abortion, bursted PT, and low germination. Surprisingly, eif3m1 exhibited improved PT integrity and a higher germination rate than wild type Col-0 when subjected to HS at 37 °C. We found that elevated levels of the paralog eIF3M2, which increased HSP70 mRNA expression levels transcriptionally, were strongly linked to the enhanced thermotolerance of the eif3m1 PT. A direct overexpression of eIF3M2 increased HSP70 expression, while an eif3m2 mutant increased PT bursts and decreased HSP70 levels under HS conditions. Further, we demonstrate that eIF3M2 interacts directly with HSP70.1 and HSP70.2 in tobacco leaf epidermal cells and co-immunoprecipitates with HSP70 in PT. The data show that plants use the eIF3M2-HSP70 module to control their thermotolerance and keep their PT intact. This makes it an advantage for adaptation to warmer temperatures during fertilization and seed set.

Keywords: Heat stress, translation, eIF3

How plant stress transcription factors regulate heat stress response in tomato

Rada Šućur[1], Velimir Mladenov[1], Sofija Petrović[1], Borislav Banjac[1], Teodora Feher[1], Sotirios Fragkostefanakis[2]

[1]University of Novi Sad, Faculty of Agriculture, Serbia
[2]Department of Molecular and Cell Biology of Plants, Goethe University Frankfurt, Germany

The heat-shock (HS) response in tomato (Solanum lycopersicum) serves as an effective model to explore transcription mechanisms and regulation. This protective process is transcriptionally governed by HS transcription factors (Hsfs), central to modulating transcriptome dynamics through the reprogramming of numerous genes' expression. The thermotolerance capability of Hsfs primarily relies on the regulation of various heat shock proteins (HSPs), assisting cells in managing stress-induced protein accumulation and misfolding. Three HS transcription factors stand as pivotal regulators of the tomato's heat stress response, categorized into classes A, B, and C. Class A is marked by a unique C-terminal activator domain that endows transcriptional activator functions, whereas class B maintains a conserved LFGV motif in their C-terminal domain (CTD), purportedly imparting repressor capabilities. Class C lacks both activator and repressor domains. The initiation of the response hinges on HsfA1a's activity, constantly expressed yet inactive under non-stressful conditions. HsfA1a is recognized as a master transcriptional regulator of heat stress responses in plants and is crucial for dynamically forming promoter-enhancer interactions in response to heat across multiple loci. In contrast, HsfB1 functions as a repressor of various Hsfs and as a co-activator of HsfA1a, with its suppression resulting in an enhanced leaf photosynthetic rate due to the accumulation of several Hsfs. HsfB1 is noted for its rapid degradation when operating as a repressor, dependent on a repressor domain. The heat stress response (HSR) encompasses signal detection, activation of heat stress transcription factors (Hsfs), and synthesis of heat shock proteins (HSPs). During the STSM the synergistic activity of HSFs was examined and the contribution of the histone acetyltransferase in the formation of co-activator complexes was explored. The analysis was done by transiently expressing the factors in tomato protoplasts and analyzing their activity using a GUS reporter system. The results show that the co-expression of HSFA1a, HSFB1 and HAC1 results in very strong induction of HS-responsive genes and likely has a positive impact on thermotolerance.

Keywords: tomato, heat stress (HS), HS transcription factors, thermotolerance

A Crosstalk Between ABA Signalling and Epigenetic Regulators of Gene Expression Modulates Pine Response to Fusarium circinatum

Glória Pinto[1], Beatriz Santos[1], Joana Amaral[3]

[1]Centre for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro, Aveiro, Portugal
[2]Lancaster University, Lancaster, United Kingdom
[3]Lancaster Environment Center, Lancaster University, Lancaster, United Kingdom

Fusarium circinatum, causing pine pitch canker (PPC), affects conifers productivity and health worldwide. Selection and breeding for resistance arise as the most promising approach to fight PPC. The accumulation of ABA (Abscisic Acid) in needles of symptomatic Pines was reported by our team as being associated with susceptibility to *F. circinatum* and explained the impairment of photosynthesis observed after stomata closure. Although *P. radiata* activates ABA catabolism conceivably to control the pool of bio-active ABA, this mechanism seems insufficient to fight *F. circinatum* infection at later stages of disease development. Besides, the conversion of ABA into the weakly ABA-like active PA may be a key defence response in *P. pinea*. To highlight the importance of ABA-signalling in PPC outcome a proteomic analysis was performed. We evaluated the dynamics of the needle proteome of a susceptible (*Pinus radiata*) and a relatively resistant (*Pinus pinea*) species upon *F. circinatum* inoculation by GeLC-MS/MS. Integration with physiological data and validation of key genes by qPCR allowed to identify core pathways regulating these contrasting responses. In *P. radiata*, pathogen effectors may target both the secondary metabolism to negatively regulate immune response; and chloroplast redox proteins to increase energy-producing pathways for amino acid production in its favour. In contrast, chloroplast redox regulation may assure redox homeostasis in *P. pinea*, as well as non-enzymatic antioxidants. The presence of membrane trafficking-related proteins exclusively in *P. pinea* likely explains its defence response against *F. circinatum* effectors. We suggest that pine response to *F. circinatum* is modulated by a crosstalk between ABA and epigenetic regulation of gene expression. Considering these promising results, further research is needed to depict the role of ABA-mediated epigenetic reprogramming during pine response against *F. circinatum* and to design innovative tools to support breeding programs aiming to achieve PPC resistance.

Keywords: *Pinus-Fusarium circinatum* interactions, biotic stress, proteomics, epigenic regulation

Investigating the Impact of Micro-Nanoplastic Exposure on Arabidopsis thaliana: Unraveling Novel Long Non-Coding RNAs and Differential Expression Profiles

Orazio Romeo[1], Federico Martinelli[2], Roberta Galbo[1], Letterio Giuffrè[1], Maria Rosa Felice[1], Domenico Giosa[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy
[2]Department of Biology, University of Florence, Florence, Italy

This study delves into the effects of micro-nanoplastic (MNP) exposure on Arabidopsis thaliana, revealing novel insights into long non-coding RNAs (lncRNAs) through RNA-seq analysis. We explore differential gene expression profiles triggered by transparent (Tr-PET) and blue (Bl-PET) polyethylene terephthalate MNPs, broadening our understanding of molecular mechanisms governing plant responses to environmental stressors. Ten-day-old seedlings underwent random allocation to one of three treatment conditions: i) control (C), ii) Tr-PET, and iii) Bl-PET. After a 17-day growth period under controlled conditions, roots were collected in biological triplicates for RNA extraction and NGS TruSeq Library preparation. Raw reads were quality-checked, mapped to the TAIR10.1 genome, and novel transcripts were reconstructed using stringtie. Transcript-level expressions were quantified using salmon, and differential gene expression analysis was conducted with edgeR. A total of 323 novel lncRNAs were identified, expanding the A. thaliana transcriptome. Differential expression analysis revealed 281 (116 up, 165 down), 1425 (795 up, 630 down), and 280 (103 up, 177 down) transcripts with altered expression levels in “C vs Bl-PET”, “C vs Tr-PET”, and “Tr-PET vs Bl-PET” comparisons, respectively. Notably, 15 novel ncRNAs showed differential expression in pairwise comparisons: 1 up and 2 down in “C vs Bl-PET”; 8 up and 6 down in “C vs Tr-PET”; 1 up and 1 down in “Tr-PET vs Bl-PET”. Most of the novel annotated ncRNAs exhibited opposite expression levels to their interacting mRNA counterparts, suggesting a modulatory role in gene expression. This study enhances our comprehension of MNP-induced responses in plants, offering valuable insights into environmental stress adaptation mechanisms.

Keywords: micro-nanoplastic, RNA-Seq, lncRNAs

Bioengineering desiccation tolerant seedling using the seed desiccation process

Naoto Sano[1], Jaiana Malabarba[1], Jerome Verdier[1]

[1]University of Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, Angers, France

Mature dry seeds have a particular mechanism that allows them to survive with loss of >90% of water content called desiccation tolerance (DT). The seed-DT is tightly regulated, being acquired during seed development and lost shortly after germination. The timing of loss of DT after germinated can be extended by exogenous stimuli e.g., a mild osmotic stress (re-induction of the DT to germinated seeds). The molecular processes involved in DT acquisition/loss and re-induction have been well studied at the transcriptomic level, which resulted in identification of DT-associated gene networks. However, our understanding of molecular mechanisms that switch-on/off the genetic networks of seed-DT remains fragmentary, especially from epigenetic aspects.
In this study, we identified DT-core genes (a cluster of genes specifically associated with DT re-induction) in root tissues of germinated Medicago by RNA-Seq, followed by analyses to determine the chromatin status of the loci of these DT-core genes. ATAC-Seq revealed that the chromatin state of genomic regions containing the DT-core genes is clearly linked to their expression levels and phenotypes. Additionally, ChIP-Seq for histone repressive marks detected a prominent signal of H3K27me3 on the DT core-gene sequences at the later stage tissue with condensed chromatin, suggesting that silencing of DT in post-germination developmental programs will be mainly due to the H3K27me3 marks by the action of the PRC2 complex. As a result of this investigation, we bioengineer the desiccation process acquired during seed maturation to reinduce this process during seedling establishment allowing an improved seed germination and seedling establishment under extreme drought conditions.

Keywords: desiccation, seed, seedling, epigenome

WEDNESDAY, JUNE 5TH

PLANT BREEDING, BIOTECHNOLOGY AND EPIGENETICS IN CROPS

KEYNOTE SPEAKERS

Between ecology and evolution, the selection of standing epigenetic variation

Benoit Pujol[1]

[1]CNRS, Perpignan, France

Evolution by natural selection can occur when organisms harbor inherited phenotypic variation, and phenotypic variants have differential fitness. Transgenerational epigenetic variation exists for fitness-related traits and both theory and population epigenetic variation predict that selection can act on this variation alone without a contribution of genetic variation. Remarkably, this hypothesis has not previously been tested by using a direct selection experiment. I will present experimental selection results showing that selection can act on transgenerational epigenetic variation, implying its role as an additional source of standing variation available for short-term adaptation.

