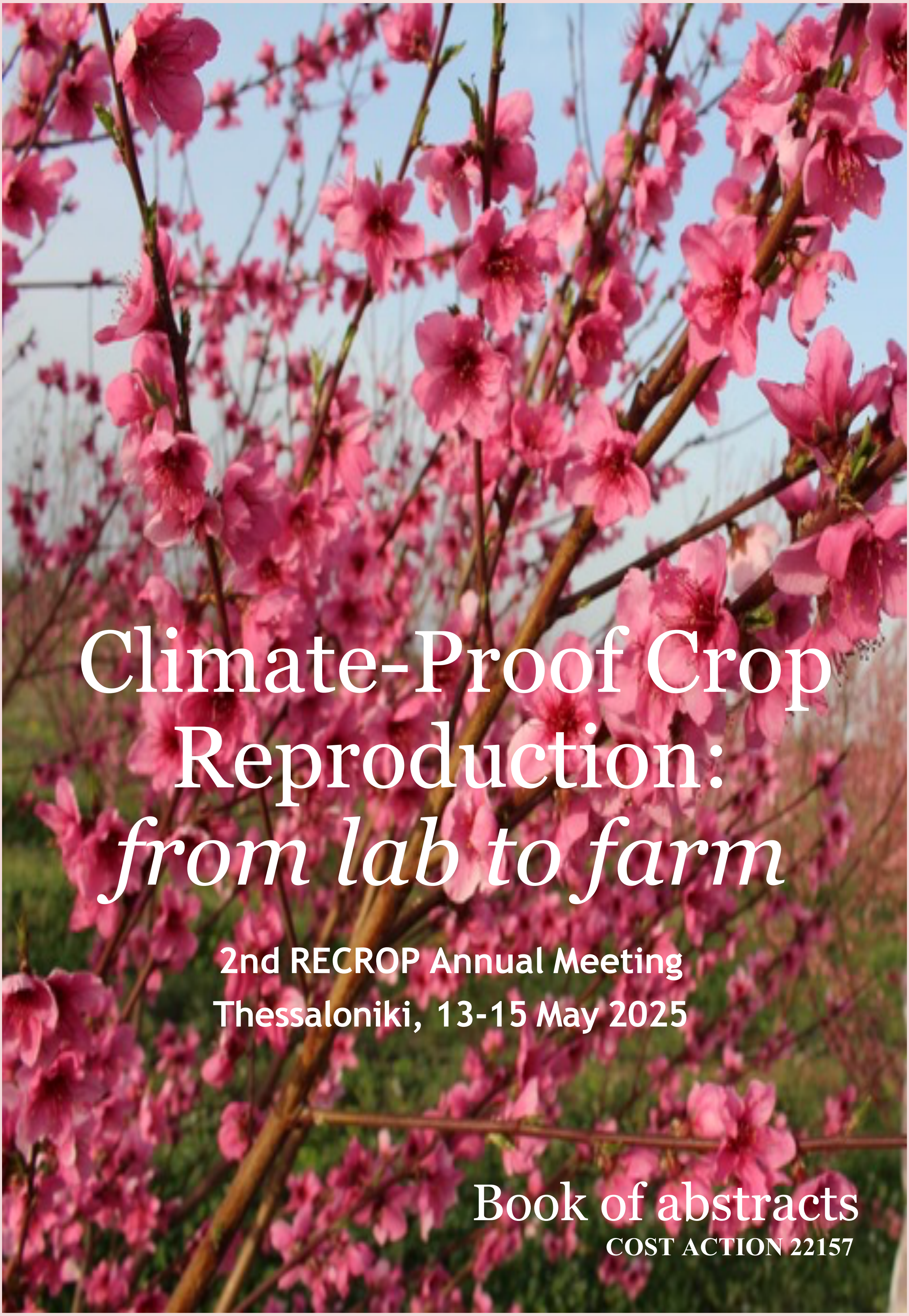

Climate-Proof Crop Reproduction: *from lab to farm*

2nd RECROP Annual Meeting

Thessaloniki, 13-15 May 2025

Book of abstracts

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ABOUT RECROP

Our Mission

RECROP, an initiative supported by the European Cooperation in Science and Technology (www.COST.eu), stands at the forefront of combatting the increasing threat of climate change on global food security. Our diverse consortium of experts collaborates to confront the challenges posed by climate change on crop resilience. We are committed to deciphering the basis of crop sensitivity during reproduction and developing sustainable strategies to boost yields in the face of a changing climate.

Our Approach

By integrating expertise in genetics, molecular science, plant physiology, bioinformatics, agronomy, and engineering, RECROP aims to uncover the genetic and physiological factors influencing crop sensitivity to extreme environmental stresses. Through sharing experience and information from state-of-the-art methodologies, we seek to identify key genetic markers and pathways, laying the groundwork for the development of robust crop varieties.

Our Commitment

RECROP fosters a culture of collaboration among researchers in Europe and beyond. We provide a platform for the exchange of knowledge and actively support the next generation of scientists, fostering innovation in the field of crop resilience.

Our Working Groups

- **WG1: Tools to decode stress response and tolerance of crop reproduction**
- **WG2: Effects of abiotic stresses on reproductive tissues and their relevance for yield**
- **WG3: Improvement of crop yield under suboptimal environmental conditions using genetic approaches**
- **WG4: Dissemination, Training and Stakeholder Engagement**

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SESSION I
Plant Reproduction Under Abiotic Stresses

Chairs: ***Michal Lieberman-Lazarovich & Ariola Bacu***

KEYNOTE SPEAKER 1

Anja Geitmann

Thermotolerance and invasive growth in the male gametophyte

Madeleine Stokes[1], Anja Geitmann[1]

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The characterization of pollen performance typically employs the terms pollen viability and pollen vigor, which, however, are not necessarily used consistently across studies. To help establish consistent and relevant terminology in the context of heat stress, we defined these terms and reviewed the methodologies used to assess them. By way of example, we assayed the temperature sensitivity of pollen from early maturing varieties of soybean (*Glycine max*), distinguishing between the effects of elevated temperature on pollen germination and on pollen tube elongation. In a complementary approach we assessed the pollen tube's capacity to elongate against mechanical obstacles typically encountered in the sporophytic flower tissues.

O1. Abiotic stresses affect vegetative and reproductive development in maize differently

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Global warming strongly affects crop production and yield of grain crops like maize. It was estimated that abiotic stresses will decrease global maize production by 45% until 2080. To push the generation of stress resistant/tolerant crops, we generated a highly reproducible atlas of maize plants at various abiotic stress conditions (cold, heat and drought) and over time. RNA-seq, proteomic and phosphoproteomic analyses will be presented to show molecular responses towards the different stresses and the usage of the generated database and web interface named MaizeStressDB. The database can be used to identify, for example, hub-genes with broad overlapping functions. While seedling development is especially impacted by cold and drought stress, the reproductive phase of maize is highly sensitive to ambient temperature stresses, with even a single hot day sometimes being fatal to reproductive success. During the annual RECROP meeting 2024 we reported among others that transient heat stress for only two days during pollen development leads to pollen germination and sperm cell transport defects (Li et al. 2024), while heat stress during the pollination phase causes elevated levels of reactive oxygen species (ROS) in silks, which can be reduced by ROS scavengers partly restoring fertility (Gong et al., 2024). This year, we will report on the role of small non-coding RNAs (sncRNAs) during maize reproduction. We generated RNA-seq data from isolated meiocytes at three stages (meiosis, tetrads, microspores) of both temperate (A188) and heat-tolerant tropical (CML228) maize lines. A comprehensive analysis with a focus on phasiRNAs and miRNAs will be presented.

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Li X, Bruckmann A, Dresselhaus T, Begcy K. 2024. *Plant Physiology* 95, 2111-2128.
Gong W, Oubounyt M, Baumbach J, Dresselhaus T. 2024. *iScience* 27, 110081.

O2. Unraveling the process of thermoregulation during the seed development in *Brassica napus*

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Our research focuses on understanding how plants respond to their environment and how their growth processes are regulated at the tissular, cellular and molecular levels. We are particularly interested in early seed development in the model plant *Arabidopsis thaliana* and its relative *Brassica napus* (oilseed rape) and how high temperature affects seed production and quality. We observed that high temperatures above the optimum growth temperature resulted in the production of seeds with ruptured seed coats in *Brassica napus* cv Topas. This phenotype is associated with accelerated embryo development. However, the relationships between the three events – high temperature, embryo growth pace, and seed coat rupture – remain unclear. To investigate the occurrence of temperature-induced seed coat rupture, we combined detailed phenotyping approaches of oilseed rape seeds with transcriptomics, histology, immunolabelling, hormone and cell wall profiling. Our data suggest that high temperatures accelerate embryo growth, resulting in larger embryos but not larger seeds. Such large embryos exert a putative mechanical pressure on the seed coat cells, for which we observed a reduced cell layer thickness. The seed coat began to mature prematurely with the accumulation of demethylesterified pectin, possibly making the cell wall stiffer, which eventually ruptured. Our data present novel observations on the impact of high temperatures on seed development, tackling issues linked to seed biomechanic features.

O3. Reproductive stage sensitivity of maize to water and temperature stress across two distinct pedoclimatic regions in Bosnia and Herzegovina

Nataša Čereković[1]*, Mihajlo Marković[2], Vojo Radić[2], Sabrija Čadro[3], Benjamin Crljenković[3], Nery Zapata[4], Teresa A. Paço[5], Wilk Almeida[6], Ružica Stričević[7], Mladen Todorović[8]

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Climate change has been significantly altering maize growth patterns and reducing production in Bosnia and Herzegovina (BiH). Prolonged drought and elevated temperatures have increasingly limited the resilience of non-irrigated maize, particularly during critical growth phases. A two-year field experiment (2021-2022) was conducted to evaluate the response of the local maize hybrid BL-43 to different water regimes (full irrigation, deficit irrigation, and rainfed conditions) across two distinct pedo-climatic sites (Aleksandrovac and Butmir) in BiH using a randomized block design with three replicates. An Excel-based irrigation tool was used to manage crop water requirements and irrigation scheduling effectively. The crop's response to water availability was influenced by site-specific agronomic practices, the timing and duration of phenological stages, and their synchronization with precipitation patterns. Abiotic stress, particularly water deficit and temperature extremes during the reproductive stage, had a pronounced impact on yield performance. The effect was notably severe at Aleksandrovac, where combined water and heat stress during flowering led to substantial yield reductions. In contrast, Butmir exhibited higher total average grain yields of 38% and 27% greater than Aleksandrovac across both seasons. The effect of both irrigation regimes was more pronounced at Butmir, due to the temperature stress in the reproduction phase at Aleksandrovac. This study provides critical insights how irrigation practices influence maize grain production under diverse pedo-climatic conditions of BiH. The findings highlight the urgent need to enhance knowledge of climate change impacts on productivity, particularly the role of reproductive-stage abiotic stress in yield variability.

Keywords: *Abiotic stress, reproduction; Zea mays L.; irrigation; maize yield; water use efficiency; climate change.*

O4. Regulation of Ovule Number and Fertility by DRM Methyltransferases During Drought Stress

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As global temperatures rise, agricultural productivity is threatened, which poses a serious risk to food security. Since seeds are a major food source, understanding how plants adapt to drought stress, particularly in the reproductive stages, is essential. Seeds are produced from ovules after fertilization, and the number of ovules in the pistil determines the potential seed yield, a critical agronomic trait. We have recently discover that DRM methyltransferases (*DRM1* and *DRM2*) could play a key role in managing the plant's response to environmental stress, including drought. In this study, we focused on how these methyltransferases influence ovule number and fertility under drought conditions. Our findings demonstrate that moderate drought stress significantly reduces ovule number and pistil length in wild-type plants, whereas *drm1drm2* double mutants showed increased drought tolerance. Further investigation through methylome and transcriptomic analysis, including small RNA sequencing, revealed an elevated expression of miRNAs in the *drm1drm2* mutants. These miRNAs are involved in copper homeostasis, ABA signaling and reactive oxygen species (ROS) signaling, which may contribute to enhanced drought resistance during critical reproductive phases. These results suggest that DRM methyltransferases play a pivotal role in regulating ovule number and seed production under drought stress, potentially offering a new avenue for improving drought tolerance in crops.

O5. From Gametophytes to Sporophytes: Investigating Plant Motif Distribution with GOLEM

Lukáš Nevosád[1], Božena Klodová[2], Jiří Rudolf[1,3], Tomáš Raček[1,3], Tereza Přerovská[1,3], Alžbeta Kusová[1,3], Radka Svobodová[1,3], David Honys[2], and Petra Procházková Schrumpfová[1,3]*

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GOLEM (Gene regulatOry eLEMents, https://golem.ncbr.muni.cz) is a user-friendly tool for visualizing gene regulatory motifs in plant promoters, specially of the genes showing higher expression in male reproductive tissues or leaves, across the selected plant genomes within the plant evolution (streptophyte algae, mosses, ferns, basal angiosperm, monocots and dicots). We demonstrate GOLEM's utility with motifs associated with male gametophyte development (e.g., LAT52, MEF2, and DOF_core), hormone-responsive elements (e.g., GCC-box, ARR10_core), and conserved motifs (e.g., TATA-box, ABRE, TC-element, I-box, and DRE/CRT element). Promoter analysis using GOLEM revealed that TATA-box-containing promoters are linked to genes expressed during late pollen development but not early pollen development in dicot plants. It was also shown that the LAT52 motif, motif associated with late pollen development, is preferentially located in the 5′ UTR. Moreover, GOLEM demonstrated that the ethylene-responsive element (GCC-box) exhibits a conserved pattern downstream of ATG throughout evolution, even in streptophyte algae. In contrast, the ARR10-binding motif (ARR10_core), associated with cytokinin response, does not show a conserved distribution across evolution, starting from streptophyte algae. This aligns with the fact that components of both signaling cascades are present in land plants, however, the streptophyte algae lacks some components of cytokinin signaling pathway. Additionally, a new Omics Repository focused on plants, which is being developed to support the storage, acquisition, and analysis of omics data also in crop plants, will be discussed.

This work was supported for by the Czech Science Foundation [21-15841S] and by the Ministry of Education, Youth and Sports of the Czech Republic under the project INTER-COST LUC24056.
Nevosad et al., TPJ, 121, 5, 2025 doi.org/10.1111/tpj.70037

O6. The role of eIF3 in the control of pollen tube growth and thermotolerance

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Pollen germination and pollen tube growth are highly sensitive to elevated temperatures, requiring precise translational control to maintain cellular integrity and reproductive success. The eukaryotic translation initiation factor 3 (eIF3) plays a crucial role in orchestrating mRNA translation dynamics, impacting both pollen tube growth under normal conditions and thermotolerance during heat stress (HS). We demonstrate that disruption of specific eIF3 subunits leads to impaired pollen tube growth and structural integrity, affecting fertilization efficiency. However, under heat stress, translational adaptation mechanisms involving eIF3 subunits contribute to enhanced pollen tube thermotolerance by regulating heat shock protein (HSP) expression. We further reveal that eIF3 interacts with specific mRNA elements, modulating their translation through a balance of repression and activation. Structural and functional analyses highlight the importance of conserved eIF3 domains and post-translational modifications in maintaining translational equilibrium essential for pollen tube membrane integrity and sustained growth. Collectively, our findings uncover a pivotal role of eIF3-mediated translational regulation in ensuring pollen fertility under both optimal and stress conditions.

Keywords: *Heat stress, Pollen development, Pollen tube growth, Male gametophyte, Translation initiation, eIF3, Cellular integrity*

Funding

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O7. Histone Acetyltransferase GCN5 and the Associated Coactivators ADA2 - From Evolution of the SAGA Complex to the Biological Roles in Plants

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Histone acetyltransferases (HATs) catalyze the acetylation of lysine residues within the N-terminal tails of core histone proteins, thereby modulating chromatin structure and gene expression. In *Arabidopsis thaliana*, the General Control Non-derepressible 5 (GCN5) protein functions as a HAT, specifically targeting lysine residues in the N-terminal tail of histone H3. GCN5 is implicated in various biological processes, including cell division, differentiation, meristem function, and development of roots, stems, leaves, and flowers. Additionally, it plays a role in orchestrating responses to environmental stimuli. GCN5 operates within two distinct transcription adaptor complexes in yeast: the Alteration/Deficiency in Activation 2 (ADA) complex and the Spt-Ada-Gcn5-Acetyltransferase (SAGA) complex. In plants, ADA2b interacts with GCN5 within these complexes, thereby enhancing the histone acetylation activity of GCN5. Mutational studies have revealed significant phenotypic effects associated with GCN5 and ADA2b function disruption. Notably, *ada2b* mutants exhibit shortened stamens relative to carpels, resulting in reduced fertility. Similarly, *gcn5* mutants display similar defects in floral morphology, indicating the critical role of GCN5 and its interaction with ADA2b in regulating flower development and reproductive success in Arabidopsis. This work monitored the effect of ADA2b and GCN5 proteins on Arabidopsis thaliana inflorescences' gene expression. Several differentially expressed genes (DEGs) were identified. DEGs involved in the stamens developmental process are down-regulated in *ada2b–1*, exhibiting up to 10-fold change of the expression compared to wild-type stamens. Approximately 41.66% of DEGs related to pollen exine formation demonstrate distinct expression alterations only in *ada2b–1* mutant. These findings suggest that ADA2b is a positive regulator in the stamen developmental process. At the same time, the GCN5 protein functions as a gene-specific regulator in this biological process. Furthermore, ADA2b and GCN5 are positive regulators of the pollen exine formation.

O8. Unveiling the molecular function of the nascent polypeptide-associated complex subunit beta in *Arabidopsis thaliana*

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The nascent polypeptide-associated complex (NAC) is represented by a heterodimeric complex composed of an alpha and a beta subunit, that binds the newly synthesized polypeptides emerging from the ribosomal exit tunnel. Its molecular functions were thoroughly described in metazoans and yeast. However, the knowledge about its function in plants remained limited. The alpha subunit of NAC complex is encoded by 5 homologous genes whereas the beta subunit is encoded by 2 homologues in *Arabidopsis thaliana* genome. The knock-out/down mutant in both *A. thaliana* NACβ subunits (*nacβ1nacβ2*) was acquired, which showed notable phenotypic changes compared to the Col-0 wild type (wt) plants, namely delayed development for 14–21 days, aberrant numbers of flower organs, shorter siliques with a significantly lower seed set, and lower chlorophyll amount in mutant leaves. We characterized the transcriptome and proteome of the mutant flower buds that showed significantly different abundances of many genes playing role in stress responses, male gametophyte development, and photosynthesis. However, the molecular function of the NAC complex remained elusive so several subsequent experiments were carried out. First, we tested the photosynthetic activity of the *nacβ1nacβ2* mutant and compared it with the Col-0 wt plants since many genes from the -omics data play their roles in photosynthesis. Then, we hypothesized that knock-out/down of both NACβ paralogues, as is the case of the *nacβ1nacβ2* mutant, will most likely influence protein sorting to chloroplasts. To test this hypothesis, a set of several other experiments was performed.

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O9. Large scale phenotyping looking at temperature stress on reproduction in Brassica diversity set

Alison Tidy, Laura Siles, Catherine Jacott, Rachel Wells, Smita Kurup, Zoe Wilson

Agriculture is facing the crucial challenge of adapting crop production in the changing climate; environmental stress during flowering has a direct and negative impact on yield. Understanding environmental sensitivity within *Brassica* is pivotal for optimising crop production in different climates and under changing environments. 94 accessions in a *Brassica* diversity set were analysed for reproductive sensitivity to short term heat stress (35°C) and cold stress (6°C) after the onset of flowering. Pollen viability/germination analysis, and ovule number/size were used to observe the effect on fertility. Final yield was also analysed to determine the effect that environmental stress had on the plant as a whole and any lasting effects it had on development; this was analysed through pod number, pod filling, pod length, seed set and seed size. Here we have shown that while cold stress had a minimum effect compared to control, heat stress had a significant effect on almost all variables measured. Negatively affecting pollen traits, final yield, pod filling and seed set. We have identified 28 genotypes that appear tolerant to heat stress, with 5 maintaining high yields despite the stress, these therefore would be interesting for breeding heat-tolerant lines. GWAS analysis on this data and QTL mapping, could also allow identification of markers used for breeding suitable genotypes to sustain crop productivity under climate change scenarios.

O10. **Heat stress response in commercial tomato varieties – from lab to farm**

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Elevated ambient temperatures pose significant environmental challenges to crop plants, by causing heat stress conditions. Exposure to temperatures 10-15°C above the optimum of each species, which can occur more frequently with global warming, subsequently limit crop productivity, and present a food security issue. Tomato (*Solanum lycopersicum*), a major vegetable crop, is highly susceptible to elevated temperatures. Under heat stress conditions, tomato plants exhibit various developmental, physiological and molecular defects, such as bud drop, reduced pollen viability, and activation of stress-related transcriptional networks. Importantly, fruit-set is dramatically reduced under heat stress conditions, leading to significant yield losses. Therefore, various approaches were taken in an effort to develop new varieties with enhanced tolerance to heat stress conditions. Despite decades of scientific research dedicated to understanding the physiological and molecular mechanisms involved in heat stress response and resilience, only a few discoveries have successfully transitioned from the lab to the field and been applied in agriculture. One reason for this challenge is that testing commercial tomato varieties in agronomic settings can yield different results compared to laboratory growth conditions. This discussion will present several lessons learned from real-life heat stress experiments, underscoring the importance of such trials in advancing the development of heat stress-resilient tomato cultivars.

O11. The *Arabidopsis thaliana SPATULA*, *CLAVATA1* and *GCN5* genes interact to regulate gene expression and gynoecium development

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The gynoecium is one of the most complex plant organs. The spatiotemporal action of transcription factors, hormone dynamics, and chromatin modifications regulates gynoecium development. GENERAL CONTROL NONDEREPRESSIBLE (GCN5), a histone acetyltransferase, has been previously shown to interact with the CLAVATA (CLV) pathway, regulating gynoecium development potentially through modulating auxin and cytokinin and affecting the expression of the transcription factor *WUSCHEL* (*WUS*). SPATULA (SPT) is a bHLH transcription factor involved in gynoecium development by affecting both auxin and cytokinin responses; *clv1gcn5* double mutants exhibit abnormal gynoecia with reduced or absent valves and enlarged stigma. To identify potential genetic interaction between *GCN5, CLV1* and *SPT,* the *sptclv1gcn5* triple mutant was generated. Mutations in *SPT* partially reversed the gynoecia phenotype of *clv1gcn5*, restoring ovary development. Gene expression analyses in inflorescences and gynoecia showed that in *clv1gcn5*, genes involved in auxin and cytokinin homeostasis, as well as the formation of the apical-basal, mediolateral and abaxial-adaxial axes of the gynoecium are misexpressed and this misexpression is partially alleviated in *sptclv1gcn5*. Tissue-specific analysis of *WUS* showed that *GCN5* and *CLV1* synergistically suppress its expression in immature developing gynoecia at stages 8-9, with *SPT* having a limited effect on that stage, but *SPT* affects *WUS* expression in mature gynoecia at stage 13, especially at the stylar region. This study sheds light on the intricate interplay among *GCN5*, *CLV1*, and *SPT*, highlighting their pivotal roles in orchestrating gene expression, polarity determination and hormone dynamics during *Arabidopsis thaliana* gynoecium development.

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P1. Winter chill relatedness with bloom time and yield in apricot

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To quantify the effects of recent climate change on apricot fruit production in Naoussa northern Greece, this study aimed at determining long-term trends in winter chill and relate with apricot bloom and yield. Winter chill was calculated since 1967 while bloom phenology (beginning and duration) and yield were monitored in 18 apricot cultivars with varying chill requirements (ranging between 43 and 73 Chill Portions (CP), during 2016-2020. Results showed that winter chill reduced by 11%, from a mean of 86 CP during 1969-1989, to a mean of 77 CP in the last five years. Yet, a dramatic increase by 38% in the Growing Degree Hours during the winter months was found, that may also become a challenge for the apricot growers, leading to elevated risk of damages from usually occurring frosts. Chill accumulation was negatively correlated with bloom duration (r= -0.509) and positively with yield efficiency (r= 0.490), whereas less important correlation was found with the beginning of bloom. For a first time, an important historical loss in winter chill has been documented in northern Greece. Moreover, prolonged bloom is a trait related to insufficient chill in apricot.

P2. HSP90-mediated stress resilience in male gametophyte of *Arabidopsis thaliana*

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Although protective heat shock proteins (HSPs) accumulate during male gametophyte development, pollen remains sensitive to high temperatures. In order find the molecular basis of this heat sensitivity, we performed transcriptome analysis during five pollen developmental stages—uni-nuclear (UN), early bi-cellular (EB), bi-cellular (BC), tri-cellular (TC), and mature pollen (MPG) — under normal and heat stress (HS) conditions. Our results reveal that most heat stress-induced gene expression changes are stage-specific, with the BC stage showing the highest number of induced differentially expressed genes (>4000), suggesting a complex, stage-dependent heat stress response potentially influenced by HSP levels. To explore stage-specific influences of HSP90s in pollen development, we characterized a knockdown RNAi line, under normal and stress conditions in early- and late-stage RNAi lines using stage-specific promoters pJASON (*JA90R*) and pLAT52 (*L90R*). The *hsp90* background leads to lower germination rate in both RNAi lines that is more pronounced under heat stress (H), caused by significant alterations in heat stress control via impaired ABA signalling or ER stress response. Heat stress conditions also lead to a high percentage of nuclei shape and orientation defects in the late *L90R* line pollen, pointing to the higher sensitivity of late stage development. We show this defect is linked to the downregulation of DNA metabolism genes. Our complex dataset provides insight into stage-specific stress response on the level of single cell undergoing developmental changes.

Keywords: *Heat stress, Pollen development, Male gametophyte, HSP90 knockdown, Genetic stability, Cellular integrity*

Funding

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P3. Grafting eggplant onto various rootstocks: assessing performance under stress and optimal conditions

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Grafting commercial cultivars onto tolerant rootstocks presents a promising strategy for mitigating issues arising from both abiotic and biotic stress factors in horticulture. In an initial study comparing several rootstocks, including two *Solanum lycopersicum* rootstocks Optifort and Emperador, and four *Solanum* rootstocks; *Solanum grandiflorum* × *Solanum melongena*, *Solanum torvum*, *Solanum melongena* × *Solanum integrifolium*, and *Solanum integrifolium*. Optifort rootstocks showed the longest roots, while *Solanum* spp. rootstocks exhibited the largest root volume. Grafting onto *Solanum Torvum* (3.94 kg/plant), *Solanum grandiflorum* × *Solanum melongena* (3.36 kg/plant), and *Solanum integrifolium* (3.34 kg/plant) significantly increased marketable yield compared to self-rooted plants (1.65 kg/plant). Rootstocks with larger xylem widths and higher cortex cell numbers promoted greater yield in grafted eggplant. Subsequent experiments assessed the salinity tolerance of Madonna eggplants grafted onto *Solanum grandifolium* × *Solanum melongena* (SH) and *Solanum torvum* (ST) versus self-grafted (SG) and self-rooted (SR) controls in a soilless cultivation. Grafted plants, especially those on SH rootstocks, exhibited higher levels of photosynthetic pigments under saline conditions. Additionally, SH-grafted plants showed enhanced total fruit yield due to increased average fruit weight. Both SH and ST rootstocks facilitated Na^+ partitioning, resulting in decreased Na^+ concentration in above-ground parts, thereby enhancing salinity tolerance and improving fruit yield and quality. In conclusion, grafting onto SH and ST rootstocks offers protection against salinity stress, leading to improved fruit yield and quality through enhanced photosynthetic pigment concentration and reduced Na^+ accumulation.

P4. Unveiling the Effects of Heat Stress on Plant Reproduction: Insights from Arabidopsis to Rice

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In angiosperms, double fertilization produces an embryo and endosperm, both crucial for viable seed development. With rising global seed demand, tackling climate change (CC) driven crop declines, is urgent. *Oryza sativa* represents an important species in the agricultural sector and one of the most consumed foods worldwide. CC is causing shifts in temperature patterns, leading to increased occurrences of extreme heat events, impacting rice reproduction and yield. Our main goal was to investigate the influence of heat stress (HS) on reproduction by studying genes potentially involved in heat tolerance in rice during anthesis, using *Arabidopsis thaliana* as a model, with the aim of transferring the knowledge obtained into rice. A set of genes (*OsACO1*, *OsACO2*, *OsEXPB11*, *OsRLCK100)* previously identified in an RNA-seq performed in a heat-tolerant rice variety was analysed and validated. Orthologues were identified in Arabidopsis (*AtACO1*, *AtACO4*, *AtEXPB2, AtCRCK2)* and T-DNA insertion lines selected. Wild-type and mutant plants were submitted to HS and the reproductive performance was assessed, as well as gene expression patterns. Anomalies in pollen tube growth along the pistil were detected, impairing their ability to reach the ovules, preventing double fertilization and subsequent seed formation. Counting viable seeds and unfertilized ovules confirmed a significantly higher percentage of unfertilized ovules under HS conditions than in controls. In all cases, HS drastically impacts pollen tube growth and seed development. All the genes studied may play important functions during reproduction, however in most cases, the absence of these genes renders the plant more heat-sensitive.

P5. Domains Rearranged Methyltransferase influences ovule number and stomatal opening in *Arabidopsis thaliana*

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The increase in temperatures and the irregularity of rainfall due to climate change decrease the availability of water in the soil, and agricultural production struggles to feed an increasing population. Among the multiple mechanisms through which plants manage environmental stresses, epigenetic modifications, including methylation, enable rapid and reversible gene responses. In particular, Domains Rearranged Methyltransferases (DRM) are involved in responses to abiotic stresses. In *Arabidopsis thaliana*, we investigated the responses of the drm1drm2 double mutant to drought stress, with a focus on female reproductive development. We recently discovered that the *drm1drm2* double mutant produces fewer ovules than wild-type plants under control conditions. However, under drought stress conditions, while the number of wild-type ovules proportionally decreased with increasing the stress severity, *drm1drm2* plants remained unaffected by the stress, maintaining their ovule production. Furthermore, *drm1drm2* plants also maintained the number of viable seeds under severe drought stress. By investigating the molecular mechanism, we found that in *drm1drm2* several miRNAs were upregulated, including miR397, miR398 and miR408, impacting the silencing of miRNA's target genes. Since changes in stomata are one of the main responses to water shortage in plants, we analysed stomatal number, size and aperture, founding that *drm1drm2* leaf stomata were more closed. The results of the phenotypic, molecular and physiological analysis suggest that drought stress responses are constitutively active in drm1drm2 plants. Although many aspects remain to be clarified, these findings represent a small step in understanding the role of methylation in water stress tolerance.

P6. PrOryza: Development of Thermotolerant Portuguese Rice – Genetic and Reproductive Analysis

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Rice (*Oryza sativa L.*) is an essential crop for human nutrition and is widely consumed in Portugal, the largest European consumer, with a per capita consumption four times higher than the European Union (EU) average. The country is also the fourth-largest producer in the EU, with about 30,000 hectares cultivated and 2,000 farmers dedicated to the sector. However, national production is not sufficient to meet domestic demand, resulting in high volumes of imports. Climate change, particularly the increase in temperature and prolonged periods of drought has significantly impacted rice production in Portugal. This crop is highly sensitive to heat stress, especially during the anthesis phase, and high temperatures compromise reproductive processes and fertilization, reducing the number of grains formed. It is estimated that each 1°C increase in temperature causes a 10% reduction in rice productivity. To mitigate the effects of water scarcity, farmers have been testing new irrigation systems, such as drip irrigation and sprinkling. However, these practices can intensify heat stress, worsening floral sterility and further reducing production. The PrOryza project focuses on evaluating the reproductive performance of rice varieties produced in Portugal – Caravela (100% Portuguese), Ariete, Ceres, Teti, and OP2115 (a new variety to be registered in 2025) – when exposed to high temperatures, as well as the genetic analysis of these varieties, facilitating the selection of genotypes more resilient to current climatic instability. The project aims to expand the National Variety Catalogue and strengthen national production, promoting the sustainability and competitiveness of Portuguese rice.

P7. The histone acetyltransferase GCN5 and the transcriptional adaptor ADA2b modulate endothecium lignification in *Arabidopsis thaliana*

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Anther dehiscence involves lignin accumulation in the endothecium of anthers, septum, and stomium lysis, releasing pollen grains to enable pollination. GENERAL CONTROL NON-DEREPRESSIBLE 5 (GCN5) and ALTERATION/DEFICIENCY IN ACTIVATION 2b (ADA2b) are members of the SAGA complex and regulate gene expression through histone acetylation, affecting several developmental procedures during plant growth. Aberrations in endothecium lignification result in male sterility, and although *gcn5* and *ada2b* mutants are partially or completely sterile, the involvement of GCN5 and ADA2b in regulating anther dehiscence remains obscure. Our data show that anther dehiscence occurrence is affected in *gcn5-1* and *ada2b-1*. In particular, only late-opening flowers of *gcn5-1* partially release their pollen grains, while *ada2b-1* anthers are indehiscent and do not release pollen. The pollen grains are viable in both mutants and do not differ significantly in their morphology from the wild-type counterparts. Anthers of *gcn5-1* and *ada2b-1* have altered endothecium cell wall lignification, which may be responsible for their anther indehiscence. Furthermore, transcriptomic analysis suggests that key transcription factors involved in endothecium lignification and several other hormone-related genes, implicated in anther dehiscence and development, are down-regulated in both mutants. Overall, our results support that GCN5 and ADA2b are positive regulators of anther dehiscence in Arabidopsis thaliana, regulating endothecium lignification.

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P8. Histone acetyltransferase GCN5 and transcriptional coactivator ADA2b regulate gibberellin homeostasis in inflorescence and flower meristem of *Arabidopsis thaliana*

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Gibberellins (GAs) are plant hormones that promote the cell elongation of stem cells in the inflorescence through a negative feedback loop involving their repressors, DELLA proteins. General Control Non–derepressible 5 (GCN5), a histone acetyltransferase (HAT), is associated with cell division and differentiation, meristem function, root, stem, foliar and floral development, and environmental responses. GCN5 forms two transcription adaptor complexes, Alteration/Deficiency in Activation (ADA) and Spt–Ada–Gcn5–Acetyltransferase (SAGA) complex, along with ADA2b, which enhances its histone acetylation activity. Phenotypic analyses of *ada2b–1* flowers and early *gcn5–1* mutant flowers revealed stamen shorter than pistil and thus reduced fertility compared to wild-type plants and abnormal gynoecium development. Our previous findings demonstrated that loss-of-function mutations in the DELLA protein RGA can suppress the floral defects of *gcn5* but not *ada2b* mutant. This research investigates the role of ADA2b and GCN5 in regulating GA metabolism, transport and signaling in inflorescence and floral meristem of *Arabidopsis thaliana*. RNA-sequencing and gene ontology analyses revealed significant down-regulation of genes associated with GA biosynthesis, transport, and catabolism, accompanied by decreased gene expression encoding DELLA proteins. Liquid Chromatography – High-Resolution Mass Spectrometry (LC-HRMS) confirmed reduced levels of bioactive GA_4 and accumulation of non-bioactive GAs in both mutants. Together, these results suggest that ADA2b and GCN5 act as positive regulators of GA homeostasis by modulating the expression of key genes in the GA-DELLA signaling feedback loop, thereby influencing inflorescence and floral meristem development in *Arabidopsis thaliana*.

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P9. High throughput isolation of male gametophyte cells of *Solanum lycopersicum* var. Micro-Tom by FACS to study the progamic phase and improve heat stress resilience

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Climate change, characterized by rising global temperatures and increasingly frequent heatwaves, poses a significant threat to the yield of fruits and seeds that are essential food sources for human populations. Heat stress, particularly during pollen development, is known to reduce pollen viability. While the progamic phase, in which the pollen tube grows through the pistil to deliver two sperm cells for double fertilization, is critical for successful reproduction, its heat stress response remains poorly understood. To investigate heat-induced transcriptomic and proteomic changes during the reproductive phase of *Solanum lycopersicum* (tomato), we developed a dual fluorescent marker line. It combines mTurquoise and mScarlet-I fluorescent proteins, driven by promoters with preferential activity in generative and sperm cells, respectively. In combination with fluorescence-activated cell sorting (FACS), this marker line enables the isolation of generative and sperm cells from pollen grains and pollen tubes grown through the pistil. These tools provide unprecedented access to the male gametophyte transcriptome during the progamic phase in tomato. Furthermore, we optimized protein extraction protocols from semi-*in vivo*-grown tomato pollen tubes for proteomic analysis. Together, these innovations offer powerful new resources for studying tomato male gametogenesis and will support efforts to enhance the resilience of plant fertilization processes under climate stress conditions.

Session II

Abiotic Stress Sensing, Signalling, and Response

Chairs: ***Marta Mendes & Konstantinos Vlachonasios***

KEYNOTE SPEAKER 2

John H. Doonan

High Throughput Phenotyping for Weather-Resilient Crop Development at the National Plant Phenomics Centre, Aberystwyth

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The National Plant Phenomics Centre is based on the western seaboard of the UK, an area well known for its highly variable weather patterns and is associated with several pre-commercial breeding and research programs. We employ mechanised high throughput phenotyping under controlled conditions, utilizing glasshouse-located conveyor-type systems to simulate defined single and multiple stress scenarios, including drought, heat, and plant-plant competition. Examples include longitudinal (seed-to-seed) studies on crops (such as wheat, oat and canola) and model species. We also integrate phenotypic data with metabolomic analyses to elucidate the biochemical pathways affected by these stresses. By examining the stress responses at both the physiological and metabolic levels, we aim to identify key traits and biomarkers associated with resilience. In addition to high throughput phenotyping, we conduct high-resolution analyses of harvested crops, focusing on grain and seed quality. This comprehensive approach allows us to assess the impact of stress conditions on yield and nutritional value, providing valuable insights for our associated breeding programs. Looking forward, we aim to extrapolate CE findings to field conditions, bridging the gap between controlled environment studies and real-world agricultural practices. Combining drone-based phenotyping with existing traditional, analytical capabilities and AI, holds promise for accelerating breeding crops can thrive in diverse and challenging environments, ensuring sustainable food production for the future.