Keywords: methylation, selection, experiment, plant

A new strategy to enhance phenotypic variation in plants

Hidayah Alotaibi[1], Korawit Opassathian[1], Thomas Dean[1], Ashley Garrison[1], Ryan Merrit[1], Javier Antunez-Sanchez[1], Jose Gutierrez-Marcos[1]

[1]School of Life Sciences, University of Warwick, United Kingdom

Being sessile organisms, plants are exposed to a wide range of environmental stress conditions. Recent studies have shown that plants can store information about environmental stresses and access this associated memory to mount a primed response that offers protection from subsequent stress events. This 'stress memory' is thought to be mediated by epigenetic modifications, which in turn modulate gene expression, physiology and metabolism. However, these environmentally-directed epigenetic changes although they are integrated into somatic cells are short-lived and/or actively reset during sexual reproduction. We have recently found that clonal plants generated using zygotic transcription factors display epigenetic and transcriptional features present in the founder cells used for regeneration. Moreover, these molecular signatures are stably transmitted over multiple generations of sexual reproduction, creating distinct phenotypic variants. Therefore, we hypothesised that cloning from somatic cells exposed to distinct environmental stimuli could be used to engineer specific primed responses in plants. To test this hypothesis, we have generated clonal lines from different tissues exposed to short stress pulses and propagated clonal progenies over multiple generations under stress-free conditions. Our data reveal that these plants display stable stress memory responses, which are underpinned by distinct epigenetic states. Using this knowledge, we have identified some of the molecular mechanisms implicated in the integration, storage and retrieval of the acquired stress memory enabling the targeted engineering of specific primed responses.

Keywords: memory, epigenetics, priming

ORAL AND POSTER PRESENTATION

A High-Tech Methylation Detection Method for Epigenetic Analysis: High Resolution Melting

Kaan Hürkan[1]

[1]Department of Agricultural Biotechnology, Faculty of Agriculture, Iğdır University, Turkey

DNA methylation is a fundamental epigenetic mechanism whereby methyl groups are added to cytosine nucleotides, typically occurring in CpG dinucleotides. This modification plays a crucial role in regulating gene expression, influencing various cellular processes such as development, differentiation, and disease susceptibility. By altering chromatin structure and accessibility, DNA methylation serves as a dynamic mechanism for cells to establish and maintain different gene expression patterns without altering the underlying DNA sequence. Epigenetics, including DNA methylation, thus provides a mechanism for cells to respond to environmental cues and ensure proper gene regulation throughout development and in response to external stimuli. Although detection of DNA methylation with traditional approaches especially bisulphite sequencing is a gold standard, it requires sophisticated laboratory skills, budget and extended period of laboratory time. Hence, High Resolution Melting (HRM), which is a technique added to the end of polymerase chain reaction (PCR), helps researchers to detect DNA variants with less time, less cost and less laboratory skills. The technique can also easily be employed to a standard molecular biology laboratory. Since it doesn't require cloning or sequencing, it is a pioneering technique for DNA methylation studies. In this presentation, we would like to give detailed information about the technique including benefits and drawbacks.

Keywords: HRM, CG - CHG and CHH methylation types, melting kinetics, DNA variants.

The quest for epigenetic variation hideouts

Sofija Petrović[1], Aleksandra Radanović[2], Borislav Banjac[1], Mirela Matković Stojšin[3], Velimir Mladenov[1], Teodora Feher[1], Rada Šućur[1]

[1] University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia
[2] Institute of Field and Vegetable Crops, Novi Sad, Serbia
[3] Tamiš Research and Development Institute, Pančevo, Serbia

The complexity of interactions within population has been high, already, and epigenetics is rising it to the whole new higher level. Bread wheat was studied, in order to diminish genetic variance, because of its genetic homogeneity within variety. As self-pollinated, wheat varieties are of high homozygosity, almost a pure lines. The uniformity of genetic constitution in population, as well as, phenotypic homogeneity should be a good starting point to single out phenotypic variation caused by other causes than genetic constitution variation. On the bases of multi-environment long term trial, conducted simultaneously at the locality with stressful high sodicity, clay rich solonetz soil type, as well as, at the locality with normal or even favorable conditions of chernozem soil type in four growing seasons, where the first one was characterized by enormous precipitations, causing a water abundance stress, the second one was stressful due to temperature extremes, and drought, the third one was extremely favorable season, and the fourth one was a normal growing season. For wheat varieties of the Green Revolution ideotype have been under the scope. Particularly their reaction to solonetz soil type stress in different growing seasons, from stressful to favorable meteorological conditions. The starting point of deductive analysis were the existing models for total trial variance partitioning and quantifying. Since there are no models that could bring out epigenetic variance and quantifying it from total Multi Genotype and Environment Trial (MGET), the quest for epigenetic variance hideouts was an endeavor within the limits of today's quantitative genetics.

Keywords: wheat, stress, epigenetics, climate changes, food security

Exploring the role of FAT genes in Solanaceae species through genome-wide analysis and genome editing

Sibel Bahadır[1], Musa Kavas[1]

[1]Ondokuz Mayis University, Faculty of Agriculture, Department of Agricultural Biotechnology, Turkey

Plants produce numerous fatty acid derivatives, and some of these compounds have significant regulatory functions, such as governing effector-induced resistance, systemic resistance, and other defense pathways. This study systematically identified and characterized eight FAT genes, four in the S. lycopersicum and four in the S. tuberosum genome. Phylogenetic analysis classified these genes into four distinct groups, exhibiting conserved domain structures across different plant species. Promoter analysis revealed various cis-acting elements, most of which are associated with stress responsiveness and growth & development. miRNA analysis identified specific miRNAs, notably miRNA166, targeting different FAT genes in both species. Utilizing CRISPR/Cas9-mediated knockout, mutant lines for SlFATB1 and SlFATB3 were successfully generated and exhibited diverse mutation types. Biochemical evaluation of selected mutant lines revealed significant changes in fatty acid composition, with linoleic and linolenic acid content variations. The study also explored the impact of FAT gene knockout on tomato leaf architecture through SEM, providing insights into potential morphological alterations. Knocking out of FAT genes resulted in a significant reduction in both trichome and stoma density. These findings contribute to a comprehensive understanding of FAT genes in Solanaceous species, encompassing genetic, functional, and phenotypic aspects.

Keywords: fatty acids, FAT genes, CRISPR/Cas9, leaf architecture

System biology analysis discovers key cold-regulated gene transcription regulators of the ERF-family in olive tree

Christina Skodra[1], Michail Michailidis[1], Adrian Mehmeti[2], Martina Samiotaki[3], Ioannis Ganopoulos[4,5], Georgia Tanou[5,6], Christos Bazakos[4,5,7], Sotirios Fragkostefanakis[2], Athanassios Molassiotis[1]

[1]Laboratory of Pomology, Department of Horticutlure, Aristotle University of Thessaloniki, Greece
[2]Plant Molecular and Cell Biology, Goethe University, Frankfurt am Main, Germany
[3]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece
[4]Institute of Plant Breeding and Genetic Resources, ELGO-DIMITRA, Thessaloniki-Thermi, Greece
[5]Joint Laboratory of Horticulture, ELGO-DIMITRA, Thessaloniki-Thermi, Greece
[6]Institute of Soil and Water Resources, ELGO-DIMITRA, Thessaloniki-Thermi, Greece
[7]Max Planck Institute for Plant Breeding Research, Department of Comparative Development and Genetics, Cologne, Germany

Cold temperatures significantly affect the growth of olive (Olea europaea L.) trees during colder seasons, yet the mechanisms by which olive plants maintain balance within their environment during cold acclimation are not fully understood. In this study, we examined how olive plants (cv. Chondrolia Chalkidikis) respond to cold conditions. One-year-old olive trees were subjected to a gradual decrease in temperature, ranging from 15 to -3°C, for 35 days in a growth chamber. Simultaneously, a control group of trees was maintained under consistent light and humidity conditions at 20°C. We assessed the physiological status of both cold-stressed and non-stressed groups using various morphological and physiological assays. Additionally, we conducted comprehensive whole-genome DNA methylation sequencing, transcriptomic, proteomic, and metabolomic analyses on leaves exposed to temperatures of 5, 0, and -3°C. By integrating and analyzing the -omics data, we elucidated the molecular pathways activated during cold acclimation in olive trees. Our findings identified key transcription factors (TFs) involved in the cold acclimation process, with particular emphasis on two ethylene response transcription factors (ERFs), ERF1a and ERF5, which showed notable transcript differentiation. Functional analysis of these TFs revealed their regulatory role in enhancing the viability of olive protoplasts during cold exposure. Finally, through genetic approaches and promoter activation assays (such as DNA affinity purification sequencing), we unveiled cold-responsive genes linked with ERF1a and ERF5. This work offers a comprehensive understanding of the molecular mechanisms driving olive tree adaptation to cold temperatures.

Keywords: cold stress, epigenetic, ethylene response transcription factors, olive, system biology

Identifying heat stress recurrence specific features for plant acclimation: a case study in oilseed rape

Sophie Brunel-Muguet[1]

[1]Université Caen Normandie, INRAE, Caen, France

The expected increased frequency of heat waves is a major threat to field crops that complete their reproductive phase over spring and summer in temperate regions. Although many studies investigated the effects of high temperatures during the crop reproductive stage on yield and seed quality, the responses of plants to repeated stressing events are much less documented. However, these new climatic features raise the following questions (i) how plants face stress recurrence during their life cycle, (ii) can stress recurrence induce stress memory and even acclimation, and (iii) are specific mechanisms implemented. Over the last few years, our group performed several heat stress experiments to characterize the effects of different temperature profiles that differed in terms of intensity, duration, timing and frequency of the stressing events, in oilseed rape. The leading objectives were to identify thermopriming protocols and the associated molecular regulations, mainly at the epigenetic level. Our results indicated that most yield components and quality criteria (e.g. fatty acids concentration and composition) were negatively impacted by heat whether intense or moderate but to different extents according to the pods cohort, thus meaning that the timing of stress application is determining. No alleviating effect (i.e. priming) of an early stress followed by a 5 day-recovery phase prior to heat peaks was observed whereas a gradual increase prior to the heat peaks helped reduce their negative effects. These last effects were associated to several histone post translational modifications, and at the proteomic level, to sulfur-metabolic processes and oxidative stress. Our analyses led to conclude that (i) the effect of a succession of heat stress events does not match the sum of the individual effects, which reflects stress memory, (ii) a gradual increase prior to an intense stress helps the plants acclimating while priming-induced processes can be reset after a too long duration of recovery. Overall, these datasets acquired at different scales of analysis will provide useful information to improve crop performances predictions by either implementing process-based crop models or developing data-mining approaches

Keywords: stress memory, heat, oilseed rape, seed

Using epigenetics to manage plant pathogens: A case study on viruses and fungi

Monika Götz[1], Khalid Amari[2]

[1] Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Urban Green, Braunschweig, Germany
[2] Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Biosafety in Plant Biotechnology, Quedlinburg, Germany

Plant pathogens, like viruses and fungi, remain a persistent and costly threat to crop yields. In response, plants have developed epigenetic mechanisms to regulate their growth and adapt to changing environments. Our research has shown promising results in creating resistant plants using these mechanisms. We have successfully used epigenetics to silence susceptibility genes for viruses and powdery mildew in Arabidopsis and tobacco plants, using both transgenic and transgene-free methods. By methylating the promoter of selected host genes, we were able to downregulate the expression of susceptibility factors and provide resistance to future generations of plants, even without the transgene. Our findings highlight the potential of epigenetics in the field of plant protection.