O12. Biochemical and molecular adaptations of two wheat genotypes to waterlogging stress and recovery

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Many ecosystems are increasingly affected by extreme precipitation, prolonged flooding, and waterlogging. Waterlogging is now a major stress in wheat, severely disrupting metabolism, particularly during reoxygenation. However, the molecular mechanisms, especially the roles of ROS and antioxidative genes in hypoxia, remain poorly understood. To address this, we investigated oxidative and antioxidative metabolism in two wheat genotypes, Nova Bosanka (B) and Julija (J), under waterlogging (eight days) and reoxygenation (six days). We analyzed antioxidative enzymes (superoxide dismutase (SOD), catalase (CAT), Class III peroxidase (POX)), glutathione, and gene expressions linked to antioxidative defense, anaerobic metabolism (lactate dehydrogenase (LDH)), and stress responses (ethylene-responsive transcription factor 1 (ERF1) and RAP2.3 (ERF RAP2.3)). Waterlogging induced genotype-specific antioxidant responses rather than significant oxidative stress. J upregulated *Cu/ZnSOD* and *CAT* early in waterlogging, while B primarily increased *POX* expression aligning with hydroxycinnamate content fluctuations. Differential *ERF* gene expression indicated distinct regulatory strategies: J upregulated *ERF RAP2.3*, while B induced *ERF1*. Reoxygenation triggered stronger antioxidant activation in both genotypes, with upregulation of *POX*, *Cu/ZnSOD*, and *MnSOD*. B further increased *CAT* expression, while J exhibited stronger *ERF1* induction, suggesting enhanced ethylene-mediated recovery. B's transcriptional response to reoxygenation was weaker, indicating less efficient recovery. Notably, *LDH1* expression was significantly upregulated exclusively during the post-reoxygenation phase, highlighting its critical role in metabolic recovery. This findings highlight genotype-specific adaptive strategies to waterlogging and reoxygenation, emphasizing the roles of antioxidative defense, phenolic metabolism, and ethylene signaling. These insights provide a foundation for breeding wheat with improved resilience to transient flooding stress.

***Keywords**: anaerobic fermentation, antioxidants, ethylene, hydroxycinnamic acids, reoxygenation, waterlogging*

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O13. Detecting effects of protein-coding variants by 3D structure prediction: application in a maize nested association mapping panel

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In plant breeding, DNA sequencing has proven valuable for detecting genes associated with key traits and rapidly screening for promising individuals. However, current genomic models still struggle to fully capture the mechanistic effects of genetic variants. To bridge this gap, artificial intelligence models have been developed to predict gene activity from biological sequences, offering insights into the biological consequences of genetic variation. Among these models, structure prediction tools like AlphaFold2 have proved valuable, as they can predict the 3D structures of proteins from their amino acid sequences. However, questions remain regarding the accuracy of these predictions and their utility for genomic analysis. In this presentation, I will describe our methodologies for predicting variant effects using AlphaFold2. We leveraged the maize pangenome to identify protein variants in a nested association mapping panel (about 5,000 lines from 25 bi-parental families) and used AlphaFold2 to predict their 3D structures. We then validated the impact of these predicted structures on 32 phenotypic traits through proteome-wide association studies (PWAS) and genomic prediction. Our findings show that protein structure prediction using AlphaFold2, combined with an appropriate metric for quantifying structural similarity between protein variants (TM-score), enables higher statistical power in PWAS and improved accuracy in genomic prediction, compared to using sequence-based similarity alone. These results suggest that protein structure prediction models like AlphaFold2 can provide valuable biological insights into the effects of protein-coding variants and improve our ability to predict and understand the functional consequences of genetic variation.

O14. Unravelling the role of CaM/CMLs in early pollen development

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Ca^{2+} signals are decoded by Ca^{2+} sensor proteins such as Calmodulin (CaM) and Calmodulin-like proteins (CMLs). Emerging evidence suggest that CaM/CMLs may directly interact with LRR-RK receptors to regulate downstream signalling. We seek to understand the role of CaM/CMLs during early pollen development. Specifically we focus on the role of calcium signalling and CMLs in the LRR-RK EMS1/SERK1-associated pathway that regulates tapetum formation in the anther. Firstly, we use the calcium indicator R-GECO1 to image and quantify the cytosolic Ca^{2+} variations that occur after TPD1 ligand perception by EMS1 complex. We next analysed available RNA-seq data and identified a few CaM/CML proteins that are expressed specifically in tapetum and meiocytes in Arabidopsis. In a targeted screen using split luciferase assay, we found that CAM3 and CML37 interact with the EMS1 and SERK1 and using Alphafold predictions coupled with mutagenesis and protein-protein interaction assays we mapped interaction sites on EMS1. Genetic studies are followed to show the functional relevance of this interaction in the EMS1 pathway. Interestingly, CML37 is upregulated by temperature elevations and future investigation will focus on examining the role of CML37 in response to heat stress. Altogether this work will provide insights on the role of calcium signalling in the tapetum and in response to heat stress.

O15. An Evaluation of Drought Stress Responses in Mutant Carrot Callus Tissue

Damla Turan Büyükdinç

Recep Tayyip Erdogan University

The present study set out to investigate the effects of drought stress on callus tissues of the black carrot (*Daucus carota* L. ssp. *sativus var. atrorubens* Alef.) genotype, with particular attention paid to the impact of varying levels of gamma rays on enzyme activity. The callus tissues of the black carrot were exposed to gamma irradiation at various doses, including 0 Gy, 7 Gy, 8 Gy, and 9 Gy, to evaluate their response to drought stress. The antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), along with other biochemical parameters such as hydrogen peroxide (H2O2), lipid peroxidation (MDA) and soluble protein content were quantified in the callus tissues. In response to drought, callus production ceased entirely at a 15% polyethylene glycol (PEG) concentration, whereas a gradual reduction in callus production was observed at 5% and 10% PEG concentrations. Subsequent analysis of enzyme activities revealed that the activities of CAT, SOD, and APX enzymes exhibited significant increases in mutant carrot callus tissues under drought stress when compared to control tissues. Furthermore, it was observed that the levels of H2O2 and soluble protein content increased under drought stress and that the levels of lipid peroxidation (MDA) increased significantly due to drought stress.

O16. The Curious Case of a Class C HSF in *Pisum sativum* (Garden Pea): A Molecular Enigma

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Plants, unable to escape stress, have developed ingenious techniques to overcome obstacles. The Heat Shock Transcription Factor (HSF) family is essential for responding to heat stress and other abiotic stresses. Our analysis discovered 38 members of the pea HSF gene family. Their chromosomal locations, intron-exon architectures, conserved motifs, probable sumoylation and acetylation sites, and cis-acting regulatory elements were defined. Phylogenetic analysis divided these individuals into three representative classes: A, B, and C. Expression study revealed upregulation of PsHsfA2a, A3, A6b, A7a, A7b, A9, B1a, B2a, B2c, and B2d in response to a variety of abiotic stimuli, confirming the importance of HSFs in distinct abiotic stress conditions in pea. The activation domain is responsible for transactivation in HSFs, our study discovered three spliced variants of PsHsfA2a. Despite sharing the identical AHA domain, only full-length PsHsfA2a was capable of showing transactivation. Aside from that, Class C HSF lacks the AHA domain but demonstrates transactivation. We identified the exact sequence responsible for this transactivation by making targeted deletions from the C-terminal region. We hypothesise that post-translational changes may have a significant impact on transactivation. Our continuing study focuses on the role of these changes in modifying the functional features of transactivation-related proteins. Furthermore, we discovered that PsHsfC confers resistance to various abiotic stresses through interactions with PsHsfA3, A6b, and B2a. Aside from that, PsHsfC1 can form homodimers, as demonstrated by the Y2H assay.

O17. Surfing the ROS wave: HDA6's journey from chromatin to cytosolic stress granules. Unveiling redox-regulation of protein phase separation during heat stress.

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Histone deacetylases (HDAs) remove acetylation from histones and are responsible for chromatin compaction and gene silencing. In animals, HDAs are regulated by oxidative post-translational modifications (oxi-PTMs) of conserved cysteines. In this presentation, I will show that AtHDA6 undergo oxi-PTMs including disulfide bridge formation and S-glutathionylation. Mutation of these cysteines to redox-insensitive amino acids impairs plant survival to heat stress, suggesting that redox regulation of AtHDA6 is involved in the heat stress response. Using colocalization experiments and cellular fractionation, we found that AtHDA6 is relocalized to cytosolic stress granules (SGs) during heat stress in a redox-dependent manner. This suggests a link between redox signalling, lysine acetylation, and stress granule formation during plant adaptation to heat stress. Building up on preliminary data, I was recently awarded an MSCA postdoc fellowship. The proposed project explores the intriguing possibility of reactive oxygen species (ROS) regulating the formation of SGs during heat stress. ROS accumulation and SG formation have traditionally been viewed as independent events in the heat stress response. However, recent studies have shed light on the significance of Oxi-PTMs as regulators of protein LLPS in plants. The REPHASE project aims to test this hypothesis by investigate the dynamics of the redox state within SGs and mapping the landscape of protein Oxi-PTMs in SGs using proteomics. By bridging the gap between redox signalling and SGs, the REPHASE project promises to provide valuable insights into the mechanisms underlying plant adaptation to environmental stress.

O18. Investigation of different colored carrot cultivars in response to single and combination of drought and heat stresses

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Carrot (*Daucus carota*) is a highly nutritious vegetable crop; however, it is highly sensitive to abiotic stresses, which significantly affect yield and quality. Among these, drought and heat stress, both individually and in combination, pose major threats to carrot production. The combined effect of these stresses is particularly devastating, leading to severe physiological and biochemical disturbances. The present study aimed to quantify the physio-biochemical traits and transcript-level changes in response to single and combined stresses. Four different coloured carrot cultivars (red, black, purple, and orange) were evaluated under controlled growth chamber conditions. Seeds were sown in 5L pots containing a 3:1 peat and perlite mixture, and plants were grown under controlled conditions (25°C Day/15°C night temperature, 40-70% relative humidity, and artificial lighting). At the taproot formation stage, plants were subjected to four treatments: control, drought stress, heat stress, and combined drought/heat stress. Separate growth chambers were used to impose drought and heat stress treatments effectively. Results revealed differential responses among the cultivars, with some exhibiting greater resilience through enhanced physiological adaptation and biochemical regulation. Notably, purple carrot cultivar showed higher stress tolerance, making them valuable candidates for further screening and breeding programs aimed at developing climate-resilient carrot cultivars. This study provides novel insights into stress adaptation mechanisms in coloured carrot cultivars, contributing to future breeding efforts and sustainable agricultural practices in regions facing increasing climate variability.

Keywords: *Carrot, Combined stress, Physio-Biochemical traits, Transcriptomic analysis, Climate adaptation*

O19. Mitochondrial Calcium Signaling in Plants: Insights into Stress Response Mechanisms

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Plants constantly face biotic and abiotic environmental challenges that threaten their growth and survival. Effective adaptation relies on rapidly detecting stress signals and initiating appropriate responses. While the plasma membrane is traditionally considered the primary sensor of environmental changes, mitochondria, being particularly stress-sensitive, may act as primary "sensors" triggering retrograde signaling—communication from organelles to the nucleus. This involves metabolites, reactive oxygen species (ROS), calcium ions (Ca^{2+}), kinases, and transcription factors to restore cellular homeostasis. In Arabidopsis thaliana, mitochondria-derived ROS are considered primary triggers of retrograde signaling, but Ca^{2+} ions may also play a role. The mitochondrial Ca^{2+} uniporter complex (MCU and MICU) is a key regulator of Ca^{2+} uptake. When cytosolic Ca^{2+} is low, MCU remains closed, but when Ca^{2+} reaches a critical threshold, MICU activates MCU, allowing Ca^{2+} entry across the inner mitochondrial membrane. To investigate the possible role of mitochondrial Ca^{2+} in retrograde signaling, in response to environmental challenges, we developed a dual-sensor Arabidopsis line co-expressing a mitochondrial-localized red-shifted Ca^{2+} indicator and a cytosolic-localized green-shifted Ca^{2+} indicator. In a plant challenged with external stimuli, this approach allows *in vivo* analysis of Ca^{2+} dynamics simultaneously with cellular resolution. Focusing on hypoxia, which affects oxidative phosphorylation in mitochondria, we analyzed mitochondrial and cytosolic Ca^{2+} dynamics in leaf cells. Additionally, we are generating *micu* mutant lines and promoterMICU-GUS lines to assess MICU's role in hypoxia response. Our findings aim to enhance understanding of mitochondrial Ca^{2+} uptake mechanisms and their role in plant stress resilience, ultimately contributing to improved plant productivity.

O20. Investigating the role of SERK1 co-receptor in regulating plant response to temperature elevations

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Plasma membrane localized receptors, such as LRR-RKs, regulate every aspect of plants life from growth and development to immunity and response to stress. Yet, there is little information on the role of receptor-associated pathways in response to temperature elevations. Pollen development, especially during the early stages of tapetum development, is vulnerable to heat stress, which negatively impacts crop yield. In a candidate approach, we selected LRR-RK that are expressed in the tapetum and meiocytes and screened for the sensitivity of loss-of-function mutants to heat stress in Arabidopsis. We found that the co-receptor SERK1 positively regulates plant response to temperature changes, both in flowers and seedlings. This regulation depends on the SERK1 kinase activity. Using a confocal-temperature system, we currently investigate the dynamics of receptor localization upon temperature changes. Following a proximity labelling coupled with proteomics approach, we aim to identify the SERK1-interacting proteins in response to heat stress in the tapetum. Altogether this work will provide insights on the role of LRR-RK signalling in response to heat stress in the tapetum.

O21 Development of the Adult Plant Projective Imaging (APPI) System: Democratizing Imaging for Non-Model Plants

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Through their lifespan, plants face various abiotic and biotic stresses. Unable to escape from unfavourable conditions, they have evolved complex mechanisms to perceive and respond to various stimuli employing a battery of signaling molecules and second messengers. *In vivo* imaging experiments with *Arabidopsis thaliana* plants expressing Genetically Encoded Fluorescent Indicators (GEFI) revealed that mechanical stimuli trigger long-distance apoplastic glutamate and cytosolic Ca^{2+} waves propagating from leaf-to-leaf or root-to-leaf. Importantly, these signals are needed to induce the upregulation of stress marker genes and the synthesis of stress hormones in systemic tissues. While Arabidopsis has been instrumental in uncovering these processes, it remains unclear whether such mechanisms are conserved in larger plant species. To answer this question, we focused on adult *Nicotiana benthamiana* plants expressing different GEFIs to study *in vivo* the dynamics of Ca^{2+} and glutamate. To perform imaging experiments with *N. benthamiana* plants, we developed the Adult Plant Projective Imaging (APPI) platform, a custom-made large field-of-view fluorescence imaging setup, that allows the simultaneous orthogonal imaging of shoot and root of plants with sizes up to tens of centimetres. Thanks to the APPI system we were able to confirm the existence of local and systemic glutamate and Ca^{2+} signals propagation in the shoot in response to mechanical damages. By leveraging the orthogonal view, and the design of custom-made rhizotrons, we could also visualize root-to-shoot Ca^{2+} waves triggered in response to salt stress. Altogether, APPI represents a revolutionary imaging system for understanding how big plants perceive and face to current environmental stresses.

O22. The effects of Dynamic Controlled Atmosphere in physiological and quality traits of *Hayward* kiwifruit during cold storage

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Cold storage of *Hayward* kiwifruit in Dynamic Controlled Atmosphere (DCA) is a newly adapted technique aiming to maintain fruit quality parameters, by monitoring Respiratory Quotient (RQ) on daily basis. This study evaluated the effects of two different DCA conditions (2% O_2 & 5% CO_2 and 2% O_2 & 4.5% CO_2) on kiwis stored at 0 ± 1 ºC and 95% RH. Fruit quality parameters, including firmness, peel and flesh color, total soluble solids (TSS), pH and titratable acidity (TA) in juice, along with ethylene and CO_2 production rates evaluated after 3 and 6 months of storage, as well as during shelf-life at ambient temperature (0, 5, and 15 days). The results showed that both DCA conditions were more effective in preserving firmness, reducing ethylene production, and maintaining stable CO_2 levels during cold storage compared to controls. During shelf-life, fruit stored under DCA evaluated with higher values of firmness, L^* and h^o color parameters of peel compared to controls, up to 15 days. As expected TSS values increased during cold storage in fruit of all treatments. Both DCA conditions and particularly 2% O_2 & 4.5% CO_2 were effective in reducing ethylene production, and maintaining fruit firmness, making DCA a promising approach for extending storability and marketability of *Hayward* kiwifruit.

P10. Identification of Heat Tolerant Tomato Genotypes by Physiological and Gene Expression Analysis

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Tomato (*Solanum lycopersicum L.*) has significance importance as a food crop all around the world as well as in Pakistan. Both conventional and non conventional methods have been used by breeders for many years to improve several varieties of tomato against biotic and abiotic stresses such as insect pests, diseases, temperature extremes, salinity and drought. Among all stresses high temperature stress is a major environmental stress in agriculture worldwide which reduces crop yield and quality. The effect of heat can be minimized by growing heat tolerant tomato genotypes. In the present study our objective was to screen heat tolerant tomato genotypes by physiological parameters and gene expression analysis. Ten tomato genotypes were exposed to heat stress at 45 °C for 8 h and samples were taken. To determine the effect of heat stress, the physiological data were recorded and analyzed by measuring proline content, cell membrane stability, osmatic potential ($\pi \Psi \pi$), and chlorophyll content before and after heat stress treatment. Under thermal stress genotype Red ruby and Roma showed relative resistance while Robin and Saffal exhibited susceptible behavior. For gene expression studies, the most and least heat tolerant genotypes on the basis of physiological response were selected for total RNA extraction by TRIzol method. The gene expression analysis was done by reverse transcriptase polymerase chain reaction (RT-PCR) for selected genes followed by band scoring after agarose gel electrophoresis. Level of temperature stress tolerance and its physiological and genetic implications in selected tomato genotypes formed the basis for the future identification of tomato genotypes better adapted to heat stress through physiological as well as molecular techniques.

***Keywords**: Tomato; Heat stress; Physiological factors; Gene expression*

P11. Memory of wheat to repeated Heat Stress during pre-anthesis could be responsible for improved tolerance

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Risk imposed by high temperatures (HT) to the quality and yield of cereals, requires evaluation of naturally resistant resources, and finding of methods to improve it. Here were evaluated the tolerance to HT of 19 winter wheat cultivars (*Triticum aestivum* L.) in use in Albania, and the possible memory gained to the repeated stress, before anthesis. Biometric (root, shoot, leafs length), physiological (fine root cells death, Relative Water Content-RWC), and biochemical parameters (*chla*, *chlb*, carotenoids and xantophylls, and total carbohydrates) were measured, and the impact of a short shock (SS) at 42°C/2hrs versus a longer treatment (LT) at 38-35°C/24hrs on pigment synthesis, and on the expression of rubisco activase (*Rca1*) coding gene were investigated. A classification system was built to describe the tolerance to HT, and cultivars were grouped via UPGMA, and PCoA. Results show that SS impacted pigment synthesis more than LT, while expression of *Rca1* was cultivar-specific; In a group of 19 cultivars under two treatments (T1, T2) at 30°C, the vulnerable: moderately tolerant: tolerant were 4: 11: 5, and 3:9:7, respectively. Values were cultivar-specific for single parameters, yet a general trend was evident for some. Improved tolerance to repeated stress was described as gained stress memory.

Keywords: *biochemical synthesis, gene expression, primed stress memory, hierarchical clustering*

P12. Impact of acclimation and increasing cold on the biosynthesis of polyamines in winter oilseed rape

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Cold stress is among the most important environmental factors reducing the yield of crops. Research has shown that the combination and duration of natural phenomena creating autumn-winter conditions are critical for the quality of winter oilseed rape's overwintering in the temperate climate. This study aimed to expand the scientific knowledge on the mechanism underlying the plant response to adverse environmental conditions by investigating the influence of increasing low-temperature stress on the metabolism of polyamines in acclimated and non-acclimated winter oilseed rape. The study was carried out under controlled conditions in the laboratory. The winter oilseed rape hybrid 'Visby' was used in the experiment. Both acclimated and non-acclimated plants were exposed to –1 °C on the first day and to –3 °C on the second day of the increasing cold treatment. The results of the study showed a decrease in putrescine, spermidine, and spermine content during cold acclimation and a decrease in putrescine and spermidine levels at sub-zero temperatures. Acclimated plants exhibited higher ADC2 expression levels compared to non-acclimated plants following the acclimation treatment and maintained a stable expression level under increasing cold conditions. These findings enhance our understanding of plant stress responses under specific environmental conditions. Further research on stress mechanisms across different plant species and environmental contexts is anticipated to address key questions and contribute to developing new strategies for improving crop resilience.

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P13. Exploring the molecular networks of cold acclimation in kiwifruit

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Kiwifruit, as a climacteric fruit, exhibits elevated ethylene sensitivity and respiration rate, which contribute to quick softening and senescence, thereby shortening its postharvest life. To sustain quality and extend shelf life, cold storage is widely used, however, due to its subtropical origin, prolonged exposure to low temperatures could induce cold-related physiological disorders. Cold stress triggers extensive transcriptional reprogramming, altering numerous biochemical pathways. Our objective was to elucidate the cold stress acclimation mechanisms in kiwifruit (*Actinidia chinensis var. deliciosa cv. 'Hayward'*) utilizing multi-omics and functional approach. To achieve this, harvested kiwifruit were cold stored (0°C and 95% RH) for 15 and 90 days. A comprehensive characterization of cold response mechanisms was achieved through extensive metabolomic, proteomic, transcriptomic, and whole-genome bisulfite sequencing analyses performed on pericarp and placenta tissues at harvest and during postharvest timepoints. This integrative, system-based approach uncovered distinct cold-associated transcriptional, epigenomic, proteomic, and metabolic signatures, revealing shared pathways modulated in both tissues. Integrated analysis of multi-omics data using advanced bioinformatic models enabled the identification of several transcription factors (TFs) implicated in kiwifruit acclimation to cold stress. Subsequent biotechnological approaches facilitated the functional characterization of key cold-responsive TFs, including members of the C2H2, GARP-G2-like, HMG, NAC, and GRAS families. Overall, our findings offer novel insights into the complex molecular strategies deployed by kiwifruit during cold exposure, and they suggest potential biomarkers that could be exploited in breeding programs aimed at enhancing postharvest resilience.

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P14 Proteomic responses to transient drought in bread wheat: Unveiling mechanisms of tolerance

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Water scarcity during anthesis significantly reduces bread wheat yields. This study investigates the drought response of two bread wheat cultivars, contrasting a drought-tolerant and a drought-sensitive variety, by analyzing photosynthetic efficiency, water status, and oxidative stress markers during a transient drought at flowering. The sensitive cultivar exhibited impaired water management and a greater reduction in photosynthesis. In contrast, the tolerant cultivar appeared to utilize photorespiration for photoprotection, displayed earlier activation of superoxide dismutase, and maintained higher photosynthetic rates. These protective mechanisms in the tolerant cultivar resulted in less yield reduction and a more stable seed proteome. Proteomic analysis revealed that the tolerant genotype exhibited rapid proteome adjustments early in the drought, while the sensitive genotype showed minimal initial proteomic changes compared to controls. Both genotypes displayed significant proteomic changes at the end of the drought, with more pronounced alterations in the sensitive variety. Upon re-watering, differences between treated and control plants diminished, but remained more evident after the drought period. The tolerant genotype's greater plasticity in response to changing conditions highlights its superior drought resilience. Future research will focus on characterizing the contrasting proteomic and redox post-translational modifications in flag leaves of these cultivars during drought and recovery.

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P15. Salt stress impacts the photosynthetic activity of *Amaranthus cruentus*: an -omic and physiological analysis

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Amaranthus sp. (amaranths) is a genus of plants in Amaranthaceae family. Several species, like *A. cruentus*, are minor crops cultivated for either seed production or for their edible leaves. They are renowned for their tolerance to biotic and abiotic stress and their excellent nutritional quality, making them promising crops in challenging environmental conditions. Soil salinity is one of the main abiotic stress constraining crop growth, mostly in (semi-)arid regions. Most of the crops used today are glycophyte (i.e. salt sensitive); finding salt-tolerant cultivars or new species is therefore a priority for sustainable agriculture. Photosynthetic activity of *Amaranthus cruentus* (red amaranth, a plant with C4-type photosynthesis) was studied in response to a moderate saline stress (75 mM) in controlled conditions. Analysis of photosynthesis parameters, recorded with an infrared gas analyzer coupled with a fluorimeter (IRGA), showed a deleterious effect of salt stress on the light-independent reactions of photosynthesis (net carbon assimilation), but not on the light-dependent reactions (electron transport rate and quantum yield of photosystem II). Transpiration and stomatal conductance were also affected by salt. Transcriptomic and proteomic analysis were also performed on leaves. Differentially expressed genes and differentially accumulated proteins involved in metabolic pathways related to photosynthesis were used to explain photosynthetic activity data. Finally, chlorophylls and carotenoids content in leaves were compared to IRGA and -omics data.

P16. MicroRNA profiling of the leaf in Italian rice genotypes reveals novel insights into the response to salinity

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Salinity and drought are a major constraint threatening rice growth and production worldwide. Herein, we aimed to find molecular markers associated with drought and salt tolerance through miRNA-seq in Italian rice varieties. Salt responsive miRNAs were identified in four Italian rice varieties . Totally, 58 known and novel miRNAs (DE-miRNAs) were differentially identified in the second leaf of four genotypes. The most number of DE-miRNAs were found in Selenio and Baldo. Remarkably, 16 out of 20 DE-miRNAs identified in Selenio were belong to osa-miR395, and Osa-miR1861 was found to be the most dominant family in Baldo. Osa-miR395 contains 25 members within the rice genome that exhibit high conservation across species. Prediction of target genes of osa-miR395 revealed that these genes mainly encode sulfaterelated genes. Sulfur plays significant role in helping plants cope with drought and salt stresses. Intrestingly, overexpression of miR1861h has been demonstrated to enhances tolerance to salinity in rice. Building on this knowledge, we are currently investigating the potential targets of miR1861h. Among the predicted targets, a subset of transcription factors will be selected for genome editing, with the aim of validating their role in improving salt tolerance in rice. In parallel, we are developing experimental protocols to expose these rice varieties to salt and drought stress during the reproductive phase. The ultimate goal of this research is to generate novel rice miRNA signature datasets. These datasets will provide valuable insights into the miRNA-mediated molecular mechanisms underlying rice resilience to drought and salinity stress during the reproductive process.

Keywords: *Oryza sativa L., Salt stress, miRNA*

P17. Metabolomics, a tool to enhance understanding of the alterations in primary and secondary metabolites of Albanian wheat cultivars (*Triticum aestivum* L.) under drought conditions

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Environmental stressors, such as drought and salinity, significantly affect various aspects of plant morphology and physiology, including chlorophyll content, photosynthetic parameters, biomass, and yield in food crops. The role of metabolites is essential in the growth and development of plants, as they facilitate cellular integrity, energy conservation, cell signaling, the formation and structure of membranes, and the allocation of resources across the plant. Therefore, metabolomic assessments are important for gaining a deeper understanding of the specific mechanisms through which drought may act as a stressor in plant-environment interactions. The objective of the study was to identify and compare the metabolic changes in Albanian cultivars wheat leaf subjected to drought stress during the germination and seedling stages. The technique of gas chromatography-mass spectrometry (GC-MS) metabolomics was applied to derive primary metabolic profiles, whereas high-performance liquid chromatography (HPLC-MS) was used to analyze secondary metabolic profiles. Both methods were effective in identifying metabolites associated with polyphenols, flavonoids, organic acids, amino acids, and sugars. Over 27 compounds of primary metabolites and 36 of secondary metabolites were detected that varied between controls. Metabolite pathway analysis revealed that although there are no substantial changes, differences can be noted among the various cultivars concerning certain constituents like syringic acid, coumaric acid, gallic acid, and caffeic acid. The integration of these data with additional physiological and molecular research substantiates a complex, physio-metabolic response of *Triticum aestivum* L. to drought stress.

Keywords: *metabolomics, primary and secondary metabolism, drought, wheat*

P18. Priming Drought Resilience in Tomato Seedlings: Metabolic and Transcriptomic Responses Induced by *Pseudomonas putida* SAESo11 strain

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Drought stress is a major global challenge, severely impacting agricultural productivity, crop yield, and plant health. As climate change intensifies, water scarcity becomes an even greater threat to food security. In this context, we previously identified a drought-tolerant strain *Pseudomonas putida* SAESo11, exhibiting various plant-growth-promoting (PGP) traits, including phosphate solubilization, indole-3-acetic acid (IAA), siderophores, exopolysaccharides and biofilm production abilities. Inoculation with SAESo11 effectively alleviated drought-induced stress in tomato seedlings by priming them for enhanced systemic responses. This priming was evidenced by increased antioxidant activity, including elevated levels of hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and catalase (CAT) activity under control conditions. Such changes in antioxidant responses were indicative of metabolic and transcriptomic reprogramming in both leaves and roots, which prepared seedlings for better stress resilience. Using high-throughput RNA sequencing, we revealed that inoculated seedlings exhibited a significantly more stable leaf transcriptome under drought stress compared to non-inoculated plants. Moreover, inoculated plants maintained a more balanced metabolic state, particularly in sugar and amino acid metabolism, which was significantly affected in the absence of inoculation. Notably, in the roots, inoculation under control conditions enhanced sugar and amino acid metabolism, and under drought stress, it facilitated better overall metabolic regulation. Targeted qRT-PCR analysis of roots revealed a strong activation of polyamine metabolism exclusively in the inoculated seedlings under drought stress, consistent with elevated putrescine levels. These findings highlight the ability of *Pseudomonas putida* SAESo11 to modulate both metabolic and transcriptomic responses, enhancing drought resilience in tomato seedlings by establishing a primed physiological state.

P19. Heat priming-induced transgenerational thermomemory in common bean (*Phaseolus vulgaris* L.)

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The ability of plants to endure and thrive under adverse environmental conditions is facilitated by intricate signalling pathways that activate stress-responsive genes and proteins. Yet, the development and inheritance of heat-stress memory, as well as its effectiveness in recurring heat-stress events, is not well understood, especially for thermosensitive crops like *P.* vulgaris L. Herein, we investigated transgenerational thermotolerance in uni- and/or multi-generationally heat-primed common bean plants using morphophysiological and integrative transcriptome and methylome analyses. Our data showed that heat priming (30ºC) enhanced physiological resilience and significantly increased shoot branching and leaf number under heat stress (35°C) in G2, demonstrating enhanced thermotolerance. Dosage- and lineage-dependent processes were identified in heat-primed G2 plants at 25°C and 35°C. Multi-generational priming significantly enriched processes, including ABA-dependent stress tolerance, stress-response mechanisms, photosynthesis-related pathways and detoxification processes, protein homeostasis and heightened expression of HSPs, developmental adaptations and systemic-acquired cross-stress tolerance. Under normal growth conditions, transgenerational heat priming promoted a readiness state indicative of stress memory development, which led to enhanced thermotolerance. Methylome analysis revealed that heat stress enhances methylation levels mainly in a priming-dependent manner. Highly enriched differentially methylated functional pathways included DNA repair, autophagy and terpenoid metabolism. Commonalities between transcriptome and methylome-enriched pathways suggest a shared regulatory strategy in developing heat-stress memory towards enhanced thermotolerance. The findings of this study are essential for understanding the mechanisms of transgenerational thermomemory and could support epi-breeding strategies for enhancing non-model crop resilience to heat stress.

Keywords: *Heat priming, thermomemory, heat stress, transcriptional response, DNA methylation patterns, common bean, Phaseolus*

P20. Alterations in Dark Glands and Expression Levels of Hypericin Biosynthetic Genes during the Flowering Period in Leaves of *Hypericum perforatum*: A Two-Year Study

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Hypericin is a key secondary metabolite of pharmacological importance within the *Hypericum* genus. It is localized in the plant's dark glands (DGs), whose size and number positively correlate with hypericin content. Although the complete biosynthetic pathway of hypericin remains unresolved, several genes involved in its synthesis have been identified. However, studies investigating hypericin biosynthesis in natural populations remain limited. This study aimed to examine the number and surface area of DGs in the leaves of native *H. perforatum* plants alongside the expression levels of two hypericin biosynthetic genes during the flowering period. Leaf samples were collected regularly over two consecutive flowering seasons (2023 and 2024) from a natural population in the Seich-Sou area (Thessaloniki). Leaf images were analyzed to quantify DG number and surface area, followed by RNA extraction and expression analysis of two key biosynthetic genes, *HpPKS2* and *POCP2*. A comparison of the data from the two years revealed consistent seasonal trends in DG surface area, which increased during flowering and declined thereafter. In both years, expression levels of *HpPKS2* and *POCP2* decreased in response to rising temperatures and the end of the flowering period. However, no consistent pattern was observed in the DG number across the two years.

Keywords: *Hypericin, Hypericum perforatum, Dark Glands, expression of biosynthetic genes*

P21. Acclimation Responses in Greek Olive Varieties Under Drought Conditions

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The olive tree is considered one of the most suitable and best adapted species to the Mediterranean climate. However, according to projections, climate change will worsen with drought becoming more severe. The aim of the present study is to investigate the acclimatization to water stress of two Greek olive varieties, 'Lefkolia Serron' and 'Chondrolia Chalkidikis' after cis-priming. The experiment consisted of two drought cycles - the first one serving as the priming event - followed by a recovery period to simulate the changes in water supply. It was conducted in greenhouse conditions, using three irrigation treatments: (i) control plants with constant irrigation (80% FC, Field Capacity); (ii) primed plants exposed to two drought cycles (15%, 80% and 15% FC); and (iii) non-primed plants, exposed to one drought cycle (80% and 15% FC). The morpho-physiological parameters used to evaluate plant performance were, relative chlorophyll content (RCC), chlorophyll *a* fluorescence (F_v/F_m), photosynthetic rate (A), transpiration rate (E) stomatal conductance (gs). Our results revealed varietal differences with 'Lefkolia Serron' following a more conservative management of water resources, while 'Chondrolia Chalkidikis' appeared to exhibit more pronounced changes, with greater sensitivity to water scarcity and a faster recovery upon rewatering. At the end of the experiment, we studied gene expression levels in the leaves, to detect changes in stress-related gene families, such as protein kinases (*MAPK5),* transcription factors *(WRKY11),* membrane transport proteins *(AQP4),* osmotic regulation (*DEH10),* ABA receptor *(ABARPYL4-9),* primary metabolism *(MI3PS2 and OesSUSY)* and ROS scavenging *(SOD2).* The results showed differences between varieties and indicated that priming triggered different responses between Primed and Non-primed plants. The identification of stress-resistant varieties, combined with the exploitation of priming dynamics, will ensure the sustainability of the olive preserve valuable genetic resources.

Keywords*: Olea europaea; climate; drought priming, gene expression*

P22. ADA2b regulates the brassinosteroid-specific transcription factor BZR1 expression in *Arabidopsis thaliana* root development

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GENERAL CONTROL NON-DEREPRESSIBLE 5 (GCN5) is a histone acetyltransferase involved in plant growth and development. *gcn5* mutants display developmental defects such as dwarfism, loss of apical growth, abnormal function of shoot and root and defective responses to hormones. GCN5 interacts with ALTERATION/DEFICIENCY IN ACTIVATION 2b (ADA2b) within the SAGA complex of *Arabidopsis*. Loss of function of *ADA2b* mutant plants are characterized by dwarfism, abnormal root growth and floral sterility. Brassinosteroids are steroid plant hormones involved in several developmental processes. Specifically in the root, they regulate meristem size, cell elongation, and lateral root initiation. Preliminary data show that brassinosteroid biosynthesis and signaling are downregulated in inflorescence and shoots of seedlings in *ada2b* mutants. BZR1 is one of the two main transcription factors of the brassinosteroid signaling pathway directing the transcription of multiple genes in *Arabidopsis*. This study aims to characterize the expression pattern of transcription factor BZR1 in the roots of *gcn5* and *ada2b*. For this purpose, *gcn5;BZR1np::BZR1-CFP* and *ada2b;BZR1np::BZR1-CFP* plants were generated, and confocal microscopy was used to analyze BZR1 expression in the roots. BZR1 was expressed in all tissues and developmental zones of the root tip, and its expression was not spatially affected by mutant lines compared to wild-type. However, BZR1 levels appeared significantly lower in the epidermis and cortex of the meristematic and transition zone and in the endodermis and pericycle in all developmental zones of *ada2b* roots. These results suggest that ADA2b acts as a positive regulator of BZR1 in the development of *Arabidopsis* root.