Keywords: plant pathogens, susceptibility genes, methylation, resistance

Multi-omic analysis during fruit development and cold storage in sweet orange

Christina Skodra[1], Michail Michailidis[1], Vaia Styliani Titeli[1], Elpida Nasiopoulou[1], Martina Samiotaki[2], Georgia Tanou[3], Ioannis Ganopoulos[4], Vasileios Ziogas[5], Athanassios Molassiotis[6], Christos Bazakos[7]

[1] Laboratory of Pomology, Department of Horticulture, Aristotle University of Thessaloniki, Thessaloniki-Thermi, Greece
[2] Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece
[3] Institute of Soil and Water Resources, ELGO-DIMITRA, 57001, Thessaloniki-Thermi, Greece
[4] Institute of Plant Breeding and Genetic Resources, ELGO DIMITRA, Thessaloniki-Thermi, Greece
[5] Institute of Olive Tree, Subtropical Crops and Viticulture, ELGO-DIMITRA, Chania, Greece
[6] Laboratory of Pomology, Department of Horticulture, Aristotle University of Thessaloniki, Thessaloniki-Thermi, Greece
[7]Institute of Plant Breeding and Genetic Resources, ELGO DIMITRA, Thessaloniki-Thermi, Greece

Sweet orange (Citrus sinensis), belonging to the genus Citrus within the Rutaceae family, is popular in the market and among consumers for its sweet flavor and superior texture. The fruit is a rich source of vitamins, carotenoids, fiber, flavonoids, phenolic compounds, and other bioactive elements. Sweet orange, accounts the 60% of global citrus production and is significant not only as a fresh fruit but also for its juice consumption. Recent advances in next-generation sequencing technologies and analytical methods have paved the way for an in-depth exploration of the chemistry and genetics underlying fruit development across pre- and post-harvest (cold storage) stages. The unveiling of the chromosome-level phased 'Valencia' sweet orange genome marks an important advancement in comprehending the unique genetic attributes of sweet orange fruits, facilitating the seamless integration of multi-omic analyses to study fruit development and response to cold storage. In this study, we present an integrated analysis encompassing whole-genome, transcriptome, epigenome, proteome, and metabolome data for the Greek-oriented 'Sanguine Gouritsis' sweet orange. We aim to uncover genes and regulatory networks involved in fruit development, adaptation to cold storage, and the maintenance of fruit quality. Through this comprehensive approach, we aim to elucidate the complex molecular mechanisms that contribute to the nutritional quality of sweet orange fruits, thereby offering insights that could enhance breeding efforts in this economical impost fruit species.

Keywords: multi-omic, fruit development, cold, breeding

Breeding for resilience: enhancing sunflower tolerance to drought stress

Sandra Cvejić [1], Boško Dedić[1], Aleksandra Radanović [1], Milan Jocković [1], Nemanja Ćuk[1], Jelena Jocković [1], Sonja Gvozdenac[1], Dragana Miladinović[1], Siniša Jocić [1]

[1]Institute of Field and Vegetable Crops, Novi Sad, Serbia

Sunflower is an economically important oil crop, mostly grown in arid and semi-arid regions where drought stress is a major limiting factor. Although sunflower is generally considered tolerant, drought stress still significantly hampers its productivity and nutritional quality across its major cultivation areas. Therefore, understanding the impact of stress and plant response is vital for enhancing the sunflower's drought tolerance. Mitigating the adverse effects of drought on productivity and fostering the development of tolerant sunflower genotypes have emerged as strategic objectives in sunflower breeding. Breeders use all available resources and methods to increase genetic variability, thereby enhancing the probability of generating highly productive genotypes. Several mechanisms (morphological and physiological) have been developed that help mitigate the effects of drought and adapt to arid or water-limited environments. Recognizing that phenotype manifestation depends on genotype-environment interactions, enhancing genetic variation in plant architecture is imperative for optimizing productivity under prevailing environmental conditions. Genomic and transcriptomic studies have elucidated the complex genetic mechanisms underlying sunflower's response to drought stress, facilitating the discovery of novel genes and pathways involved in drought tolerance. This molecular insight has enabled breeders to accelerate the introgression of favorable alleles and genomic regions conferring drought resilience into elite sunflower germplasm.

This work is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2022-14/ 200032, by the Science Fund of the Republic of Serbia through IDEAS project “Creating climate smart sunflower for future challenges” (SMARTSUN), grant number 7732457, by the European Commission through Twinning Western Balkans project CROPINNO, grant number 101059784, by Center of Excellence for Innovations in Breeding of Climate-Resilient Crops - Climate Crops, Institute of Field and Vegetable Crops, Novi Sad, Serbia, and based upon work from COST Actions PlantEd (CA18111) and EPI-CATCH (CA19125), supported by COST (European Cooperation in Science and Technology). www.cost.eu.

Keywords: sunflower, drought, adaptation, phenotyping, genetic mechanisms

A comprehensive approach to enhancing sunflower drought tolerance

Aleksandra Radanović[1*], Sandra Cvejić[1], Boško Dedić[1], Milan Jocković[1], Siniša Jocić[1]; Jelena Jocković[1], Sonja Gvozdenac[1], Nemanja Ćuk[1], Nada Hladni[1], Ana Marjanović Jeromela[1], Ankica Kondić-Špika[1], Dragana Miladinović[1]

[1]Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia

Agricultural production faces a significant reduction as a result of unpredictable weather events due to global warming. Among the most significant abiotic stresses contributing to crop yield loss are drought, extreme temperatures, and soil salinity. Sunflower, the fourth most important oil crop worldwide, is considered to be moderately drought tolerant. However, recent studies showed that drought can cause up to 51% yield loss. Breeders are thus facing a complicated challenge - breeding for drought resistance which is a quantitative trait causing complex changes on all levels: morphological, physiological, and molecular. Institute of Field and Vegetable Crops is situated in the Pannonia region which is characterized as one of the European regions that will be the most affected by extreme climate changes such as drought. Within our breeding programs and project activities, we are conducting sunflower phenotyping at the germination stage in in vitro conditions and rhizothrons to identify traits that can be exploited in drought-tolerant sunflower breeding. This research is accompanied by an analysis of the expression of genes identified as of potential interest in enhancing drought tolerance. Further studies will include analysis of lncRNAs and small RNAs to obtain a comprehensive knowledge of mechanisms involved in drought tolerance on a molecular level. The final goal is to identify key drought-tolerant genes as well as epigenetic-targeted genes.

Acknowledgment: This research is supported by “Epigenetic Mechanisms of Crop Adaptation to Climate Change” (EPI-CATCH) - CA19125, Twinning project CROPINNO Grant No. 101059784 and Horizon Europe HelEx N°101081974 funded by the European Commission and “Creating climate smart sunflower for future challenges” (SMARTSUN) Grant No. 7732457 funded by the Science Fund of the Republic of Serbia, through IDEAS call. This work was done as a part of activities of Center of Excellence for Innovations in Breeding of Climate-Resilient Crops - Climate Crops, Institute of Field and Vegetable Crops, Novi Sad, Serbia. The research was supported by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, grant number: 451-03-66/2024-03/200032.

Keywords: Helianthus annuus L., abiotic stress, small RNA, lncRNA, epiQTLs

Biochemical response of sunflower inbred lines inoculated with Macrophomina phaseolina

Nemanja Ćuk[1], Biljana Kiprovski[1], Sandra Cvejić[1], Boško Dedić[1], Brankica Babec[1], Miloš Krstić[1], Siniša Jocić[1], Vladimir Miklič[1], Jelena Jocković[1], Milan Jocković[1], Boško Dedić[1], Velimir Mladenov[2]

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

Macrophomina phaseolina, the causative agent of charcoal rot, affects a wide array of plant hosts, including sunflower. This disease thrives in warm, arid conditions, leading to symptoms such as the wilting, drying, and premature ripening of sunflower plants. This study aims to explore the biochemical responses of 15 inbred lines, each exhibiting varying levels of resistance, to uncover potential correlations between resistance levels and biochemical reactions in sunflower inbred lines. The investigation focused on: total protein content, lipid peroxidation intensity (as a marker of membrane integrity), reduced glutathione, superoxide-dismutase activity, and total phenolic content (as antioxidant compounds). These parameters were assessed 10 days following the laboratory inoculation of inbred lines with the pathogen. Correlations between resistance levels and the results of these five assays were analyzed in conjunction with disease severity observed in the inbred lines. Remarkably, after the 10-day assessment period, only the total phenolic content showed a significant positive correlation with the resistance of inbred lines ($r=0.712$; $p<0.05$). This finding identified inbred lines PB 21 and L 1 as the most resilient among the tested varieties. In conclusion, it was observed that different inbred lines exhibit distinct responses to Macrophomina phaseolina. However, in the majority of cases, an increase in total phenolic content was noted in sunflower plants following inoculation. This suggests a potential defensive mechanism triggered by the pathogen. Further studies can analyze more precisely into the molecular intricacies of sunflower resistance to charcoal rot and validate these findings across broader genetic backgrounds.