P23. Heat and salinity stress induce time-dependent cytoskeleton rearrangements in *Arabidopsis thaliana*

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Heat and salinity are major abiotic stress factors, severely affecting the viability and productivity of plants. The effects of these stressors on plant cytoskeleton during the early stages of exposure are poorly studied. In the present study, mitotic microtubule organization as well as F-actin dynamics were assessed in *Arabidopsis thaliana* seedlings exposed to short-term heat or salinity treatment. Both stresses induced time-dependent alterations. Specifically, phragmoplast microtubules of *A. thaliana* root cells exposed to 37°C for 20 min – 1 h became elongated and phenocopied the phenotype of *fra2* p60-katanin mutant. Longer exposure (2 h) resulted in total mitotic arrest. Salinity exerted a milder effect on microtubules. Exposure to 250 mM NaCl for 20 min induced phragmoplasts to phenocopy the *fra2* phenotype, whereas after 1 h of exposure typical phragmoplast organization was restored. F-actin dynamics were monitored in transgenic *A. thaliana* hypocotyls, overexpressing the actin biomarker LifeAct-GFP. High concentration of salinity (500 μM NaCl) resulted in decreased F-actin dynamics and bundling and increased anisotropy of filament organization. In transgenic lines of *A. thaliana*, expressing the chimeric protein KTN-GFP, short exposure to heat or salinity depleted the p60-katanin signal. At longer treatments, katanin signal intensity was restored in both cases of stress. The observed differences in KTN-GFP localization were, however, not reflected in the expression levels of p60-katanin, which remained stable in all cases. Consequently, the decrease in KTN-GFP signal could be attributed to post-translational modifications. Overall, our findings showcase plant cytoskeleton as a target of short term heat and salinity stress.

P24. A serine/arginine-rich like protein modulates the heat stress response and thermotolerance in tomato

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Tomato (*Solanum lycopersicum*) is one of the most economically important crops worldwide, but its productivity is increasingly threatened by heat stress caused by global warming. Understanding the mechanisms underlying tomato's heat stress response is essential for developing strategies to enhance its resilience in the face of climate change. A key aspect of the heat stress response is the activation of genes encoding heat shock proteins (HSPs), which act as molecular chaperones to prevent protein misfolding and toxic aggregate formation. This process, along with the transcription of numerous other thermotolerance-related genes, is driven by heat stress transcription factors (HSFs). Pre-mRNA splicing is an important mechanism of regulation of gene expression, with many genes undergoing alternative splicing in response to heat stress. However, the mechanisms by which alternative splicing is regulated under high temperatures and the contribution of splicing factors in thermotolerance remain poorly understood. Members of the Serine/arginine-rich (SR) proteins are considered as the core regulators of alternative splicing not only in plants but in mammals as well. We have identified SR-like protein (SR46a) which is highly induced by HS, this presentation highlights the critical importance of SR46a in RNA splicing and its broader implications for gene expression in tomato plants under heat stress. A CRISPR/Cas9-mediated mutation causes altered expression of HSFs and HSPs and reduced thermotolerance. Moreover, through RNA-seq data analysis we were able to identify differentially expressed genes and alternatively spliced genes that are regulated by SR46a under heat stress treatment. These findings provide new insights into the molecular mechanisms underlying stress adaptation and identify SR46a as a potential target for enhancing stress resilience in plants.

P25. Evaluating Salinity and Drought Stress Adaptation in Sea Fennel (*Crithmum maritimum* L.)

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Crithmum maritimum L. (sea fennel) is a facultative halophyte species which possesses high nutritional value. This study examines the physiological and growth responses of sea fennel to different levels of salinity and drought stress. Therefore, plants were exposed to 100, 200, and 400 mM NaCl and at the same time, irrigation levels were set at 100% (control), 70%, 40%, and 10% of saturated substrate (peat:perlite, 3:1). In order to access any stress responses, parameters such as biomass, dry weight, plant height, and chlorophyll fluorescence were estimated. Results showed that high salinity treatment (400 mM) significantly reduced growth and photosynthetic efficiency. In specific, under 400mM salinity plants averaged 22.2 g of fresh weight compared to control (55.7 g) and resulted in a statistically significant reduction in Fv/Fm value (0.79), while moderate salinity (100 mM) showed no differences. Drought stress led to severe degradation in chlorophyll efficiency, especially under extreme deficit (10% saturation) resulting in 0.75 Fv/Fm, 1.6 RC/ABS and 4.19 PI values, respectively. However, plants endured 70% water uptake, indicating a moderate drought resistance. The outcomes of this study suggest that *C. maritimum* L. can thrive in low-saline (200mM) and semi-arid environments, enhancing its potential use in sustainable agriculture against malnutrition.

P26. Integrative analysis of wheat responses to combined drought, elevated temperature, and increased CO_2 stressors in simulated climate scenarios

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Climate change is anticipated to exacerbate environmental stressors such as drought, elevated temperatures, and increased CO_2 levels, posing a significant threat to crop productivity. We investigated the morpho-physiological acclimation and transcriptional responses of hexaploid wheat (*Triticum aestivum* L.) under both single and combined stress conditions. Wheat plants were subjected to drought (D), elevated temperature (eT), and elevated CO_2 levels (eC) individually, as well as in double (eT+D, eC+D, eC+eT) and triple (eC+eT+D) stress combinations. Our findings reveal that the eT+D combination resulted in the most severe reductions in growth and yield, while elevated eC conditions partially mitigated these effects by enhancing biomass production and water-use efficiency. Transcriptomic analyses identified key regulatory networks, including specific protein-coding genes, transcription factor (TF) families, and potential marker genes associated with stress adaptation. Stress conditions were associated with upregulation of genes involved in various pathways, such as transmembrane transport, plant hormone signal transduction or carbohydrate metabolism. Overall, our study provides new insights into the complex genetic and physiological mechanisms underlying wheat resilience to multifactorial environmental stress. These findings highlight valuable molecular targets for breeding strategies aimed at improving wheat tolerance to climate change-induced stress conditions.

P27. Molecular and Physiological Insights into Cadmium Stress Response in MTP1-Edited *Populus alba* Lines

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Poplar (*Populus*) is an important forest tree belonging to the *Populus* genus, which comprises around 35 species within the Salicaceae family (1). It has become a key species for global afforestation and shelterbelt projects due to its rapid growth, high biomass yield, and ability to thrive under various environmental conditions. Cadmium (Cd) is a heavy metal commonly introduced into arable soil through industrial processes and agricultural practices. It has been ranked among the top toxic heavy metals (2). All parts of the plant are susceptible to Cd accumulation, leading to chlorosis, stunted growth, leaf epinasty, and inhibition of photosynthesis, as well as reductions in chlorophyll and flavonol content and deactivation of CO_2 fixation enzymes. Therefore, understanding ionic and osmotic homeostasis is essential for developing plant tolerance to cadmium. Our work aimed to investigate the role of *Metal Tolerance Protein 1* (*MTP1*) in poplar under Cd stress. This gene encodes a membrane-bound protein belonging to the *Cation Diffusion Facilitator* (*CDF*) family and it is located in the vacuolar membrane of plant cells. To this aim, mtp1 knock-out lines of *Populus alba* L. "Villafranca" were generated by CRISPR/Cas9 technology. The study involved characterizing physiological and biochemical responses of *mtp1* knock-out lines and wild-type under control (0 μM $CdSO_4$) and 100 μM $CdSO_4$ treatments. After six weeks, phenotypic symptoms of chlorosis were observed in the apical parts of transgenic lines treated with 100 μM $CdSO_4$. Physiologically, reductions were noted in all analyzed parameters, including photosynthesis, chlorophyll, and flavonol content, particularly in the symptomatic regions across all treated groups, with the *mtp1* knock-out lines being the most affected. Moreover, the *mtp1* knock-out lines accumulated less Cd in all parts of the plant compared to both the treated wild-type and the control plants.

Keywords: *Cadmium, Populus alba L. "Villafranca" clone, MTP1, Knockout lines.*

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P28. Impact of Low-Temperature and Controlled Atmosphere Storage on the Secondary Metabolites of *Actinidia chinensis* 'Dulcis' fruit

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"Dulcis" (*Actinidia chinensis var. chinensis*) is a newly introduced kiwifruit cultivar that combines the aromatic complexity typical of the species with the distinctive green flesh coloration. To extend its commercial life, targeted postharvest studies are required to define optimal storage protocols that maintain key quality traits driving sensory appeal of kiwifruit. Cold storage (CS) and controlled atmosphere (CA) are well-established techniques to slow down the metabolism of fleshy fruits, including kiwifruit, thereby preserving critical quality attributes (1). Due to its recent introduction to the market, the impact of cold and hypoxic stresses on the technological and quality properties of "Dulcis"—particularly aroma— remains poorly understood. Moreover, their potential role in triggering storage-related disorders (SBDs) has yet to be fully assessed. In this study, "Dulcis" kiwifruits stored under CA and CS conditions were compared at the same firmness level (10 N), reached after 4 months in CA and 3 months in CS, followed by one week of shelf-life. In CA-stored fruit, the low-oxygen environment favored the preservation of complex phenolic compounds—such as proanthocyanidins and kaempferol derivatives—by limiting oxidative degradation. Conversely, CS fruit showed high levels of quercetin-3,4-O-diglucoside, likely as a result of stress-induced antioxidant responses. Volatile Organic Compounds (VOCs) analysis revealed that CA-treated samples accumulated higher levels of esters and alcohols—compounds typically associated with full ripening—whereas CS fruit retained more aldehydes, indicative of early ripening stages. Notably, in our trials, the incidence of SBDs was low overall, with CA conditions affecting only 4% of the fruit after three months, compared to 8% in CS-stored samples. The combination of higher ester content and optimal eating firmness in CA-stored fruit suggests an enhanced sensory profile and greater consumer appeal.

Keywords: *Actinidia chinensis***,** *cold, hypoxia, secondary metabolites*

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P29. Cold stress treatment of fresh walnuts added to the packing line prior to drying in a pilot experiment – Effect of orchard on moisture, color and antioxidants in the kernels

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Walnuts are highly nutritious due mainly to antioxidants and fatty acids. It has been shown that the antioxidants in fresh walnuts are higher than dried ones, and they can be further increased after a short cold stress before drying. Kernel moisture in fresh walnuts should be at least 17% (w/w), whereas in dried should be 5-8%. Therefore, fresh kernels/in-shell kernels deteriorate easily and cannot be marketed for long in contrast to the common dried form, although they have a unique taste and gain popularity. The aim of this work is to investigate the effect of a cold (4 °C, 80% R.H., 10-d) stress step added to de-hulled whole walnuts before drying on kernel color, moisture, total phenolics (TP) and total antioxidant capacity (TAC) in comparison to the conventional drying method in a pilot experiment. "Chandler" walnuts were harvested from 15-year-old trees from three different orchards in Domoko, Greece, with all trees receiving the same cultivation practices. Preliminary experiments were run to correlate the standard kernel color classes (USDA, 2017) with Minolta determinations. Results concluded that L* parameter was essential for color classification of kernel halves, with the value being greater than 60 in the superior 'extra light' class. The cold stress applied on walnuts of 'extra light' kernels did not affect kernel moisture and color, while increased averages of TP and TAC by 10% and 11%, respectively, but with large variations. Also, the color was affected by the different orchards. It is suggested that the walnuts origin should be considered in order the resulting mixed lot to be in the desired class. The final dried products, kernels/in-shell kernels, can be marketed with added value.

SESSION III

Biotechnology, Gene Editing and System Biology Approaches to Decipher Crop Resilience

Chairs: ***Antonio Granell & Olha Lakhneko***

KEYNOTE SPEAKER 3

Francesca Desiderio

Combining genomic and phenotyping approaches to study the genetic diversity for drought resilience in durum wheat

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Durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) is an important cash and staple crop for many countries of the Mediterranean area, a region where abiotic stresses, especially drought, have a significant impact on durum wheat yield and thus on food security. Taking advantage from the most advanced information available at genome and pangenome level, we are working to develop the knowledge required for selecting new adapted varieties capable of producing when water availability is less than optimal (drought) while maintaining the ability to express high production potential under fertile conditions. We aim to identify genotypes, loci/genes, and alleles that can be utilized in breeding programs. Our research includes targeted actions that dissect specific traits to understand their genetic underpinnings, as well as untargeted actions that aim to select superior genomic backgrounds, even when the individual components are not fully understood. Both actions rely on the large diversity of tetraploid germplasm (from wild relatives to landraces and varieties) well represented within the reference collections established in the framework of international collaborative initiatives (Tetraploid wheat Global Collection Maccaferri et al. Nature Genetics 2019, Global Durum Panel, Mazzucotelli et al. Frontiers Plant Science 2020; and Wild Emmer Panel, Mastrangelo et al. Plant Genome 2025). Among many traits contributing to water use efficiency (WUE) we focus on those related to stomata, specialized structures on leaves that regulate gas and water exchange. Density stomata and size are being phenotyped by a high-throughput approach utilizing a handheld digital microscope coupled with imaging analysis based on artificial intelligence (AI) for automatic object recognition. In parallel we are applying phenomics to identify drought tolerant genotypes. To this end, we conduct field trials in multiple locations with varying water availability to quantify yield stability and drought experiments in a multi-sensor gravimetric-based platform (PlantArray®), to define the drought response profile of selected diverse genotypes. Through these efforts, we are demonstrating that durum wheat breeding can effectively address the challenges posed by climate changes by leveraging on key adaptive traits and new natural diversity recovered from the tetraploid germplasm.

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O23. A tomato prolyl 4 hydroxylase 3 and Fascilin-like Arabinogalactan proteins 1 and 2 are involved in tomato abscission

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Organ abscission is controlled by highly regulated molecular mechanisms. The position of the tomato abscission zone (AZ) is strictly regulated and defined by the ratio of the proximal to distal part of the pedicel. This ratio was altered due to a shift in the position of the AZ which was attributed to shorter and longer pedicels of tomato SlP4H3 RNAi and OEX lines due to changes on cell division and expansion in AZ and distal part. This might be associated with LM2- and JIM8- AGPs which increased in OEX and decreased in RNAi lines throughout the pedicel. The JIM13 AGPs were downregulated in the flower AZ of OEX lines, pointing to a role on abscission regulation. FascilinLike Arabinogalactan proteins, FLA1 and FLA2, were identified which showed physical interaction with SlP4H3 while VIGS-induced suppression resulted in shorter distal pedicel indicating involvement in the shift of the AZ closer to the subtending flower. The overexpression lines of FLA1 and FLA2 were also characterized in relation to the position of the abscission zone. Moreover, ethylene-induced flower abscission was accelerated in the RNAi lines and delayed in OEX lines, while exactly the opposite response was observed in the red ripe fruit AZs. This was partly attributed to alterations in the expression of cell wall hydrolases. Overall, these results indicate that P4Hs might regulate molecular and structural features of cell walls in the AZ as well as abscission progression by regulating the structure and function of FLAs.

O24. Determination of metabolic biosignatures by cell physiological phenotyping to assess predictors of crop resilience and improve the lab to field translation

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The physiology of the host plant is the key interface to integrate the impact of plant/biostimulant intervention to sustainably improve crop resilience within the complex genotypes x environment x management interaction. Although the physiology is the key determinant of the phenotype, the classical methods used are inherently slow. To facilitate the integration of cell physiological phenotyping into a holistic functional phenomics approach and to complement molecular OMICs techniques we have established a semi-highthroughput analytical platform to determine the activity signatures of 33 key enzymes of carbohydrate, antioxidant and nitrogen metabolism in a microtiter plate format. The suitability of this approach was verified in case studies through assessment of the response of model and crop plants to beneficial microbes (BM). Stimulation of tomato growth by BM of the genus *Pseudomonas* correlate with distinct local and systemic biosignatures. Notably the strain G20-18 also primes for enhanced drought stress responses. Determination of activity fingerprints revealed that tomato growth promotion by a fungal endophyte is associated with sucrose de-novo synthesis in roots and other distinct metabolic effects. Seed coating of maize with a *Bacillus* increases water use efficiency and resulted in distinct impacts on carbohydrate and antioxidant enzymes. Finally, this strain was also used for a comparative analyses of greenhouse and field experiment with the same three soil types. The obtained biosignatures correlate with the impact of the *Bacillus* on growth and yield related traits and allow predictions on field performance. Thus, a proof of concept was obtained that enzyme activity profiling is a robust predictor of resilience mediated by BM and has a potential to improve the success of lab to field translations and for ground truthing of remote phenotyping methods.

O25 Decoding the molecular basis of superficial scald development in apples using a multi-omics and functional approach

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Superficial scald (SS) is considered a significant physiological disorder affecting apple fruit (*Malus domestica* Borkh.), marked by skin browning after extended cold storage at an early ripe stage. However, the downstream molecular processes driving SS development remain poorly understood. To explore the mechanisms behind SS occurrence, 'Granny Smith' apples were harvested at two different maturity stages (early and late) and stored at low temperature (0°C for 3 months), followed by 'shelf life' exposure at room temperature (24°C). Phenotypic and physiological analyses showed that early-harvested apples exhibited reduced ethylene-dependent ripening and increased SS phenotypes. A comprehensive multi-omic approach (including RNA-seq, whole genome bishulfite sequencing, nanoLC-MS, and metabolic analyses) on apple skin tissue identified potential genes, proteins, and metabolites associated with scald (early harvest) and non-scald (late harvest) phenotypes at both the pre-symptomatic (during cold storage) and symptomatic (ripening) stages. In addition, *in silico* protein modification analysis suggested that protein post-translational remodeling driven especially by oxidation events contribute to SS development. This approach uncovered novel molecular interactions linked to scald including the two protagonists of SS development; a-farnesene synthase (*MdAFS*) and polyphenol oxidases (*MdPPO*). The functional roles of *MdAFS1* and *MdPPO16* in SS development were explored using biotechnological tools such as RNA interference (RNAi) and CRISPR-Cas9, during cold storage and shelf life, revealing no-scald phenotypes. This combination of large-scale -omics and functional studies may provide valuable insights into the molecular mechanisms and *AFS-PPO* interplay orchestrating SS development, contributing to future breeding attempts for post-harvest life improvement in apples.

Keywords: *Apple, superficial scald, epigenetic, multi-omic, RNA interference, CRISPR-Cas9, a-farnesene synthase, polyphenol oxidase*

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O26. Special RECROP Session: Antonio Granell

HarnesstomDB v1.0: A Comprehensive Multi-Omics Resource for Tomato Genetic Diversity

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Over the past decades, significant advancements have been made in characterizing a series of tomato diversity panels at the genetic, phenotypic, and metabolic levels in. However, this valuable information remains scattered across databases and labs, often lacking integration both among these levels and with essential (meta)data such as plant passport and experimental/environmental conditions. These gaps hinder the effective use of germplasm data in breeding efforts aimed at sustainable agriculture and food quality. To address these challenges, we developed HarnesstomDB, a comprehensive platform based in Europe (accessible at https://gateway.harnesstom.eu/) that integrates tomato genetic resources with pre-breeding bioinformatics tools. The platform uses a BrAPI-compliant, JSON-based REST API, enabling integration with other databases and facilitating data exchange. HarnesstomDB serves as a centralized hub for storing, integrating, and exploring experimental metadata, accession details, phenotypic and metabolic data, images, genetic information, genotyping, QTLs, and GWAS variants. Currently, HarnesstomDB consolidates data and metadata from major European projects like TRADITOM, TomGem, and G2P-SOL, along with data from selected INRA GWAS experiments. The database offers information of Multicrop Passport Descriptors for 17,000 accessions (including traditional, wild, and breeding lines), 170 pedigrees, 10,472 accession images, and 93,020 phenotypic observations across 79 variables. Additionally, it provides metadata for 872 metabolites, and genotypic data from five platforms (e.g., TRADITOM GBS, SPET panel, SolCap arrays across three genome versions) covering 127,809 markers, 3 million genotyping points, 21 QTLs, and 773 GWAS variants. HarnesstomDB also includes tools for exploring germplasm attributes, phenotypes, and genetic data, such as multi-filters, interactive plots, genome browsers, and the HarnessAPI. These features enable users to compare accessions, analyze genomic features, and investigate environmental and phenotypic variables in a user-friendly manner. By combining comprehensive data, advanced tools, and open access, HarnesstomDB has become the largest germplasm tomato database in the world, fostering knowledge sharing to addres global challenges in agriculture and nutrition

O27. Increase of Lateral Roots in Tomato Plants Using Crispr-Combo Technology

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Tomato (Solanum lycopersicum) is a vital food source for human health, widely produced and consumed globally. However, abiotic stress factors like high temperature, drought, and salinity, driven by global warming and climate change, negatively impact tomato production. This highlights the need for innovative approaches to preserve and enhance this economically and health-significant crop. In this study, the CRISPR Combo system was employed to boost tomato resistance to abiotic stress. Lateral roots, crucial for water and nutrient uptake, adapt under drought and salinity by growing toward moister soil areas, aiding plant survival. Accordingly, the Solyc03g121060 (IAA18) gene, a negative regulator of lateral root development, was silenced, while the Solyc09g066260 (LBD16) gene, a positive regulator, was overexpressed. Physiological, morphological, and molecular changes were assessed in transgenic (T0) and next-generation (T1) plants. Mutation analyses, gene expression levels, and root development parameters were evaluated. Mutation rates of 70–90% were observed in silenced lines, with LBD16 expression significantly elevated compared to controls. Root growth in transgenic lines surpassed that of control plants. Seeds underwent osmotic stress testing, comparing transgenic and wild-type (WT) lines via germination rates, plant height, water content, proline, and MDA levels. Transgenic lines showed increased root growth, water content, and proline, with statistically significant results, while MDA levels dropped significantly at 200 mM mannitol compared to controls. Ultimately, silencing IAA18 and overexpressing LBD16 yielded a tomato variety with enhanced lateral root development and osmotic stress resistance.

Keywords: *Lateral root, IAA18, LBD16, Abiotic stress, CRISPR-Combo, Tomato*

O28. Bio-Sensors for Precision Agriculture

Adi Avni, Aakash Jog, Ron Sverdlov, Atiya Y, Yosi Shacham-Diamand

Increasing the global crop yield in past years was primarily done using traditional farming methods. These methods lacked accurate analysis of the plant cell's biological state, which was extremely expensive and labor intensive, and obtaining the data they provided took a lengthy amount of time. Traditional methods were cheaper to maintain but heavily relied on synthetic pesticides and fertilizers and a gradual increase of their usage. This in turn resulted in serious ecological and economical global damage. New farming techniques rely on real-time analysis and provide insights into plant cell's biological state at a much lower cost. A functional electrochemical sensor was designed, built and tested to detect stress in plants induced by pathogens. We explore the integration of synthetic biology and electrical engineering to create functional biosensors using Boolean logic gates in plants. Specifically, NOT and NOR gates were implemented in Nicotiana tabacum by employing inducible promoters and Cas6 proteins as translational inhibitors.

P30. Morphological and molecular diversity of *Alternaria alternata a*ssociated to tomato *Lycopersicon esculentum* L. fruit from Hyderabad, Pakistan

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Tomato [*Lycopersicon esculentum* (L.)] lavishly cultivated in sultry areas of the globe, tomatoes contains huge dietary substances. This dietary vegetable crop is severally harmed by numerous biotic and abiotic factors from all *Alternaria* is the foremost disparaging causative agent for black rot illness of tomato fruit. The aim of this study was to isolate, recognize, and describe *Alternaria alternata* morphologically and at molecular level from tomato fruit. To achieve this, local markets of Hyderabad region of Pakistan were selected to collect diseased samples of tomatoes. Collection sites are Tandojam, Kotri, and Hyderabad city. The contaminated samples were hygenated and inoculated by agar plate method. CTAB method was used for DNA extraction; ITS regions were sequenced and analyzed. Results showed six fungal species. From all, the dominating pathogen was *Alternaria alternata* with the frequency of (30.6%). The morphological variations were noted in the length and breadth of conidia, and numbers of horizontal, vertical, and oblique separations. From the backside of petri plates the colonial color was appeared to be brown, dark brown and brown to black. Surface region showed gray-green, lettuce green and olive green color with white boundaries and surface texture had white rough crystals forming different shapes. Apart from morphological studies, for authentic identification at molecular level one isolate was selected from each location for ITS region sequencing. Results showed that the sequenced isolate of Tandojam, Kotri, and Hyderabad had 574, 570 and 570 fragments of nucleotide base pairs correspondingly. Sequences were blasted on NCBI that validated that all studied isolates are affiliated with *Alternaria alternata* as they shared 98-99% resemblance.

Keywords: *Tomato, Alternaria alternata, PCR, ITS.*

P31. Sugarcane carries relatively larger family of CAMTA transcription factors, active against drought stress

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As the highly demanding complex genome of the hybrid sugarcane cultivar became publicly available recently (2023), it opened avenues to further study this important crop at molecular level. We are interested in digging the multiple stress responsive transcription factors family, the Calmodulin-Binding Transcription Activator (CAMTA) of sugarcane. This manuscript presents a comprehensive study of *ScCAMTA* family based on the latest sugarcane genome sequence information. Within the 10 gb genome, through HMM model prepared from sorghum CAMTA common domains, we found 48 genes, 46 out of which carry all the CAMTA-associated domains including CG-1, TIG, IQ and Ank. The phylogenetic analysis clustered then into seven classes. Keeping sorghum as reference, we named them as *ScCAMTA1 – ScCAMTA7*, while each one representing a class having 5-7 copies such as *ScCAMTA1A – ScCAMTA1E*, present in each sub-genome (chromosome) within the hybrid sugarcane. In parallel to determining their physico-chemical attributes, miRNA targets, protein interaction network and genome collinearity, we observed evolutionary conservation in gene structures, protein domains, and motif organization across the phylogenetic classes. Promoter analysis revealed the presence of multiple stress-responsive cis-regulatory elements, such as MBS (drought) and MYB (salinity), suggesting their direct involvement in stress adaptation. MicroRNA target analysis predicted 20 unique miRNAs targeting *ScCAMTA* transcripts, highlighting potential post-transcriptional regulation. Additionally, protein-protein interaction networks indicated functional connections to stress signaling pathways. Finally, their expression patterns under drought stress were determined using RNA-seq data, which revealed that *ScCAMTA7* is highly active under drought conditions, underscoring its potential role in drought response. This study furthers the insights into complex sugarcane genome and will assist in developing its drought-tolerant varieties.

P32. Comparative Analysis of Gene Delivery Efficiency: Selenium Nanoparticles vs. Functionalized Single-Walled Carbon Nanotubes in *Arabidopsis thaliana*

Gülnur Şener[1], Ravzanur Yazıcıoğlu Başaran[1], Zehra Çobandede[1], Melike Çokol Çakmak[1], Stuart J. Lucas[1], Nihal Öztolan Erol[1]

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Efficient gene delivery is crucial for genetic modifications aimed at enhancing plant resilience and productivity. Conventional methods suffer from inefficiencies, laborious tissue culture protocols, host limitations, genomic integration of transgenes, and cytotoxic effects. Nanoparticle-mediated gene delivery offers a promising alternative by providing high efficiency, low toxicity, transgene-free, and *in planta* application. Carbon nanotubes have demonstrated effectiveness in gene transfer, but their application is limited due to phytotoxicity. Selenium nanoparticles (SeNPs), widely utilized in biomedical research, have not been extensively explored in plant biotechnology. This study compares the gene delivery efficiency and toxicity of chemically synthesized SeNPs (~20 nm) and polyethyleneimine-functionalized single-walled carbon nanotubes (PEI-SWNTs) in *Arabidopsis thaliana* leaves and roots. Both nanoparticles were loaded with two *Green Fluorescent Protein* (*GFP*) gene forms: plasmids and linear gene cassettes. Phytotoxicity assessments revealed that SeNPs were less toxic than PEI-SWNTs. Gene transfer efficiency was evaluated by monitoring the *GFP* gene expression in leaf and root tissues. Results showed that SeNPs were more effective in delivering linear DNA, while PEI-SWNTs exhibited superior plasmid DNA delivery. These findings suggest that SeNPs hold potential as a safer and more efficient alternative for plant gene delivery, particularly for linear DNA transport. Their biocompatibility and reduced phytotoxicity make them a promising tool for plant genetic engineering. Further research should explore their applicability in diverse plant species and their role in enhancing plant resilience to environmental stressors.

P33. A systems biology approach to characterizing greek varieties and exploring the evolutionary dynamics of the NF-YA Transcription Factor for enhanced breeding and conservation

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Legumes, including lentils (*Lens culinaris*) and chickpeas (*Cicer arietinum*), are essential for food security and sustainable agriculture due to their ability to fix nitrogen, improve soil health, and provide essential protein and micronutrients for both humans and livestock. Legume development is highly regulated by transcription factors (TFs). The Nuclear Factor Y (NF-Y) TF, a heterotrimer composed of NF-YA, NF-YB, and NF-YC subunits is of particular interest as high-level developmental regulator. In particular, the NF-YA subunit due to its DNA-binding domain, recognizes the CCAAT box in promoters of target genes initiating their transcription. In this study, we conducted a comparative analysis of greek and international varieties of lentils and chickpeas, examining their physiology and growth characteristics, while also determining the variation of seed microfeatures. Additionally, we focused on the evolutionary dynamics of the NF-YA TF, by conducting phylogenetic analysis which enabled the construction of evolutionary trees, illuminating the relationships among NF-YA subtypes within legumes. Through PCR and HRM techniques, DNA sequence variations were identified in the *NF-YA* gene among the selected greek varieties. This systems biology framework has the dual purpose of laying the foundation for genome editing strategies, while also identifying novel genetic markers for legume breeding and variety conservation.

P34. Validation of the DRO1 Gene in Tomato Under Drought Stress: Insights into Root Architecture and Drought Adaptation

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Drought stress significantly reduces crop productivity, emphasizing the need for strategies to enhance water use efficiency without compromising yield. Optimizing root architecture, particularly through genes like *Deeper Rooting 1 (DRO1)*, offers a promising approach to improve drought resilience. *DRO1*, a key quantitative trait locus (QTL) in rice and Arabidopsis, promotes deeper root growth, enabling access to groundwater during drought. However, root traits vary by species, necessitating further exploration of *DRO1* in other crops. This study focuses on functionally characterizing *DRO1*-mediated root architecture in tomato (*Solanum lycopersicum L.*), a globally important crop, using an omics-driven approach combining phenomics and genomics. Root system analysis revealed decreased root length and fine root fraction after 30 days of drought, while root mass ratio increased, indicating a shift in resource allocation. Imaging techniques showed stable root surface area and fineness, while near-infrared (NIR) imaging confirmed reduced water content in drought-stressed plants. These findings highlight key root traits and spectral indices for assessing drought adaptation. qPCR analysis demonstrated that *SlDRO1* expression in roots remained stable initially but increased significantly after 20 days of drought, while stem expression decreased, suggesting tissue-specific regulation. *In situ* hybridization localized *SlDRO1* expression in both roots and stems, providing spatial insights into its role during drought adaptation. Ongoing efforts aim to generate knockout (KO) and overexpression (OEX) lines of *SlDRO1* in tomato, enabling full functional characterization. These findings will enhance understanding of root system plasticity and contribute to improving drought resilience in tomato and other crops.

Keywords: *Root architecture, Drought, CRISPR-Cas9*

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P35. *Breed-Epeautre-Omics* European project: Genomics and genetics approaches of Spelt for a sustainable agriculture facing global warming.

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The European project 'Breed-epeautre-omics' aims to study the genetics and quality improvement of spelt (Triticum spelt) in a context of ecological transition and global warming as a source of alternative crops for producers in France (Hauts-de-France) and Spain. A context in which, for example, 2020 was the hottest year in the history of France, and crop's yields (e.g. wheat, peas, etc.) were considerably reduced strongly impacting the income and competitiveness of producers. In 'Breed-epeautre-omics' we propose an innovative solution to this challenge integrating genetics, quantitative genetics, transcriptomic studies, genomic prediction, and high-throughput qualitative analysis. 'Breed-epeautre-omics' is a collaborative initiative that integrates all stakeholders of the cereal sector composed of public and private research institutions, regional public agencies, cereal transformation stakeholders, seed breeding companies, and growers.

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P36. CRISPR/Cas9-Mediated miRNA Knockout for Climate-Resilient Crops: Enhancing Drought Tolerance in Tomato

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Tomato is a major crop worldwide, suffering yield losses due to drought, intensified by climate change. Recent studies highlighted microRNA as the key regulators of gene expression under the abiotic stresses. Unfortunately, their role under drought stress remains under-investigated. This research aims to investigate the role of microRNA in drought tolerance and to develop potential drought tolerant tomatoes by CRISPR/Cas9 system. In this study, two drought-responsive miRNAs (sly-miR5302b & sly-miR9477) were selected from previously present transcriptomics data. sgRNAs specific to these miRNAs were designed and inserted into pHSE401 vector using golden gate cloning system. The designed plasmid was transferred to *Agrobacterium tumefaciens* using the electroporation method. Ten days old cotyledon leaves of tomato variety Crocker were transformed using *Agrobacterium–mediated transformation* method. After the treatment, the explants were cultured on the selection media to induce callus formation. The regenerated shoots were then transferred to rooting media for root induction and then transferred to greenhouse to acclimatize to external environment and fruit formation. After transgenic lines confirmation by PCR and sanger sequencing, three lines were selected to analyze drought tolerance and gene expression. Comparative analysis between transgenic and wild type plants under drought stress demonstrated the tolerance of transgenic–lines, reinforcing their potential for climate resilient agriculture. Gene expression of miRNA targeted genes will be examined by qPCR in transgenic plants. This study provides significant role of miRNA in conferring drought tolerance, emphasizing the broader potential of non-coding RNAs as crucial regulatory molecules, offering potential breakthroughs for developing drought resilient tomatoes.

Keywords: *CRISPR/Cas9, Knockout, MicroRNA, Drought, Tomato*

P37. CRISPR-Cas9 Genome Editing for Photosynthesis and Chlorophyll Biosynthesis Modifications in Plants

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Genome editing technologies have transformed agricultural biotechnology by enabling precise modifications to crop genomes. Among these technologies, CRISPR-Cas9 has emerged as a powerful tool for enhancing key agronomic traits through targeted genetic changes. Here, we present a comprehensive framework for using CRISPR-Cas9 to edit genes involved in photosynthesis and chlorophyll biosynthesis in three crops: tobacco (*Nicotiana tabacum*), tomato (*Solanum lycopersicum*), and lupin (*Lupinus* spp.). Specifically, we report the identification and selection of the target genes across these crops, the design and selection of single-guide RNAs (sgRNAs) to target the respective coding sequences, and the preparation of primers to amplify the edited regions. We also describe the genotyping pipeline used to confirm the absence of genetic variants at the sgRNA target sites. To assess the feasibility of agro-infiltration, preliminary experiments indicate the successful transient expression of reporter genes in leaf tissues. Ongoing validation experiments include evaluating sgRNA efficiency *in vitro*, in protoplasts, and *in planta* through agro-infiltration and *Agrobacterium*-mediated transformation. By introducing precise mutations, we aim to evaluate the functional impact of these genes and validate genome editing methodologies across multiple crop species. Our expected outcomes include the identification of gene-edited plants with altered chlorophyll content or photosynthetic efficiency, laying the foundation for future crop improvements. This study establishes a proof-of-concept framework for targeted genome editing in various crops, which can be expanded to modify additional genes influencing critical agronomic traits. Ultimately, this approach will contribute to the development of more resilient and productive crops, supporting sustainable agriculture through precision breeding.

P38. *De novo* domestication of wild plant species of *Solanaceae* family

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The wild representatives of *Solanaceae* family are perspective for creation new-generation crops. The aim of this work was to analyse all gene editing achievements of wild *Solanaceae* species: *Physalis grisea, P. pruinose, P. pubescens, Solanum nigrum, S. americanum, Lycium ruthenicum, S. pimpinellifolium, S. peruvianum*. The analysis was conducted in PubMed using the keywords "editing" + species name. The list of selected 16 articles was prepared and all data was organized in the form of table. The following analysis parameters were selected: edited species; explant type; explant age; edited genes; *Agrobacterium* species and strains; genetic vectors; types of mutations; method of editing tools delivery; editing efficiency; types of changes. The comparative analysis was performed in Flourish application and the Sankey diagrams were built.
The experiments were conducted mainly with *S. pimpinellifolium* and *P. grisea*, 14-day old cotyledons and leaves were used. The usage of *A. tumefaciens* LBA4404 (pTC321 genetic vector) or pCAMBIA1300 strain (different vectors) and AtCas9 or Cas9 resulted in 54.55-100% editing efficiency and obtaining knockout mutants. The following genes were edited: *APETALA2, CLE9H, CycB, DHNA, FAS (CLV3), FW2, GGH, GGP1, HG18103, MULT (S), O, PDS, PFCRC, PgAN1, PgDEF, PgEJ2, PgER, PgGLO1, PgGLO2, PgLIN, PgMPF2, PgMPF3, PgRIN, PgSP, PgTAG1, PgTAGL1, PgTM6, Ppr-CLV1, Ppr-SP, Ppr-SP5G, Rx4, SnS, SnAN2, SnLazy1, SnMYB1, SnSP, SP, SP5G, WF2, WUS, Y*. In the majority of experiments the mutants with plant architecture changes and fruit size were obtained. Thus, the *de novo* domestication of wild *Solanaceae* species opens the new horizons for application of genetic engineering.