Keywords: charcoal rot, disease severity, resistance, total phenolic content

Transient knockdown of a grapevine DNA demethylase gene for improved resilience against downy mildew

João Proença Pereira[1], Rita B. Santos[1],Vicente Vives-Peris[2], Aurelio Gomez-Cadenas[2], Walter Chitarra[3], Luca Nerva[3], Andreia Figueiredo[1]

[1]Grapevine Pathogen Systems Lab, BioISI, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal
[2]Department of Biology, Biochemistry and Natural Sciences, Universitat Jaume I, Castelló de La Plana, Spain
[3]CREA – Council for Agricultural Research and Economics, Research Centre for Viticulture and Enology, Conegliano, Italy

Grapevine downy mildew poses a significant threat in vineyards worldwide, which renders urgent the search for novel ways to combat this disease. Among these, epigenetic modifications, and specifically DNA methylation, have gained novel attention in the field of plant-pathogen interactions, mainly for its many roles in gene expression regulation and possible transgenerational inheritance. In this work, we set out to characterize the family of grapevine DNA demethylases both bioinformatically and at the gene expression level, and elect a candidate gene for transient gene silencing using the Spray-induced gene silencing (SIGS) platform. Expression patterns revealed a clear distinction between incompatible and compatible interactions, with an early-onset upregulation of all detected transcripts in the former, while the latter only showed significant upregulation in the later timepoints, linking early onset activation of DNA demethylases to tolerance. From this assay, VvROS1-like was elected for further characterization using SIGS. Expression data revealed sucessful knockdown of the target gene, with no notorious compensation effects by other DNA demethylase paralogs. Expression of two genes responsible for jasmonic acid (JA) synthesis - VvLOX13 and VvJAR1 - were also assessed, with expression patterns revealing concomitant downregulation with VvROS1-like knockdown, implying a possible role for VvROS1-like in regulating the expression of these genes. Global DNA methylation abundance and phytohormone quantification were also performed, to better understand the systemic roles of VvROS1-like in the immune response of grapevine towards downy mildew.

Keywords: grapevine downy mildew, DNA methylation, DNA demethylase, phytohormones

Improvement of phenotypic predictions by integration of epigenetic data into genomic models: case study in black poplar

Alexsandre Duplan[1], Harold Duruflé[1], Leopoldo Sanchez-Rodriguez[1], EPITREE Consortium[1], Stéphane Maury[2]

[1]Laboratoire de Physiologie, Écologie et Environnement - P2E (at Orleans university), Orleans, France
[2]BioForA - INRAe Val de loire, Orléans, France

A major challenge in biology is to understand the interplay between different omics layers (e.g. genomics, epigenomics, transcriptomics.) and how these interactions can improve our understanding of variation in complex phenotypic traits. The objective of this study is to improve the accuracy of prediction models of complex traits by integrating epigenetic data layers into existing models, and to test new statistical prediction models combining multiple layers. For this purpose, we used a collection of 200 individuals of Populus nigra from 8 populations in Western Europe. Trees were grown in a common garden in Orléans (FRANCE) for 4 years and different phenotypic traits were measured as height or wood circumference. Multi-omics data were generated for the whole population to obtain a collection of SNP, gene expression data and DNA methylation (SMP= single methylation polymorphism). Spectral data obtained by NIRS are also available. Descriptive analyses (PCA) for the intra- and inter-populations variability of the methylome have been conducted. Association analyses were also done for epiQTL, which aim to identify specific regions of the genome that appear to play a role in regulating epigenetic features, or epiGWAS, to study the associations between SMPs and phenotypic traits. The ultimate goal of these analyses was to develop prediction models through statistical modeling or machine learning, integrating multi-omics layers to predict complex traits. Another output will be to explore the relationships between molecular information (omics) and phenotypic traits, to better understand how omics influence the phenotype of an organism.

Keywords: predictive model, multi-omics, population, trees adaptation

R program for data analysis in fruit breeding

Milena Lakićević[1], Sandra Bijelić[1]

[1]University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia

R program and its interface RStudio are widely used for data processing in agricultural science. One of the main features of the R program is the possibility to apply different packages suited for specific analysis when processing laboratory and field data. This paper presents the most suitable R packages for data processing in fruit breeding. There are R packages suitable for numerical data analysis in general and some of the most commonly applied ones are: (1) „dplyr“ – developed for data filtering, transformation and summarization, (2) „ggplot2“ – suitable for advanced graphical visualization of results and (3) „agricolae“ – developed for a detailed set of statistical tests and statistical analysis. In addition, there are packages exclusively developed for processing results in fruit breeding and some of the most commonly applied ones are: (4) „fitPoly“ – designed for genotype-trait association tests and a detailed identification of genetic markers associated with desirable traits such as fruit flavor, size, and nutritional content, and (5) “ASMap” – developed for constructing linkage maps from marker data, which helps in understanding so-called “genetic architecture” and important fruit traits in marker-assisted selection. The paper analyzes the suitability of each package in specific fruit breeding tasks.

Keywords: R packages, data processing, visualization of results, „fitPoly“, “ASMap”

Comparison of DNA methylation landscape between Czech and Armenian vineyards show their unique character and increased diversity

Miroslav Baránek[1], Kateřina Baránková[1], Jana Raddová[1], Anna Nebish[2]

[1]Mendeleum Department, Faculty of Horticulture, Mendel University in Brno, Lednice, Czech Republic
[2]Department of Genetics and Cytology, Yerevan State University, Yerevan, Armenia

Grapevine is a worldwide crop and it is also subject to global trade in wine, berries and grape vine plants. Various countries, including the countries of the European Union, emphasize the role of product origin designation and suitable methods are sought, able to capture distinct origins. One of the biological matrices that can theoretically be driven by individual vineyards' conditions represents DNA methylation. Despite this interesting hypothesis, there is a lack of respective information. The aim of this work is to examine whether DNA methylation can be used to relate a sample to a given vineyard and to access a relationship between a DNA methylation pattern and different geographical origin of analysed samples. For this purpose, DNA methylation landscapes of samples from completely different climatic conditions presented by the Czech Republic (Central Europe) and Armenia (Southern Caucasus) were compared. Results of the Methylation Sensitive Amplified Polymorphism method confirm uniqueness of DNA methylation landscape for individual vineyards. Factually, DNA methylation diversity within vineyards of Merlot and Pinot Noir cultivars represent only 16% and 14% of the overall diversity registered for individual cultivars. On the contrary, different geographical location of the Czech and Armenian vineyards was identified as the strongest factor affecting diversity in DNA methylation landscapes (79.9% and 70.7% for Merlot and Pinot Noir plants, respectively).

Keywords: grapevine cultivar; geographical origin, plant adaptation, epigenetic changes, MSAP

Salicylic acid as biostimulant for secondary metabolite production in wheat sprouts

Ružica Ždero Pavlović[1], Boris Popović[1]

[1]University of Novi Sad, Faculty of Agriculture, Serbia, Novi Sad

Wheat (Triticum aestivum) is one of the most consumed grain in the world. Also, wheat is one of the most important food staples in Serbia with an average annual production of 2.5 million tons. The growing interest in agriculture to increase crop yield has led to a boom in the number of biostimulant products in market. Biostimulants, including seed priming agents, are a promising choice for sustainable agricultural production. Recently, consuming of wheat sprouts has become popular because it can bring more health benefits than consuming wheat grain. During the sprouting of wheat seeds, many valuable processes are activated that accumulate various bioactive compounds, such as vitamins, amino acids and phenolic acids. Salicylic acid (SA) is one the most important plant phenolics that affects many process in plants. The aim of this study was to assess the effect of SA (50 mg/L) as a seed priming material on wheat growth parameters and the production of pharmacologically attractive secondary metabolites in wheat sprouts. Wheatgrass grown from SA primed seeds exhibited better performance in terms of seed germination traits, growth and antioxidant parameters. The results showed that SA enhanced the biomass accumulation and phenolic acid accumulation in wheat sprouts.

Keywords: wheatgrass, salicylic acid, seed priming

Addressing Major Challenges: Evaluating Advanced Apple Selections in Turkey

Ayşe Nilgün Atay[1], Şerif Özongun[2], Burak Kunter[3], Ersin Atay[1]

[1]Burdur Mehmet Akif Ersoy University, Food Agriculture and Livestock School, Department of Crop and Livestock Production, Horticulture Programme, Burdur, Turkey.
[2]TAGEM Fruit Research Institute, Egirdir, Isparta, Turkey
[3]Turkish Energy Nuclear and Mineral Research Agency, Nuclear Energy Research Institute (TENMAK-NÜKEN), Ankara, Turkey

Apple breeding programs are instrumental in developing new apple cultivars tailored to consumer preferences and industry challenges, including disease resistance and climate adaptability. In Turkey, our breeding initiative targets the development of resilient apple varieties with superior taste, texture, appearance, and extended storage capabilities. Utilizing nuclear techniques, we have established a mutant pool derived from the esteemed local 'Amasya' cultivar to produce enhanced variants. Our crossbreeding endeavors involve prominent cultivars such as 'Braeburn,' 'Gala,' and 'Granny Smith.' During the initial phase (2011-2015), we established plantations, introducing approximately 4000 full-sib offspring into our institution's gene pool. Subsequently (2016-2020), fruit evaluations were conducted, with individuals showing promise advancing to grafting onto M.9 rootstock. Fourteen full-sib offspring progressed to the second stage of selection. Concurrently, mutation breeding studies identified effective mutation doses, resulting in a plantation with 857 putative mutants grafted onto M.9 rootstock. Detailed fruit observations and morphological measurements from 2016 to 2020 identified 13 mutants advancing to the second stage of selection. Further studies will assess additional commercial properties such as yield and storage life. Looking ahead, our focus will encompass investigating the impact of abiotic stresses on these superior genotypes to elucidate underlying molecular mechanisms. This holistic approach underscores our dedication to advancing apple breeding in Turkey, committed to meeting evolving consumer demands and environmental challenges.

Keywords: fruit quality, mutation, selection, yield, sensory analysis, offspring

Analysis of differentially methylated DNA sequences in potato plants exposed to French marigold essential oil

Jelena Savić[1], Milica Milutinović[1], Milan Dragićević[1], Mladen Seničar[2], Sofija Stupar[1], Sanja Šajkunić[1], Nina Devrnja[1]

[1]Institute for Biological Research "Siniša Stanković"- National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia
[2]Institute for Artificial Intelligence Research and Development of Serbia, Novi Sad, Serbia

Volatile organic compounds (VOCs) are important for tropospheric chemistry and have diverse roles in ecosystems. Plants 'sense' VOCs to efficiently adapt and respond to their environment. Volatiles of neighboring plants through alternations in the receiver plant's physiology contribute to the induction of direct plant defense against pest organisms and elevate immunity status by translating plants to 'primed' status when they are ready for rapid and energy-efficient response to upcoming attacks. Induced epigenetic changes, on the level of DNA and histones, could be involved in this response. Plant essential oils (EOs) have shown promising prospects as novel priming inducers, but a little is known about their potential to induce previous epigenetic alternations. In this study, potato (Solanum tuberosum L.) plants were exposed to French marigold (Tagetes patula L.) EO during 3 consecutive days for 8h each day and samples were collected 10 days after EO removal. Whole-Genome Bisulfite Sequencing (WGBS) was used to identify EO-induced differentially methylated (DM) DNA sequences. Average methylation level of whole genome and every chromosome, percentage of cytosine methylation in CG, CHG and CHH contextes, number of selected DM elements (promoters, exons, introns, 3'UTR, 5'UTR, and intergenic regions), were analyzed at differential methylation ratio between control and treated samples. Functional annotation of DM genes was done with KEGG pathway maps and Gene Ontology categories, and associated DM genes were identified for upcoming analyses of gene transcription in potato plants challenged with different abiotic and biotic stress conditions.

Keywords: DNA methylation, whole-genome bisulfite sequencing, essential oil, defense priming

Integrated multiomics approach for revealing epigenetic regulations of combined heat and salinity stress in rice cultivars

Kumar Vinay[1], Suraj Patil[1]

[1]Department of Biotechnology, Modern College of Arts, Science and Commerce (Savitribai Phule Pune University), Ganeshkhind, Pune, India

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population. However, the production potential of modern cultivars has remained stagnant. Increasing rice production in different rice-growing ecosystems is imperative to feed the increasing world population. The climatic changes and challenging environmental dynamics have added new challenges to crop sustainability and food security for the current and upcoming generations. Due to global warming and increasing sea levels, there is a gradual increase in soil salinity and atmospheric temperature, especially around coastal regions, the home to the well-explored coastal rice fields. Combined heat and salinity stresses imply additive stress impacts and are projected to drastically reduce the yield and quality of rice harvests. Therefore, understanding and exploring the in-depth mechanistic insights (especially epigenetic plasticity) of rice responses and adaptations to these combined stressors is the need of the hour. However, historically, studies are primarily focused on either of these single abiotic stresses though in nature plants rarely face single stress.

In this study, most responsive Indica rice to heat+salinity stress was selected based on screening of phenotypic and biochemical responses. To decipher the epigenetic response under single and combined stress, DNA bisulfite sequencing, transcriptomic and small RNA sequencing was performed from root and shoot of rice plants. DNA bisulfite conversion was carried out using NEBNext® Enzymatic Methyl-seq Kit, library preparation was facilitated using NEBNext UltraExpress™ for DNA and RNA respectively while, small RNA libraries were prepared using NEBNext® Small RNA Library Prep Set for Illumina. Sequencing libraries on Illumina NovaSeq 6000 system produced 145.78 million reads in bisulfite sequencing, 20 million reads in transcriptome and 50 million reads in small RNA sequencing. The results showed interesting and important findings and the differential methylation along with differential expression patterns confirm the critical roles in epigenetic regulations of heat+salinity adaptive responses in rice. This study also identified epimarks that have the potential to explored as targets for engineering heat+salinity stress tolerance in rice and other related crops.

Keywords: rice, climate change, epigenetic regulation

Exploration of solanum spp. transcriptomic and epigenetic changes after early-stage broomrape infestation

Maria Gerakari[1], Vasiliki Kotsira[2,3], Aliki Kapazoglou[4], Spyros Tastsoglou[2,3], Anastasios Katsileros[1], Dimosthenis Chachalis[5], Ar-temis G. Hatzigeorgiou[2,3], Sotirios Fragkostefanakis[6], Eleni Tani[1]

[1]Agricultural University of Athens, Laboratory of Plant Breeding and Biometry, Athens, Greece
[2]Hellenic Pasteur Institute, Athens, Greece
[3]University of Thessaly, DIANA-Lab; Department of Computer Science and Biomedical Informatics, Lamia, Greece
[4]Hellenic Agricultural Organization-Dimitra (ELGO-Dimitra), Institute of Olive Tree, Subtropical Crops and Viti-culture (IOSV), Department of Vitis, Athens, Greece
[5]Benaki Phytopathological Institute, Laboratory of Weed Science, Kifisia, Greece
[6]Institute for Molecular Biosciences, Molecular Cell Biology of Plants, Goethe University Frankfurt; Frankfurt; Germany

Tomato (*Solanum lycopersicum*) stands as a pivotal horticultural crop with significant economic value. The presence of parasitic weeds from the *Phelipanche* and *Orobanche* genera, known as broomrapes, poses a considerable challenge to tomato cultivation, imposing biotic stress and reducing yields. Therefore, it is crucial to develop tomato varieties that exhibit tolerance to these parasitic weeds to ensure sustainable agricultural farming systems. *Solanum pennellii*, a wild relative of the cultivated tomato, is considered a valuable genetic resource for breeding efforts aimed at enhancing resistance of *S. lycopersicum* to biotic and abiotic stressors. In this study, a commercial tomato hybrid along with two Introgression Lines (ILs), resulting from crosses between *S. lycopersicum* and *S. pennellii*, were utilized to identify genes and metabolic pathways associated with resistance against broomrapes. Through comparative transcriptomic analysis, a plethora of differentially expressed genes (DEGs) were identified, particularly in the roots, and the resistant genotype IL6-3 exhibited notably different expression patterns. Validation of several DEGs was performed using qPCR. Additionally, DEGs and pathway enrichment analysis revealed a diverse array of molecular mechanisms potentially involved in the host's defense response and the establishment of resistance against broomrapes. These findings hold significance for molecular breeding strategies aimed at developing resistant tomato genotypes, thereby offering alternative approaches to weed management in tomato cultivation and other agriculturally significant crops. Finally, studies of epigenetic mechanisms (ie DNA methylation of specific genes), are underway.

Keywords: -omic technologies, molecular plant breeding, tomato, broomrape, resistance mechanisms

Genetic and epigenetic factors associated with berry development and aromatic potential in grapevine

Aliki Kapazoglou[1], Theodora Pitsoli[1], Eleni Tani[2], Ioannis Lampropoulos[3]

[1]Hellenic Agricultural Organization-Dimitra (ELGO-Dimitra), Institute of Olive Tree, Subtropical Crops and Viticulture (IOSV), Department of Vitis, Athens, Greece
[2]Agricultural University of Athens, Laboratory of Plant Breeding and Biometry, Athens, Greece
[3]"IPER", Laboratory of chemical and microbiological analyses, Ioannina, Greece

The quality characteristics of grapevine berries and the aromatic potential of wine are regulated by genetic and epigenetic mechanisms encompassing an array of genes involved in the biochemical pathways of secondary metabolite synthesis. Climate change has severe consequences on grapevine productivity, yield and fruit quality, thus, studying gene networks and metabolic pathways implicated in proper plant growth, fruit development and quality is important for exploring systems towards adaptation to climatic pressures without compromising berry quality. In the current work comparative analysis of a set of genes involved in the quality characteristics and aromatic profile of grapes was performed in different grapevine varieties from Greece. In particular, the expression of genes encoding proteins such as terpene synthetases, monoterpene glycosyltransferases and transcription factors involved in the biosynthesis of secondary metabolites (like monoterpenes) have been assessed across four berry developmental stages (pre-veraison, veraison, mid-veraison and maturity. Substantial differences in gene expression dynamics were witnessed among different varieties and locations suggesting a genotype- and terroir- specific mode of gene regulation linked to aromatic profile establishment. Furthermore, DNA methylation analysis revealed differences in DNA methylation profiles, associated with variations in gene expression characteristic for each grapevine variety. Findings will be discussed in the context of epigenetic regulatory mechanisms affecting berry secondary metabolite content and ultimately wine quality.

Keywords: DNA methylation, epigenetic regulation, grapevine aromatic potential

Unveiling Genetic Diversity in Montenegrin Durum Wheat Landraces Through SNP Genotyping

Ana Velimirović[1], Zoran Jovović[1], Dragan Perovic[2], Sanja Mikić[3], Novo Przulj[4], Giacomo Mangini[5], Mariella Finetti-Sialer[5]

[1]Biotechnical Faculty Podgorica, University of Montenegro, Podgorica, Montenegro
[2]Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Julius Kuehn-Institute, Quedlinburg, Germany
[3]Institute of Field and Vegetable Crops, Novi Sad, Serbia
[4]Faculty of Agriculture, University of Banjaluka, Banja Luka, Republika Srpska, Bosnia and Herzegovina
[5]Institute of Biosciences and Bioresources, National Research Council (IBBR-CNR), Bari, Italy

The Rogosija durum wheat landraces, historically the primary cereal in Montenegro until the mid-20th century, faced a significant threat with the introduction of high-yielding common wheat cultivars after World War II, leading to their disappearance from agricultural fields. Efforts to conserve these landraces began in 1955 through the sampling of durum wheat accessions across Montenegrin regions, resulting in the establishment of a Rogosija durum collection in the Montenegro Plant Gene Bank. This collection, holding untapped genetic diversity, presents an opportunity for identifying valuable alleles crucial for enhancing wheat crop adaptability to biotic and abiotic treats.
This study aimed to assess the genetic diversity and population structure of the Rogosija collection using SNP markers, as well as to explore correlations between genetic clusters and eco-geographic conditions in Montenegro. Utilizing a high-throughput genotyping system based on the 25K Illumina SNP wheat array, 6,915 high-quality SNPs were identified and mapped on the durum genome. Principal components analysis and phylogenetic analysis delineated two distinct genetic durum clusters. Analysis of molecular variance indicated that 16% of the total variation stemmed from differences among these genetic clusters, with the remaining 84% within clusters.
To assess the relationship between genetic clusters and Montenegrin eco-geographic regions, durum accessions were georeferenced and evaluated based on ecological data from collection sites. Intriguingly, one genetic cluster comprised samples from areas surrounding Lake Skadar, while the second cluster consisted of accessions from the Montenegrin littoral coast. This suggests that the Rogosija durum collection harbored two distinct Rogosija durum populations, adapted to different eco-geographic micro-areas, highlighting the importance of preserving and studying such genetic resources for agricultural resilience and sustainability.