P39. Investigating the role and the interactome of NF-Y transcription factor heterotrimeric protein in tomato

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NF-Y is a key transcription factor (TF) involved in regulating numerous developmental pathways in plants. In tomato, the NF-Y TF family is composed of multiple homologous genes for each subunit: NF-YA, NF-YB, and NF-YC. Targeted disruption of the *NF-YA8* gene using zinc finger nucleases (ZFNs) resulted in specific developmental phenotypes, highlighting its critical role in both embryogenesis and morphogenesis. The M3 generation is presently being characterized. To identify partners of the NF-Y subunits themselves and with other proteins, yeast-two-hybrid (Y2H) assays were conducted. Specifically, tomato NF-YA8 was cloned into a bait vector (Trp+) that produces a protein fused to the Gal4 DNA-binding domain. The L1L4 protein, an NF-YB subunit, was cloned into a prey vector (Leu+) that produces a protein fused to the Gal4 activation domain. These assays confirmed the interaction between NF-YA8 and L1L4, indicating their likely involvement in the same regulatory complex. Both NF-YA8 and L1L4 were then used to screen a tomato fruit cDNA library and a yeast library, with the goal of identifying potential interacting proteins. Given the conserved nature of NF-Y function, a heterologous screen was also performed using a yeast DNA library with each tomato TF as bait. The yeast strain PJ69-4A was transformed with these constructs and initially selected for successful co-transformation on -Trp/-Leu media. Protein interactions were then validated by replica plating onto high-stringency -Trp/-Leu/-Ade/-His media supplemented with 1 mM 3-AT. The results of the screens are presented and discussed in the context of NF-Y's role in tomato development.

P40. GCN5, CLV1, and SPT Coordinate Tissue-Specific PIN3 Expression During Arabidopsis Gynoecium Development

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The development of the gynoecium, one of the most complex plant organs, is regulated through the coordinated action of transcription factors, hormone signaling pathways, and chromatin modifications. Auxin distribution in gynoecium formation is influenced by the histone acetyltransferase GENERAL CONTROL NONDEREPRESSIBLE 5 (GCN5), which has been shown to interact genetically with the CLAVATA (CLV) signaling pathway. Additionally, auxin-mediated responses involved in gynoecium development are regulated by SPATULA (SPT), a basic helix-loop-helix (bHLH) transcription factor. To investigate the role of GCN5, CLV1, and SPT in auxin-mediated gynoecium development, we analysed the expression of the auxin efflux carrier PIN3 using the *pPIN3:eGFP-GUS* reporter line across developmental stages 8 to 12 in various genetic backgrounds of *Arabidopsis thaliana*. Our findings reveal that *PIN3* expression is dynamically regulated in a tissue-specific manner, with GCN5 acting as a negative regulator in the stigma but as a positive regulator in the style and gynophore. CLV1 consistently functions as a negative regulator in the replum and gynophore, while SPT primarily promotes PIN3-mediated auxin transport in the style and stigma. Genetic interaction analyses indicate that these regulators act independently and synergistically—SPT appears to act downstream of CLV1 and GCN5 in the stigma and style, whereas GCN5 may act downstream of CLV1 and SPT in the replum. Collectively, our data support a complex regulatory network involving GCN5, CLV1, and SPT that fine-tunes PIN3 expression.

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P41. Development of transgenic lines with tissue-specific targeted silencing of the transcriptional factor WUS in *Arabidopsis thaliana* flowers

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The transcriptional factor WUS (*WUSCHEL*) is essential in organising meristems in *Arabidopsis thaliana*. In contrast to the flowers of wild-type *A. thaliana* plants, *wus* mutants develop flowers lacking the whole gynoecium. While *WUS* was proven to be not directly responsible for the development of the gynoecium, fluorescence microscopy on the carpels of pWUS::GFP-ER line plants evinced activity during the development of the gynoecium's style. Transgenic lines were created to target and silence WUS in a tissue-specific manner to reveal the role of WUS in the development of the *A. thaliana* carpels. Using the Modular Cloning and Golden Gate strategies, two different plasmids were constructed containing the *zCas9i* gene, driven by a different tissue-specific promoter each, along with a selection marker and guide RNAs complementary to the first exon of *WUS*, therefore creating a tissue-specific targeting CRISPR/CAS9 system. The promoters chosen were the promoter of the *STYLISH2* (*STY2*) gene that is expressed in the apical parts of the developing gynoecium and the promoter of the *CRABS CLAW* (*CRC*) gene that plays a major role in the development of carpels. The CRISPR/Cas9 system was introduced into *A. thaliana* plants through an *Agrobacterium tumefaciens*-mediated transformation with the floral dipping method, creating transgenic plant lines.

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P42. Characterization of *Brassica rapa* plants carrying *BrGCN5a* point mutations

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The SAGA complex contains a histone acetyltransferase module (HATm). In *Arabidopsis thaliana*, the transcriptional adaptor GENERAL CONTROL NON-DEREPRESSIBLE 5 (GCN5) of the HATm appears to participate in developmental processes. *Brassica rapa* is a diploid plant with growth similarities to *A.thaliana* and *Brassica napus*. As a result of an ancient triplication, *B.rapa* contains three paralogous genes to the *AtGCN5*, designated as *BrGCN5a*, *BrGCN5b*, and *BrGCN5c*. This study aims to characterize the phenotypes of various *B.rapa gcn5a* mutants, investigate the possible functions of BrGCN5a and compare them with the known functions of the AtGCN5. Mutagenized *B.rapa* seeds carrying point mutations of *GCN5a* were acquired from the TILLING population of John Innes Center. Bioinformatic analysis indicates that BrGCN5a is a truncated protein containing part of the N-acetyltransferase domain and the entirety of the bromodomain. Plants carrying *gcn5a* mutations exhibited belated seed germination, semi-dwarfism and reduced root length. RNA-seq analysis from wild-type, *gcn5a-2* and *gcn5a-3* mutants showed that from the total genes expressed in the root of 4-days-old seedlings, 6,6% were differentially expressed in *gcn5a-2* and 4,9% in *gcn5a-3*. Similarly, in the hypocotyl, cotyledons and shoot apical meristem of 4-day-old seedlings, 17,7% of total genes were differentially expressed in *gcn5a-2* and 8,1% in *gcn5a-3* seedlings. Therefore, BrGCN5a affects gene expression in young seedlings. The phenotypic similarities between *B.rapa gcn5a* and *A.thaliana gcn5* plants might suggest possible similarities in the function of these proteins.

This work is supported by the Hellenic Foundation for Research and Innovation (HFRI) Grant Number 3026. PI Konstantinos Vlachonasios

P43. Manipulating Tomato Seed Fatty Acid Composition via Non-Transgenic Disruption of NF-Y Transcription Factors using Zinc Finger Nucleases

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Improving tomato fruit quality and nutritional content is a key focus in modern breeding. Targeting transcription factors (TFs), which control metabolic pathways, presents a powerful strategy to achieve this. This study explores the role of the heterotrimeric TF NF-Y in tomato seed characteristics, specifically seed phenotypes, germination and fatty acid biosynthesis. Tomato seeds, often a discarded byproduct of processing, are a valuable source of oil rich in essential fatty acids. To investigate NF-Y's function, we used Zinc Finger Nucleases (ZFNs), a non-transgenic gene editing technology, to create disruption lines of *NF-YB/L1L4* (lines 7-5, 29-2) and *NF-YA8* (line 1T) in tomato. We analyzed transcriptomics data and the fatty acid composition of several tomato seed samples, revealing a high proportion of unsaturated fatty acids, predominantly oleic and linoleic acid, and significant variability between samples, highlighting their potential industrial applications. Analysis of the major fatty acid profiles in these lines showed significant alterations compared to wild-type seeds, particularly in linoleic and oleic acid levels. These results demonstrate the potential of ZFN-mediated gene editing to enhance the nutritional profile of tomato in a non-transgenic way, circumventing regulatory obstacles associated with traditional transgenic methods. Our findings emphasize NF-Y's regulatory role in lipid metabolism and its promise as a target for breeding superior tomato varieties with improved seed oil composition.

P44. Removal of sterols from cell membranes affects cell division and division plane orientation in *Triticum aestivum* and *Zea mays*

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In plants, cell division plane (CDP) orientation and cell growth directionality determine tissue patterning and organ formation of the landmark of CDP orientation is a cortical microtubule annulus, the preprophase microtubule band (PPB) which forms at the vicinity of the plasma membrane. The PPB microtubules are physically linked to the plasma membrane requiring recruitment of scaffold proteins with affinity towards specific lipids and proteins associated with microtubules. The mechanisms underlying the determination of CDP orientation are being vigorously studied in *Arabidopsis thaliana*. Sterols, are major membrane lipid constituents forming nanodomains, with vital roles in cellular processes such as endocytosis, establishment of cell polarity and cell expansion. However, the implication of sterol biosynthesis in the determination of CDP has not yet been dealt with in crop plants. Here, the effect of methyl-b-cyclodextrin (MbCD), a drug disrupting plasma membrane nanodomains, was evaluated on *Triticum aestivum* and *Zea mays*. Three to 4-d old were treated with MbCD and processed for α-tubulin immunostaining or prepared for transmission electron microscopy (TEM). Confocal imaging of α-tubulin- immunostained cells of both plants revealed double PPBs, ectopic PPBs and disruption of CDP orientation. TEM observations revealed aberrant cortical microtubule orientation and PPB organization. Overall, disruption of sterol accumulation in the plasma membrane affected CDP orientation and establishment in both crop plants.

P45. Ethylene response factor ERF1A positively regulates the UV-C induced ripening delay in peach fruit

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The ripening of climacteric fruits, such as peach, is a complex process regulated by both internal signals and external cues. Ultraviolet-C (UV-C) irradiation has been shown to delay fruit ripening, yet the mechanisms underlying this phenomenon remain poorly understood. Herein, using transcriptomic and biochemical approaches, we explored the tissue-specific responses of peach fruit to UV-C, examining the peel and flesh tissue independently. Data indicated that UV-C triggered central metabolism reprogramming and promoted anthocyanin accumulation, especially in peel tissue, consistent with changes in anthocyanin biosynthetic gene expression and fruit coloration. Interestingly, UV-C treatment delays fruit ripening by modulating ethylene signaling and altering the expression of ripening-related transcription factors, including ERF1A. UV-C stimulated DNA N6-methyldeoxyadenosine (6mA) and RNA N6-methyladenosine (m6A) in peel but did not induce cytosine methylation or mutation in *ERF1A.* RNA interference (RNAi)-mediated silencing of ERF1A supported its key role in UV-C-mediated ripening delay, as evidenced by changes in ethylene production, ripening-associated metabolites, fruit softening and the fluorescent immunolocalization of cell wall matrix components, particularly arabinogalactan, pectin and xyloglucan epitopes. Levels of auxin and salicylic acid were increased and abscisic acid was reduced in ERF1A-silenced peels, while the biosynthesis of peel-derived volatile compounds was altered. Proteomic analysis identified several ERF1A targets and highlighted that the UV-C delayed ripening could be partially reversed by ERF1A silencing. Data also supports that *PpCXE11*, *PpCXE13* and *PpSABP2* are potential *cis*-targets of ERF1A. These findings provide insights into the mechanisms underlying UV-C-induced ripening delay and highlight the pivotal role of ERF1A in regulating this process.

Keywords: *Ethylene response factor, peel tissue, ripening delay,* RNA interference*, UV-C irradiation, water loss*

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P46. Transcription factors *PaWRKY57* and *PaNAC29* regulate color and size in sweet cherry fruit

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Although the ripening process of climacteric fruits is well-documented, the signals that trigger ripening in non-climacteric fruits, such as sweet cherry, remain poorly understood. Here, we present a transcriptomic analysis during eight selected developmental stages in the late-maturing cherry cultivar 'Regina', providing a wide map of gene expression changes during fruit ripening. This study reveals transcriptomic and metabolomic reprogramming across multiple pathways, particularly those related to sugar metabolism, in pedicel during fruit development, suggesting that cherry ripening may be partially regulated by pedicel-derived mechanisms. Using our datasets, we identified key transcription factors, especially *PaWRKY57* and *PaNAC29*, as putative regulators in fruit ripening. Silencing of *PaWRKY57* and *PaNAC29* in both 'Regina' and the early-maturing cultivar 'Carmen' at color break stage resulted in delayed pigmentation and reduced fruit size. Targeted gene expression along with proteomic analysis in silenced fruit identified several *PaWRKY57* and *PaNAC29* candidates targets and revealed regulatory networks associated with anthocyanin biosynthesis. Components of central metabolism along with major anthocyanins, particularly cyanidin glucoside and cyanidin rutinoside, were reduced, while abscisic acid levels were altered in silenced fruit. Our analysis further demonstrates that *PaWRKY57* and *PaNAC29* directly bind to *PaDFR* and *PaLDOX*, thereby disrupting the flavonoid biosynthesis, while *PaNAC29* was also capable of binding to *PaPRE6* and *PaLOX5* promoters, impacting abscisic acid and volatile compound biosynthesis. This work provides a novel dataset on tissue-specific regulatory dynamics in sweet cherry, establishes a molecular framework for non-climacteric fruit ripening, and identifies key targets for enhancing cherry yield and quality.

Keywords: *Anthocyanin, fruit development, fruit ripening, pedicel, sweet cherry, transcription factors.*

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SESSION IV

Chemical Priming and Modern Practices for Increased Crop Yields in Challenging Environments

Chairs: ***Vassilis Fotopoulos & Jan Fila***

O29. The application of priming agents as a stress alleviation strategy to combat abiotic conditions in soft fruit crops

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Soft fruits comprise a diverse group of crops that belongs to different genus (Fragaria, Ribes, Rubus, Vaccinium) and thus are being characterized by different properties and requirements. The current presentation aims to shed light in the efficacy of priming agents to strawberry and raspberry plants grown both under conventional conditions and or after application of stress factors, namely salinity. Special reference will be given to strawberry cultivation and production that is currently affected by multiple challenges that threaten both yield efficiency and fruit quality parameters. The trial was implemented as a factorial scheme with three levels of salinity stress and application of five priming agents (100μM Melatonin, 0.1% w/v sodium alginate (NaA)/100μM melatonin, 2mM proline, 0.1% w/v sodium alginate (NaA)/2mM proline) with two controls (Untreated and Hydro-primed), in split-plot design with five replicates (five blocks). Considering raspberry plants, melatonin (Mel), glycine betaine (GB), NOSH, sodium slginate (SA), combination of Mel and SA, DMSO and Water (Control), were applied in 4 successive growth stages of cv. 'Vica Abril'. Overall, this study will report the main findings regarding the genes involved and/or governing the efficacy of priming agents in the alleviation of stress conditions as well as how salinity may affect the qualitative properties and phytochemical profile of strawberry fruits.

O30. Plant cell culture-based biostimulants alleviate salinity stress in tomato seeds

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The adverse climatic and soil conditions affect crop yield, especially to the germination process, the most critical phase of plant development. Seed priming has been established as a technique for providing seeds with stress tolerance and enabling their development under adverse conditions, especially when treated with biostimulants. Among all plant biostimulants, higher plant-derived biostimulants are of special interest, due to all the advantages they have, especially the diversity of bioactive compounds that compose them. We have developed bioproducts based on plant cell cultures enriched in bioactive compounds, using elicitation techniques. These bioproducts has been shown to have a biostimulant effect never described before in tomato seeds germinated under salt stress conditions. The application of low doses of these bioproducts partially reversed the alteration generated by salt stress in germination parameters, in addition to having an antioxidant and H_2O_2-scavenging effect thanks to its composition rich in phytosterols and polyphenols. Furthermore, it triggers a mechanism of action that promotes proline accumulation and stimulates the enzymatic activity of peroxidase and catalase to counteract the overproduction of reactive oxygen species. The treated seeds developed into seedlings that were less damaged by stress conditions and with a higher physiological quality index, which indicates that these bioproducts have a potential effect as biostimulants for tomato seeds against salt stress.

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O31. Fertilizer optimization using XAI

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Optimizing the nutrient supply from fertilizers, with a focus on nitrogen, in the field for different crops, including rapeseed and wheat, as well as the varieties used, is of paramount importance. This includes the development of a system for early and dynamic detection of deficiency and oversupply phenomena in plants. The basis for this system is newly identified features from image recordings and their correlation with molecular markers. The establishment of causal relationships between molecular biomarkers, environmental variables, and phenotypic imagery from drones and satellites, as well as the optimization of fertilizer use and management through local and rapid responses, is enabled by the learned ability to establish such relationships. Potential crop markers will be identified at the molecular level using next-generation sequencing (NGS) of the transcriptome and linked to phenotypic variables related to chlorophyll and nitrogen content. The development of a deep learning method based on Convolutional Neural Nets (CNNs) with eXplainable Artificial Intelligence (XAI) components is also underway. This method will allow the extraction of causal conclusions from image information regarding biomarkers and plant health, as well as the identification of the required fertilizer application. Another research focus is on optimizing methodologies for acquiring images using drones and the requisite cameras. This project's innovation lies in its identification of novel early detection features for determining the nitrogen content in plants based on molecular data using XModNN. A more sustainable use of resources has the potential to reduce the costs of over-fertilization by up to 30%. The molecular modifications in nutrient limitation that occur earlier than strong phenotypic changes, such as chlorophyll content, are detected using current measurement methods. Biomarkers could be of great benefit as a diagnostic tool in the field for automatic detection of needs (precision farming).

O32. Elucidating Pattern-Dependent Interactions, Immunomodulatory Roles, and Bioactivity Mechanisms of Chitooligosaccharides in Plants

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Sustainable agriculture requires significant reductions in the use of agro-chemicals, e.g. by partially replacing them using agro-biologics such as chitosans acting as biostimulants or biopesticides. Chitosans are linear co-polymers of N-acetylglucosamine (GlcNAc) and glucosamine (GlcN) residues, derived by partial deacetylation of the abundant biopolymer chitin. Chitosans differ in their degrees of polymerisation (DP) as well as in their fractions and patterns of acetylation (FA and PA, resp.). These structural parameters influence the functional properties of chitosans, including antimicrobial and phytostimulatory activities, but the influence of PA is least investigated owing to the difficulties in producing and characterising chitosans with well-defined PA. Here, we have performed enzymatic hydrolysis using chitinases and chitosanase on a well-defined chitosan polymer (FA 0.2, DP 800, Đ 1.5). Depending on the cleavage preferences of the enzymes, the products are partially defined in their PA, namely having defined GlcN or GlcNAc residues at and/or near their reducing and non-reducing ends. When Nicotiana benthamiana plants were treated with these hydrolysates or oligomers with DP 4, 8, or 12 purified from them, they exhibited strikingly different elicitor-, priming-, and disease resistance-inducing activities against tobacco mosaic virus (TMV) infection. The partial PA-control derived from enzymatic hydrolysis, with excellent biological activities, is presumably suitable for the development of a reliable agro-biological agent to effectively combat both biotic and abiotic stresses.