Keywords: durum wheat, genetic diversity, SNP

CROPINNO - Catching Epigenetic Variations in Sunflower Drought Tolerance

Dragana Miladinović[1], Aleksandra Radanović[1], Serena Varotto[2], Irene Luzzi[2], Alessia Ronch[i2], Renate Horn[3], Malin Alf[3], Ankica Kondić-Špika[1], Svetlana Glogova[c1], Dragana Trkulja[1], Marina Ćeran[1], Dragana Rajković[1], Ana Marjanović Jeromela[1], Boško Dedić[1], Sandra Cvejić[1], Siniša Jocić[1]

[1]Institute of Field and Vegetable Crops, National Institute of Republic of Serbia, Novi Sad, Serbia
[2]Department of Agronomy Food Natural Resources, Animals and Environment (DAFNAE) Agripolis, University of Padova, Padova, Italy
[3]Department of Plant Genetics, Institute of Biological Sciences, University of Rostock, Rostock, Germany

In the future, it is expected that integrative approaches that combine -omics technologies by using bioinformatic tools will facilitate the identification of target genes and markers for complex traits and crop adaptation to the changing environment. CROPINNO project aims to implement multi-omics tools, with emphasis on epigenomics, for increased climate resilience of sunflower, chosen model crop for the project. In order to determine epigenetic variations in sunflower drought response, effects of drought and plant recovery are analysed at chromatin and transcriptional level. Preliminary drought stress protocols that mimic field progressive stress condition were developed with the aim to characterize sunflower response to the environmental challenges at molecular level using -omics tools, such as RNA-Seq and ChIP-Seq, as well as histone modifications such as H3K4me H3K27me3. Stress experiments are performed to identify both candidate genes and their chromatin state, associated with a variable molecular response among the characterized lines and the molecular signature, in the form of a list of regulatory pathways affected.

Acknowledgement: This work is supported by European Commission through CROPINNO project , grant number 101059784, by the Ministry of Science, Technological Development and Innovations of Republic of Serbia, grant number 451-03-66/2024-03/200032, by the Science Fund of the Republic of Serbia, through IDEA project SMARTSUN, grant number 7732457, by Centre of Excellence for Innovations in Breeding of Climate-Resilient Crops - Climate Crops, Institute of Field and Vegetable Crops, Novi Sad, Serbia, and based upon activities of COST Action EPI-CATCH (CA19125).

Keywords: sunflower, stress tolerance, climate change, epigenetics

Epigenetic inheritance of stress impacts seedling performance of forest trees

Rosa Sanchez-Lucas[1], Joe He[1], Mark Raw[1], Marcella Chirico[1], Marco Catoni[1], Estrella Luna[1]

[1]University of Birmingham, United Kingdom

Plants reproduction by seeds is one of the most used propagation methods. However, climate change effects on phenology and plant responses can reduce the morpho-physiological, and biochemical quality of seeds, ultimately reducing field performance and planting value. This fact increases its relevance when we look at maternal inheritance and its effects on future progeny performance. Considering the importance of genetic-based breeding and the impact of epigenetics in plant stress adaptation, it is important to study how epigenetics affects transgenerational inheritance. Our research goal is to study how stimuli on the parental trees alter the epigenetics of parental trees and their progenies. For this purpose, we performed WGBS on leaves from oak and hazel trees and their progenies. Using these two tree species allows us to evaluate and compare the response of recalcitrant seeds (acorns) and dormant-intermediate seeds (hazelnuts). Parental trees were exposed to ambient CO2 atmospheric concentration (aCO2) and to elevated CO2 atmospheric concentration (eCO2) at Birmingham Institute of Forest Research (BIFoR) Free Air CO2 Enrichment (FACE) facilities. A total of 12 oaks and 16 hazel trees were analysed. Their seeds were collected and evaluated for size and their growth was monitored under controlled conditions. Acorns from eCO2 trees were bigger and grew faster than the acorns produced under aCO2. In contrast, hazelnuts from eCO2 trees were generally smaller and failed to germinate, in comparison to the produced under aCO2. Strikingly, low percentages of global methylation at the CHH context were found in both species (1-2%) for adults and seedlings. Interestingly, the analysis of DMR's revealed some clear patterns among parents and progenies depending on the CO2 conditions. Current analysis focusses on the identification of genomic regions that change upon parental exposure and that are transmitted to the progenies. This information, combined with the phenotypic data on seed size and seedling growth will disentangle epigenetic and maternal inheritance in forest trees in the context of climate change.

Keywords: maternal inheritance, seedling performance, stress resilience

Is transcriptional reprogramming following wheat polyploidization driven by DNA methylation changes?

Peter Civan[1], Meriem Banouh[2]

[1]INRAE/UCA UMR 1095, Clermont-Ferrand, France
[2]INRAE/UCA UMR 1095, Clermont-Ferrand, France

Bread wheat is a natural allohexaploid that can be recreated artificially, producing 'synthetic' wheat with significant use in breeding programs. The hybridization between diploid Aegilops tauschii and tetraploid durum wheat (the first step in wheat allohexaploidization) can cause transcriptional activation of some transposable element (TE) families and heritable transcriptional reprogramming of a subset of genes. Although we have recently demonstrated that such changes are much less frequent than previously thought, questions remain about the mechanism of their establishment. It has been hypothesized that DNA methylation changes could be responsible for the remodelling of the transcriptome. The changes could arise during the development of hybrid embryos, as a result of imbalanced parental genome doses. It has been proposed that endosperm-derived siRNAs could migrate to the embryo and guide de novo methylation of previously unmethylated targets, analogically to the reinforcement of TE methylation observed during gametogenesis. Here we examine whether genes differentially expressed (DEGs) between a synthetic wheat allohexaploid and its parents also differ in DNA methylation levels around their transcription start sites, using capture-bisulfate sequencing datasets. We also search for a connection between siRNAs and DEGs, and more generally, investigate the temporal dynamics of TEs and siRNAs during grain development in wheat. We found little evidence in support of the hypothesized involvement of DNA methylation in the transcriptional reprogramming of nascent wheat polyploids.

Keywords: wheat, polyploidization, transcriptome, DNA methylation, siRNAs

The Heredity of Drought: Probing Physiological and Molecular Markers of Stress Memory in Myles Chickpea Cultivar

Miriam Negussu[1], Maria Ventimiglia[1], Cristiano Soares[2], Eirini Kaiserli[3], Federico Martinelli[1]

[1] University of Florence, Florence, italy
[2]University of Porto, Porto, Portugal
[3]University of Glasgow Glasgow, United Kindom;

Drought stress poses a significant challenge to crop productivity, with chickpea (Cicer arietinum L.) being particularly susceptible to water scarcity. Understanding the long-term effects of drought stress on plant morphology across generations is crucial for devising resilient agricultural strategies. This study investigates the response of chickpea plants to drought stress and explores stress memory effects on morphology in the subsequent generation, comparing offspring from stressed plants with those from control conditions.

Chickpea plants subjected to drought stress exhibited characteristic adaptive responses, including reduced plant height, decreased leaf area, and altered root architecture, indicative of stress avoidance strategies. Furthermore, analysis of the subsequent generation revealed intriguing stress memory effects on plant morphology. Offspring derived from stressed plants displayed phenotypic plasticity, with significant alterations in morphological traits compared to progeny from control conditions. Notably, these changes included enhanced root system development, increased leaf thickness, and altered shoot architecture, suggesting transgenerational inheritance of stress memory.

In this study, we will complement these findings by employing a combination of molecular transcriptomic and methylome analyses. By integrating molecular approaches with morphological observations, we aim to unravel the underlying mechanisms driving the observed phenotypic changes. This comprehensive analysis will provide valuable insights into the molecular pathways involved in stress response and transgenerational inheritance in chickpea plants under drought conditions.

Keywords: drought stress, chickpea, transgenerational inheritance, morphological traits, stress memory

EPIBREEDING AND EPIGENOME EDITING

KEYNOTES SPEAKERS

Exploiting Genetic and Epigenetic Heterogeneity to Increase Yield

Ueli Grossniklaus[1,2], Hoda Mazaherikalaroud[1,2], Marc W. Schmid[3], Christian Heichinger[1], Deepak Tanwar[1,2], Dusan Denic[1], Alex Plüss[1,2] Bernhard Schmid[3]

[1]Department of Plant and Microbial Biology & Zurich-Basel Plant Science Center, University of Zurich, Zurich, Switzerland
[2]University Research Priority Program "Evolution in Action", University of Zurich, Zurich, Switzerland
[3] MWSchmid GmbH, Switzerland, Glarus, Department of Geography & Zurich-Basel Plant Science Center, University of Zurich, Zurich, Switzerland

Breeding more resilient crops with equal or increased yield under harsh conditions has become a major challenge in light of global climate change. Unfortunately, the genetic basis of many crop plants is very narrow as domestication and the development of modern cultivars imposed severe genetic bottlenecks. However, we could recently show that purely epigenetic variation is subject to selection and can contribute to novel phenotypes if it is sufficiently stable. Furthermore, there is ample evidence showing that diversity, either at the level of species or of genotypes within a species, leads to significant increases in productivity. Using the model plant Arabidopsis thaliana, we could show that epigenetic heterogeneity can also improve performance. Thus, we propose that increasing epigenetic heterogeneity, through mixture and/or within given genotypes, should also be considered in future breeding programs.

ORAL AND POSTER PRESENTATION

Unlocking epigenetic variation to breed sustainable crops in a changing climate

Dušan Denić[1] , Marc Schmid[2] ,Ueli Grossniklaus[3]

[1]University of Zurich, Department of Plant and Microbial Biology, Zurich, Switzerland
[2]MWSchmid GmbH, Glarus, Switzerland
[3]University of Zurich, Department of Plant and Microbial Biology, Zurich, Switzerland

Agricultural productivity must significantly increase by 2050 to meet the rising demands for food, feed, fiber, and fuel production. This necessitates the development of well-adapted cultivars that require fewer input resources and can withstand the extreme effects of climate change. Our project's long-term goal is to improve crop yield by leveraging the potential of epigenetic variation. However, the stability of epigenetic variation, particularly epialleles, their heritability, and the influence of genetic background on them, is not well-understood. In this project, we explore these aspects using the model plant Arabidopsis thaliana, focusing on DNA methylation, a prominent epigenetic mark that is easily assessable. By analyzing public data from 87 accessions of A. thaliana, we identified approximately 100 loci exhibiting a high anticorrelation between gene expression and DNA methylation. These accessions clearly segregated into two groups, some correlating with an associated phenotype. We monitored the DNA methylation status of 10–12 loci in different F1 hybrids and in recombinant inbred lines (RILs) of A. thaliana using the Chop-PCR assay. Contrary to previously published results on trans-chromosomal methylation and demethylation events, our initial findings suggest that differentially methylated alleles are stably inherited in F1 hybrids. These initial results were later substantiated by performing Whole Genome Bisulfite Sequencing (WGBS) in several F1 hybrids of A. thaliana.

Keywords: epibreeding, DNA methylation, inheritance

Modifying DNA methylation at the CNR promoter using a dCas9-M.SssI methyltransferase epigenetic editing tool

Tal Gurevich[1], Golan Miller[1], Adi Faigenboim[1], Michal Lieberman-Lazarovich[1]

[1]Institute of Plant Sciences, Agricultural Research Organization, Volcani Center , Israel

DNA methylation is an epigenetic modification essential for gene regulation and genome stability. Changes in DNA methylation were shown to be linked with various developmental and physiological processes. Loss of DNA methylation at gene promoter regions was demonstrated to induce transcription, possibly due to its effect on chromatin accessibility. Thus, DNA methylation is a target for gene expression manipulations. The COLORLESS-NON-RIPENING (CNR) gene expression is regulated by DNA methylation, as spontaneous DNA methylation within the CNR promoter inhibits fruit ripening and cause the colorless fruits phenotype of the cnr epimutant of tomato (Solanum lycopersicum). A valuable tool for DNA methylation editing is the CRISPR deactivated Cas9 (dCas9) nuclease fused to a methyltransferase that allows targeting a specific DNA sequence directed by guide RNAs (gRNAs). We characterized the cnr epimutant compared with its isogenic line Ailsa Craig (AC) on the phenotypic, gene expression, and DNA methylation levels, and detected the differentially methylated region (DMR) at the CNR promoter. We designed dCas9-M.SssI methyltransferase-gRNA systems to target this DMR and successfully hyper-methylated it in AC and MP1 tomato genotypes, which showed a DNA methylation pattern that resembles the cnr epimutant. We aim to use this approach to target other target genes known to be regulated by DNA methylation in order to study their mechanism of regulation. Furthermore, utilizing this epigenetic modification tool in tomato, a major vegetable crop, can pave the way for targeting desired genes for epigenetic editing and manipulate gene expression, in a way that is not considered a genetic modification.

Keywords: DNA methylation, editing, tomato

Deciphering the biological significance of genomic imprinting

Trezalka Budinsky[1], Martin Kovačik[1,3], Vojtěch Čermák[1,2], Adéla Přibylová[1,2], Ömer Iltas[1], Aleš Pečinka[3], Clément Lafon Placette[1]

[1] Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic
[2]Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic;
[3] The Czech Academy of Sciences, Institute of Experimental Botany (IEB), Centre of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic

Genomic imprinting, an epigenetic phenomenon where an allele is expressed based on its maternal or paternal origin, has been recognized to have an important role in mammalian development. However, its function in plants has been much more elusive. Indeed, its biological significance in plants has been a long-standing controversy, since there are few obvious phenotypic manifestations of genomic imprinting in plants and a high variability in imprinted gene identity between and within plant species. Here we investigate the effects of genomic imprinting on the seed development of a well characterized outcrosser, Arabidopsis arenosa. We consider imprinted genes in the context of gene regulatory networks, which allows us to study their functions and interactions with other genes on a genome-wide scale. We performed RNA sequencing on 20 whole seed and 18 isolated endosperm samples. Imprinted genes were identified using known parent-specific SNPs and came up to 33 maternally expressed genes (MEGs) and 38 paternally expressed genes (PEGs). We constructed coexpression networks to measure the connectivity of MEGs and PEGs with other genes and functionally characterized networks with the involvement of imprinted genes. Both MEGs and PEGs displayed significantly higher network connectivity than non-imprinted genes. Networks with high MEG and PEG involvement were functionally implicated in cell division, a process essential to seed growth. Our results suggest that imprinted genes play an important role in normal seed development. We set the stage for delving deeper into the functional consequences of imprinted genes.

Keywords: genomic imprinting, epigenetics, arabidopsis, endosperm

Correlation Analysis of Meteorological Parameters and Cherry Cross-sectional Anatomical and Histological Characteristics

Tijana Narandžić[1], Mirjana Ljubojević[1]

[1]University of Novi Sad, Faculty of Agriculture, Serbia

Plants have the ability to adapt to various stressful environmental conditions, including abiotic and biotic factors. While a portion of their plasticity is genetically controlled, recent studies highlight the importance of epigenetics in plant resilience to adverse conditions caused by climate change. This research aimed to investigate the extent to which modifications in stem anatomical and histological characteristics in cherries are influenced by genotype and environment. The plant material included genotypes from two indigenous cherry species, Prunus cerasus L. ecovar. 'Oblačinska' and Prunus fruticosa Pall., as well as one interspecific hybrid, 'Gisela 5', serving as a control. Both ungrafted plants and trees grafted with the sweet cherry cultivar 'Summit' were included in the study. The trial took place at the experimental field of the Faculty of Agriculture, University of Novi Sad, in northern Serbia (45° 20' N; 19° 50' E; 80 m a.s.l.). Stem sampling was conducted during winter dormancy for two consecutive years, with ten replications per scion-rootstock combination. Cross-sectional anatomical and secondary wood features were analyzed using standard microscopy techniques. Meteorological data was collected using the on-field installed weather station. Different degrees of correlation were observed between the cambial activity of stems among different genotypes, as well as the stems of the variety grafted onto those genotypes, concerning meteorological conditions throughout the study years. Cambial plasticity within the 'Oblačinska' genotypes was higher compared to stems of P. fruticosa species and was mostly influenced by precipitation amounts, the number of dry and tropical days, drought periods, and cumulative daily temperatures. The different responses of the investigated species to environmental stimuli were genetically controlled, as expected. The modification of cambial activity in scions grafted onto 'Oblačinska' genotypes followed the pattern observed in ungrafted plants. Scions grafted onto P. fruticosa genotypes exhibited plasticity in anatomical traits, while histological characteristics were correlated with meteorological parameters to a lesser extent.

Keywords: adaptability, autochthonous germplasm, climate chang, European ground cherry, 'Oblačinska' sour cherry

THURSDAY, JUNE 6TH

DYNAMICS OF TRANSPOSABLE ELEMENTS (TEs) IN PLANTS AND EPIGENETIC PATHWAYS IN EVOLUTION

KEYNOTE SPEAKERS

Evolving by jumping: The role of mobile DNA in the generation of adaptive variation in response to environmental challenges

Leanrdo Quadrana[1]

[1]Institute of Plant Science Paris-Saclay (IPS2), Gif-sur-Yvette, France

Transposable elements (TEs) are powerful engines of genome evolution, as illustrated by their implication in the rewiring of gene regulatory networks and the creation of new cellular functions. Short-term consequences of TE mobilization can also be particularly dramatic given that TE insertions are a unique source of large effect mutations and that transposition can be exquisitely sensitive to the environment. Despite these considerations, there is a lack of knowledge about the role of ongoing transposition to within-species variation. In this talk I will present our efforts to characterize the rate and landscape of natural TE mobilization in Arabidopsis thaliana and to assess the role of the environment both as a selective force and a trigger of TE-driven diversity.

Transposable elements in Arabidopsis natural populations: deleterious? if, when, how

Nélida Padilla-García, Audrey Le Veve, Vojtěch Čermák, Ömer Iltas, Robert Horvath, Clément Lafon Placette

[1] Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic;
[2] Albert-Ludwigs-Universität Freiburg, Freiburg, Germany

A common assumption is that most transposable element (TE) insertions are deleterious. Hence, purifying selection is expected to prevent the accumulation of TEs within their host, especially when located in and around genes and if affected by epigenetic silencing. Is it always the case? What factors can support TEs to spread and be maintained in a population? In this talk, I will show our recent efforts to assess the role of these factors, with a particular focus on demography and selective forces. To this aim, we compared the frequency and distribution of TEs in A. lyrata from Europe and North America showing different degrees of mutation load. Generally, we found that TE insertions showed a significantly lower frequency when they were inserted in or near genes, especially TEs targeted by epigenetic silencing. More surprisingly, we found that many gene-neighbouring TEs got fixed in North American populations as a result of successive colonization events and transition to selfing. Another factor was genomic imprinting: the TEs establishing genomic imprinting seemed to spread and get fixed in natural populations more than other TEs. This suggests that in A. lyrata, genomic imprinting could have been established through positive selection acting on TEs, potentially under a parental conflict scenario.

Keywords: transposable elements, genomic imprinting, natural selection, population history, Arabidopsis

ORAL AND POSTER PRESENTATION

A Strategy for Studying Epigenetic Diversity in Natural Populations

Isabelle Lesur[1], Odile Rogier[2], Mamadou Dia Sow[3], EPITREE consortium[4], Stéphane Maury[5]

[1]INRAE, Univ. Bordeaux, BIOGECO, and HelixVenture, Cestas, France
[2]INRAE, ONF, BioForA, Orléans, France
[3]INRAE/UCA UMR GDEC 1095. 5 Chemin de Beaulieu, Clermont Ferrand, France
[4]EPITREE consortium ANR France
[5]LBLGC, INRAE, Université d'Orleans, EA 1207 USC 1328, Orleans, France

These last 20 years, several techniques have been developed for quantifying DNA methylation, the most studied epigenetic marks in eukaryotes, including the gold standard method, whole-genome bisulphite sequencing (WGBS). WGBS quantifies genome-wide DNA methylation but has several inconveniences rendering it less suitable for population-scale epigenetic studies. The high cost of deep sequencing and the large amounts of data generated prompted us to seek an alternative approach. Restricting studies to parts of the genome would be a satisfactory alternative had there not been a major limitation: the need to select upstream targets corresponding to differentially methylated regions (DMRs) as targets. Given the need to study large numbers of samples, we propose a strategy for investigating DNA methylation variation in natural populations, considering the structural complexity of the genomes with their size and their content in unique as coding regions versus repeated regions as transposable elements. We first identified regions of highly variable DNA methylation in a representative subset of genotypes representative of the biological diversity in the population by WGBS. We then analysed the variations of DNA methylation in these targeted regions at the population level by Sequencing Capture Bisulphite (SeqCapBis). The entire strategy was then validated by applying it to another species. Our strategy was developed as a proof of concept on natural populations of two forest species: Populus nigra and Quercus petraea.

Keywords: DNA methylation, epigenetics, population

Transposon-mediated somatic mosaicism in Arabidopsis thaliana

Heena Ambreen[1], Hans-Wilhelm Nützmann[1], Alexandros Bousios[2]

[1]Department of Biosciences, University of Exeter, Exeter, United Kingdom,
[2]School of Life Sciences, University of Sussex, Falmer, Brighton, United Kingdom

Transposable elements (TEs) are potent endogenous mutagens empowered to alter the genomic landscape within an organism. Their dynamic movement facilitates heritable structural variation, rewiring of regulatory pathways, and generation of novel phenotypic diversity. Tracing TE activity in germline cells has been the focus of most studies due to their role in transmitting changes to future generations. In contrast, investigation of TE activity in somatic cells remains limited. While somatic cells may have little evolutionary importance, in plants, establishment of germline in late adulthood increases the likelihood of somatic changes being passed to progeny. Thus, a systematic characterization of somatic TE activity is warranted. Here, we explore TE dynamics in somatic cells of Arabidopsis thaliana leaves. We employ a TE-enrichment sequencing approach (Transposon Display Sequencing) to assess de-novo integrations under destabilized epigenomic conditions triggered by heat-stress and various methylation and RdDM mutants. We demonstrate a profoundly active and heterogenous mobilome, including ONSEN, EVADE, and ATCOPIA21. Somatic transposition rates are TE-specific and vary depending on the genotype background. Interestingly, abolishment of DNA methylation in a methylation maintenance mutant generates higher TE loads than RdDM mutants in somatic cells. We discuss TE insertion profiles in relation to integration preferences and distribution across genome. Mobilization of heat-responsive ONSEN in wildtype soma offers a case study for prioritizing understanding of environmentally-induced TEs in agriculture setups. Overall, our findings are among the first to report extensive somatic TE mobilization in plants, opening new avenues for studying impact of somatic TE activity in genome function and evolution.

Keywords: transposon, somatic transposition, Arabidopsis, epigenetics

Patterns of Transposable Elements in Centromeres across Brassicaceae Species

Estela Perez-Roman[1], Alexandros Bousios[1]

[1]School of Life Sciences, University of Sussex, Brighton, United Kingdom

Transposable elements (TEs) represent the most abundant component of eucaryotic genomes and are key drivers of genomic and phenotypic evolution. Despite their importance, several aspects of their interactions with the host genomes are still not well understood. One of them revolves around their role in the function and evolution of centromeres, regions of ultra conserved function that can, however, rapidly evolve between species. Aided by recent advances in sequencing technologies, it was recently shown that a specific lineage of LTR retrotransposons, termed ATHILA, are engaged in cycles of invasion and purging in the Arabidopsis thaliana centromeres - both, processes that drive centromere and genome evolution, and, ultimately, speciation. We now take a broader view and explore the patterns of TEs in centromeres across additional Brassicaceae species that are overall separated by ~30 million years of evolution. We show that the centrophilic behaviour of TEs is dynamic and quick to evolve, suggesting repeated convergent adaptation. The LTR retrotransposon families that have invaded the Brassicaceae centromeres are diverse but share common characteristics, such as their younger insertion age compared to their centrophobic relatives, consistent with ongoing invasion. Overall, our ongoing study offers new insights into the ways that TEs interact with and affect this distinct genomic niche.

Keywords: transposable elements, centromeres, plants

Investigating the distribution and stability of epigenetic variation in natural populations of Arabidopsis thaliana

Alex Pluess[1], Dusan Denic[1], Marc Schmid[2], Simon Aeschbacher[1], Ueli Grossniklaus[1]

[1]University of Zurich, Zurich, Switzerland
[2]MWSchmid GmbH, Glarus, Switzerland

Epigenetic marks, such as DNA methylation, are important regulators of gene expression. In plants, epigenetic variation (EV) can be meiotically heritable and, therefore, potentially contribute to adaptation. Research in our laboratory aims at understanding the evolutionary significance of EV in plant adaptation and its potential application to plant breeding.

Previous experiments in our lab have shown, that EV responds to artificial selection in a greenhouse environment. However, evidence for its evolutionary relevance in natural populations remains scarce. With this project, we want to bridge the gap between the greenhouse and outdoors to gain a better understanding of the epigenetic basis for plant adaptation.

To test whether epialleles are subject to selection in a natural environment, we have repeatedly sampled wild populations of Arabidopsis thaliana over the course of four years. We collected up to 75 plants per population per year for five populations in total. We use Chop-PCR, a combination of qPCR and methylation-sensitive restriction enzymes, to asses methylation states of a set of select epialleles. The initial monitoring of a large number of individuals showed that many of the epialleles segregate in these populations, providing an opportunity for selection. Now we are looking into how epiallele frequencies in a population change over the years. We hypothesize that a change in epiallele frequency over time, that is larger than what is expected under genetic drift, could reflect a selective pressure on observed methylation states.

Keywords: epialleles, DNA methylation, evolution, Arabidopsis thaliana, natural populations

The role of epigenetics in local adaptation in oak trees: a population epigenetic analysis

Shannon Brandt[1], Ludovic Duvaux[1], Isabelle Lesur[1,2], Odile Rogier[3], Mamadou Sow[4,5], Stéphane Maury[5], Alexandre Duplan[3,5], Harold Duruflé[5], Christophe Plomion[1], Jerome Salse[4], Jorg Tost[6], Leopoldo Sanchez[7], EPITREE CONSORTIUM

[1]INRAE, Univ. Bordeaux, BIOGECUnO,Cestas, France
[2]HelixVenture, Mérignac, France.
[3]INRAE, ONF, BioForA, Orléans, France
[4]INRAE/UCA UMR GDEC 1095. 5 Chemin de Beaulieu, Clermont Ferrand, France.
[5]LBLGC, INRAE, Université d'Orleans, EA 1207 USC 1328, Orleans, France
[6] Centre National de Recherche en Génomique Humaine, CEA-Institut de Biologie François Jacob, Université Paris-Saclay, Evry, France.
[7]AGPF - Unité de recherche Amélioration, Génétique et Physiologie Forestières, France

The current speed of global change forces species to adapt to an unprecedented pace or to die off from their current distribution areas. This change is particularly pressing for long-lived sessile organisms such as trees, as they cannot migrate and their long generation times make them sensitive to very quick environmental changes. One possible avenue to speed up adaptation is the modification of epigenetic marks, such as DNA methylation. Here, we explore the role of DNA methylation in local adaptation between populations of sessile oaks (Quercus petraea). It is expected that the epigenetic component of variation will have a stronger effect than the genetic component in the early stages of local adaptation. We studied methylation patterns for approximately 200 individuals from 23 different populations grown together in a common garden experiment. This allowed us to investigate the epigenetic differences between the various populations to gain an understanding of the possible impacts of the original population environment. Preliminary results seem to indicate that some populations have distinct methylation patterns in biosynthesis process genes that might be linked to environmental variables like summer humidity and annual temperature. This study will enable us to compare the epigenetic and genetic components of variation through the use of SNPs in the future. Which will provide insight into the differences between epigenetic and genetic adaptation in the various populations. Understanding the ways in which trees can exploit epigenetic changes for rapid local adaptation is vital to allowing us to safeguard our precious forest resources.

Keywords: epigenetics, local adaptation, sessile oak, DNA methylation

Identification of retrotransposons and horizontal gene transfer in callus and regenerants of wheat plant (Triticum aestivum L.) in vitro

Gunay Ismayilova[1], Mahira Mammadova[2], Sevgi Marakli[3]

[1]Institute of Molecular Biology and Biotechnology, Baku, Azerbaijan
[2]Institute of Molecular Biology and Biotechnology, Baku, Azerbaijan
[3]Yildiz Technical Universitety, Istanbul, Turkiye

Epigenetics is a field of science that studies the dynamism and plasticity of the epigenome of living organisms in response to the environment and its development and evolution. Epigenetic modifications are heritable changes that do not change the nucleotide sequence of DNA but can affect the phenotype. In particular, the study of transposon elements (TEs) in the genome of plant organisms helps to understand epigenetic mechanisms. Transposable elements are found in almost all plant genomes and play a major role in the evolution of plant genes and genomes.Plant retrotransposons are structurally and functionally similar to retrotransposons and retroviruses found in other eukaryotic organisms. Transposons can change the expression of genes at their new locations on chromosomes. As a result, they affect the existing genetic information and cause significant changes in gene expression. In this context, the determination of retrotransposons in different wheat genotypes with the IRAP ("Inter-Retrotransposon Amplification Polymorphism") marker method was considered. During the study, 11 primers were tested in wheat callus culture and regenerating plants, retrotransposons were detected in 7 of them. So, using the IRAP marker method, Hopi, Houba, Osr 30, Rire retrotransposon specific to rice plant, Nikita1 and Sukkula specific to barley plant, Pst1 specific to potato plant, and Sire1 retrotransposon specific to soybean plant were determined in wheat plant. These results suggest that genes are transported from one place to another through horizontal transfer due to the survival of TEs and the frequent occurrence of interspecific gene flow in ecosystems.

Keywords: epigenetics, transposon elements, in vitro callus, wheat, philogenetics