O33. Enhancing chestnut plants' climate resilience: a stress priming approach

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Castanea sativa, one of the main forest crops in Portugal, faces increasing threats from emerging diseases and climate change. With heat waves and droughts becoming more frequent across the Mediterranean, developing sustainable, effective strategies to enhance chestnut plants' resilience is crucial. In this context, stress priming has emerged as a promising approach, as plants pre-exposed to mild stresses often display greater tolerance to future adverse conditions. However, this strategy remains largely unexplored in tree species. Thus, this study assessed the potential of drought-induced stress priming to improve chestnut plants' tolerance to water stress. For this, young plants underwent a mild drought period (three weeks at 35% field capacity [FC]), followed by two weeks of recovery under optimal conditions. Then, primed plants and non-primed controls were subjected to severe drought stress (25% FC) for four weeks. Results showed that neither stress priming nor drought significantly affected most biometric traits. However, primed plants exhibited a smaller reduction in leaf area under drought. Compared to their non-primed counterparts, drought-primed plants also displayed higher photochemical efficiency, lower non-photochemical quenching, and stable transpiration, stomatal conductance, and carbon assimilation, suggesting enhanced photosynthetic performance, even under stress conditions. Analysis of redox homeostasis supported these findings, as severe drought increased reactive oxygen species accumulation and lipid peroxidation in non-primed plants, while primed plants showed no signs of oxidative stress without majorly changing their antioxidant network.
Overall, this study highlights drought-induced stress priming as a promising strategy to enhance chestnut resilience, with ongoing research exploring its underlying proteomic mechanisms.

Keywords: *Castanea sativa; abiotic stress; mitigation strategies; drought*

P47. Enhancing Crop Growth and Yield with Algae-Based Biostimulants: A Sustainable Approach

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As agriculture faces increasing demands, algae-based biostimulants have been considered a sustainable way to enhance plant growth and productivity and to reduce fertilizer use. This study investigated the effects of different algae-based extracts on rice (*Oryza sativa* var. Caravela) and tomato (*Solanum lycopersicum* var. Heinz) across developmental stages: germination, early vegetative growth, and reproductive phase. During germination, 29 extracts were tested on rice and 36 on tomato, observing species-specific responses. The most effective extracts improved the Germination Index (GI) by up to 40% in rice (N13.2* at 0.1 g/L) and 32% in tomato (N32.1* at 0.1 g/L), with lower concentrations generally being more beneficial. Higher concentrations, in general, inhibited root growth, particularly in tomato. At the early vegetative stage, 30 algae species were assessed for their biostimulant effects in rice and tomato. Regarding rice, specific algae extracts* (NG6.1, NG7.1, N14.1, and N15.1, all in 3 g/L) significantly promoted shoot growth and biomass accumulation suggesting their potential for optimizing crop productivity. The biostimulant effect of these selected algae extracts was also assessed when applications were made during the reproductive stage of rice, a soil application of these extracts (3g/L) at three time points (one at booting and two during flowering) further illustrated the biostimulant potential of algae extracts. Among the tested extracts, NG7.1* showed the most promising effects, enhancing the number of panicles per plant, and improving grain fertility parameters, including total grain count and filled grains, when compared to the control and other extracts. Regarding tomato, selected algae extracts* (NG7.1, AP11.2, and AP10.2) applied at the early vegetative stage (3 g/L) significantly enhanced biomass accumulation, increasing dry weight by up to 22.4% and fresh weight by 24.1% evaluated under controlled conditions. These findings show the potential of plant treatments with algae-based biostimulants to enhance germination, early growth, and yield. Further research is needed to understand their mechanisms, refine application methods, and evaluate their effectiveness under stress conditions.

Keywords: *Biostimulants, Algae Extracts, Sustainable Agriculture*

*Due to confidentiality agreements, the algae species are not disclosed

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P48. Enhancement of bud dormancy release and development, flower and fruit quality of kiwifruit cv.'Hayward' induced by BUD 14 biostimulant

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The aim of this research was to investigate the influence of a foliar fertilization program, consisted of the BUD 14 nitrogen-calcium commercial formulation (N: 14% w/w, CaO: 5.5% w/w) as a biostimulant, on bud development percentage, flowering rate, classification of flowers into open, closed and triple, flower and pollen quality traits and fruit quality attributes of the 'Hayward' kiwifruit cultivar. The results showed that BUD 14 induced synchronization in bud development relative to different vegetative stages including initiation of bud expansion, appearance of leaf apices covered by hair and deployment of 2-8 leaves and increased the flowering rate of open flowers. Pedicel length, ovary fresh weight, and dry weight, dry matter and length in female flowers as well as maximum pollen grain diameter and area in polar view in male flowers were significantly enhanced in the BUD 14 treatment. Fruit quality characteristics like average weight and dry mass were significantly augmented, and a 1.5-fold and 2-fold increase was recorded in canes length and number of kiwifruits per cane. The efficacy of BUD 14 as a more target-oriented and environmentally friendly alternative method of supplying plants with smaller and controlled amounts of nutrients for breaking bud dormancy and improving their development was demonstrated, enhancing flower and fruit quality, leaf nutrition, kiwifruit developmental characteristics, and finally the total production per fruit per tree.

P49. Effect of biostimulant application on yield and quality of tomato subjected to drought stress: a field study

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The application of biostimulants in agriculture has gained significant attention due to their potential to enhance crop productivity in a sustainable manner, particularly under both optimal and challenging environment. This study aimed to evaluate the effect of biostimulants on the yield and quality of tomatoes grown under water-scarce conditions. The experiment was conducted during the spring-summer season of 2024 in an open field at the Maritsa Vegetable Crops Research Institute (MVCRI), Bulgaria (42.177296, 24.760820). The tomato variety used was Prometey (MVCRI), characterized by a determinate growth habit and oval, red-colored fruits weighing 60–65 g. To simulate drought stress, water supply was reduced by 50% in half of the experimental plot throughout the growing season, while the other half received full irrigation, ensuring a controlled comparison. Two biostimulant treatments were tested: one with the bacterium *Priestia megaterium* alone and another combining *P. megaterium* with organic matter-rich products. The applied water deficit led to a 20–40% decrease in total yield, fruit weight, and the number of fruits per plant in all treatments. The biostimulants increased yield by up to 25% under optimal irrigation and 30% under water-deficit conditions. However, none of the treatments in drought stress were effective enough to match the yield of untreated plants grown under optimal conditions. Notably, the biostimulant treatments, especially under drought stress, enhanced specific quality traits, with the bacterium alone improving biochemical stability (TSS, Vitamin C), while the combination with organic matter-rich products enhanced fruit firmness.

P50. The effect of biochar in relation to genotype on the cellular development, anatomy, and morphology of seedlings in traditional tomato varieties

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Biochar's diverse properties improve soil health, boost microbial activity, enhance resource utilization, and optimize plant growth. This greenhouse study investigated the effects of a 3% biochar soil amendment on the growth of both traditional and hybrid tomato varieties. Results showed that plants grown with 3% biochar exhibited smaller root surface area, an indication of nutrient and water optimal conditions. Biochar significantly increased stem diameter in both traditional and hybrid tomato plants. Histochemical analysis revealed that biochar application subtly increased pith lignification, potentially strengthening stems and improving stress resistance. Furthermore, biochar promoted larger vessel sizes within the stem's vascular bundles. Notably, the study also identified symbiotic relationships with rhizobacteria in roots of plants grown with biochar. These findings support the use of biochar as a viable strategy for improving tomato plant development and contribute to the implementation of more sustainable agricultural practices.

P51. Impact of water stress and humic acid on early development of eggplant under greenhouse conditions

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The Mediterranean region is among the climate crisis hot spots already facing adverse conditions including drought, especially during the warmer months when several vegetables are produced. Soil amendments such as humic acids are important tools to enhance plant responses versus environmental stressors. Our objective was to assess the impact of Leonardite (humic acid) as soil amendment to ameliorate the effects of water deficiency upon transplantation of eggplant (*Solanum melongena* L.) in a greenhouse. Seedlings were transplanted in pots filled with soil. In half pots Leonardite (Humita 40) was also included, while the other half was the control treatment. Moreover, each half of the pots was irrigated with different water levels; at 100% or 60% water capacity. After 3 weeks (when blooming started), above-ground morphological parameters such as height and leaf number were significantly affected by water levels, with 100% water leading to greater values, irrespective of Leonardite presence. This was also observed for root dry weight; however root length was positively affected by the incorporation of Leonardite. Relative chlorophyll content was negatively affected by reduced water levels. On the other hand, chlorophyll fluorescence parameter PIabs (performance index of PSII) and PItot (total performance index) showed significantly greater values when treated with 60% water, and especially with absence of Leonardite. It was concluded that low water levels at the early stage of eggplant growth imposed a mild stress which positively affected root growth and photosynthetic performance, while Leonardite possibly stimulated resource allocation towards the root system versus the photosynthetic machinery.

Keywords: *Solanum melongena; humic acid; Leonardite; water stress; photosynthetic mechanism*

P52. Efficiency of freeze-drying method for the production of plant-based foods

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Dehydration is a widespread method to produce plant-based foods with a long shelf life. Conventional thermal-drying is the most widespread method of dehydration. The purpose of this study was to investigate the efficiency of freeze-drying method in dehydration of eggplant (EG), bell pepper (PE), processed olive (OL), *Stevia rebaudiana* (ST) and sea fennel (CR). Fresh tissues were freeze-dried for 48h and the dehydration efficiency was assessed by the measurements of moisture and water activity (aw). Freeze-dried samples were compared with both conventionally thermal-dried and fresh (non-dried) samples. In all products, both freeze- and thermal-dried samples showed significantly lower moisture and aw than the fresh samples. For EG, OL and ST the freeze-dried samples had similar moisture with the thermal-dried ones. For PE and CR the freeze-dried tissues had higher moisture content than the thermal-dried one. All freeze-dried products had statistically lower aw values than the thermal-dried ones. The aw values of the freeze-dried products were recorded below the upper limit of aw related with food safety (aw < 0.4) and were 0.35, 0.33, 0.36, 0.36 and 0.37 in EG, PE, OL, ST and CR, respectively. In conclusion, freeze-drying could be proposed as a promising novel method for plant-food dehydration, and aw as the most suitable index for the assessment of dehydration rate.

Key words: *eggplant; bell pepper; processed olive; Stevia rebaudiana; sea fennel; water activity*

P53. Effects of post-harvest ozone (O_3) treatments on the quality of dried figs

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The conventional dried fig production chain includes the use of phosphine for disinfestation and sulfides for fruit bleaching. The aim of the present work was the assessment of the use of ozone (O_3) in the post-harvest handling chain of dried figs for the substitution of the regularly used chemicals (phosphine and sulfides). The O_3 treated fruits (O_3-fruits) produced by the following protocol: (i) fumigation during drying with 10ppm O_3 for 24 h, (ii) fumigation of dried figs with 30ppm O_3 for 6 h and (iii) washing of dried figs for 1 min with ozonized water (30 ppm). Fruits from three different farms were included in the experiments and the O_3-fruits were compared with the conventional one (Conv-fruits). The examined attributes of the fruits were moisture (% w/w), water activity, peel and flesh color (L*, a*, b*) and infestation (percentage of infested fruits). O_3-fruits and Conv-fruits had statistically similar moisture (18.4-19.6% w/w), aw (0.480-0.486), peel L*(58.1-58.4), peel a*(8.01-8.22), flesh L*(41.5-42.4), flesh b* (8.3-9.3) and infestation levels (3.3-4.7%). O_3-fruits showed higher peel b* and a* than Conv-fruits by 1.2-folds and 1.1-folds, respectively. In conclusion, O_3 showed similar efficacy and improved color quality in comparison with conventional chemical treatments.

***Key words**: Ficus carica L.; disinfestation; water activity; color*

P54. Effects of pre-harvest sprays with mannitol on the oil content and ripening of olives

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Harvest timing of oil olives is important for oil quality and yield. The maturation and ripening of olive fruits extends over a long period where the oil accumulation occurs. Olive oil is the end product from a metabolic pathway where oligosaccharides and polyols are involved and considered as precursors. The aim of the present work was the study of the effect of the exogenous application of mannitol on the acceleration of ripening and the increase in oil content of olive fruits. Mannitol was applied once by foliar sprays at two concentrations (50 and 100 mg/L), in two different stages during ripening [September (BBCH80) and October (BBCH81)] and in two olive varieties ('Koroneiki' and 'Dopia Zakynthos'). The efficiency of mannitol sprays was assessed by the measurements of oil content (Soxhlet method) and Maturity Index (MI) of fruits. Olive trees were in an off-year (low tree load) and fruit were harvested in November (46-47 or 26-27 days after treatments depended on variety). Mannitol treatments lead to an increase in oil content in both varieties (by 7.5-13% in 'Koroneiki' and by 7.5-15% in 'Dopia Zakynthos'). Fruit ripening was accelerated by the foliar sprays showing higher MI than control. The 'Dopia Zakynthos' appeared to respond better than 'Koroneiki' to mannitol treatments. In conclusion mannitol at 50mg/L applied at BBCH 80 or 81 could be suggested as an effective treatment in years with low tree load and normal climatic conditions.

Keywords: *olives; olive oil; ripening; maturity index*

P55. Profiling PGPR-derived volatile organic compounds: Implications for plant growth promotion and biological activities

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Recent research has focused on "Plant Growth Promoting Rhizobacteria" (PGPR) due to their multiple functions as biological agents against fungi and other pests, as plant growth promoters, and abiotic stress alleviators. Among their beneficial traits, synthesis of low molecular weight volatile organic compounds (VOCs) which are easily diffused through air and soil, are of great importance. Bacterial VOCs interfere in multiple interactions among co-existing organisms, and play crucial roles in different processes, such as quorum sensing, induction of plant defenses, and growth promotion. The present study aimed to identify VOCs emitted by PGPR strains and to evaluate their impact on plant growth. Headspace GC-MS analysis of five bacterial strains performed by ITEX dynamic sampling, identified a total of 55 compounds belonging to different chemical classes, i.e. alcohols, ketones, esters, aldehydes, and sulfur-containing compounds. Individual volatiles, such as dimethyl disulfide, 1-butanol, 3-methyl-, 1-hexanol, 2-ethyl-, and 2-heptanone which have antifungal and/or growth promotion properties were detected. *In vitro* bioassays in which *Arabidopsis thaliana* was used as model plant, demonstrated that VOCs released by *Chryseobacterium aquifrigidense* strain SAESo14 significantly enhanced total plant biomass. These findings indicate the possible influence of PGPR-derived VOCs on plant growth. More research is needed to evaluate the mode of action of these VOCs, as well as their potential use as an eco-friendly alternative to agrochemicals.

P56. From Stress to Success: Enhancing Potato Plant Resilience to Short-Term Freezing Stress Through Melatonin Priming

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Chemical priming, using agents like melatonin and polyamines, has shown great potential in enhancing plant resilience to various abiotic stresses, including freezing, by improving overall adaptability to unfavorable conditions. During the early stages of potato plant growth, this approach may serve as an affordable and effective strategy not only to mitigate frost damage but also to optimize plant recovery. In this study, we aimed to explore the potential role of Melatonin (MEL) priming in enhancing the resistance of potato plants under short-term freezing conditions (-2°C) using an integrated proteo-transcriptomics approach. MEL (100 μM) was applied at 4-week potato plants by spraying, while 24 hours after, MEL-treated and control plants were transferred to freezing conditions for 2, 4 and 6 hours. Leaf samples were collected from four individual plants for transcriptomic and proteomic analysis. Results highlighted that MEL, as a pleiotropic signaling molecule, was able to minimize the negative effect of freezing temperatures on plant survival and development. Further analysis demonstrated that transcripts related to glycolysis, photosynthesis and phenylpropanoid pathways were significantly enriched in MEL-treated samples. Meanwhile, MEL accumulated during pretreatment could exert direct antioxidative effects through the activation of ROS scavenging activity. Notably, among the top accumulated proteins in MEL-treated plants were those related to fatty acid and carbon metabolism, as well as signal transduction. These findings clearly demonstrate that the exogenous application of MEL is an effective strategy for mitigating short-term freezing damage in potato plants, via boosting energy reservoirs, reinforcing structural integrity, and enhancing biosynthesis of secondary metabolites.

SESSION V

Breeding for Climate Resilience Crops

Chairs: ***Aleksandra Radanovic & Ifigeneia Mellidou***

Avraham A. Levy

New breeding technologies and applications for vertical farming in tomato

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New breeding technologies have enabled the precise modification of plant genomes. We describe here advances in genome editing in tomato and how these can be harnessed for developing new, climate-proof crop production methods. Most CRISPR-based genome editing methods start with the induction of a targeted DNA double-strand break (DSB) or nick in the genome. We have studied the fate of DSBs and found that in the majority of the cases, they can be precisely repaired, leaving no footprint, or that mutations can be induced at the DSB as a result of error-prone repair. We showed that DSBs can become hotspots for homologous DNA recombination, using an extrachromosomal donor or a homologous chromosome as a repair template, leading to gene targeting, targeted crossover, or gene conversion. We also found that, in rare instances, unrepaired DSBs can trigger large-scale chromosomal rearrangements. We have harnessed genome editing to develop tomato varieties adapted to growth under a controlled environment in vertical farms. Targeted mutations in genes determining plant size and floral induction was done using CRISPR/Cas9, leading to rapidly cycling miniature plants. We also selected plants that can grow under low light, and identified candidate genes, using GWAS and transcriptomics, that will be tested for growth under low light intensity. Ultimately, we aim to produce several generations of miniature tomato plants throughout the year, regardless of the external climate that can grow under artificial low-light LEDs, for energy saving, in several "floors" of trays.

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O34. The Potential Contribution of Orphan Crops to a More Climate-Resilient Agriculture

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Many orphan crops are characterized by unique properties, e.g., extreme stress tolerance or high nutritional value, such that they are considered crops of the future. Orphan crops could thus play a major role in contributing to sustainable food systems in light of a changing climate and in ensuring global food security. Orphan crops are typically grown Africa, Asia, and South America and play an important role in local diets, but most of them are used only locally and are not traded internationally. Consequently, they receive little attention in research, breeding, or improvement of agricultural practices to produce them. The legume *Tylosema fassoglense*, one of five species loosely known by the common name marama bean, is native to Africa. Marama bean has never been cultivated but it has traditionally been gathered by the Khoisan and Bantu peoples of Southern and Eastern Africa. *T. fassoglense* grows in deserts, open grasslands and woodlands, and can survive long drought periods and sweltering heat. The high protein and mineral content of marama bean rivals that of soybean, which, together with its drought tolerance, make it a very attractive orphan crop for potential cultivation. Despite the tremendous potential of *T. fassoglense* for domestication, there is a general lack of knowledge about this species. We will report on our progress in establishing genomic resources for marama bean and in the characterization of key characters central to its domestication and cultivation, such as traits related to reproduction and germination.

O35. Exploring orphan crops and crop wild relatives to improve agricultural resilience to climate change

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Climate change is expected to bring rising temperatures and reduced rainfall, imposing multiple stresses on agricultural systems. Expanding food sources by incorporating underutilized crops, such as pseudocereals, could help mitigate these challenges while improving food security. Among them, buckwheat (*Fagopyrum esculentum* and *Fagopyrum tataricum*) and amaranth (*Amaranthus cruentus*) are gaining attention for their nutritional and medicinal benefits. However, the physiological mechanisms underlying their resistance to heat, drought, and salinity remain largely unexplored. Another promising approach to enhancing crop resilience involves leveraging wild relatives as sources of stress-resistant genes. Within the tomato clade, *Solanum chilense* stands out due to its high genetic variability and remarkable tolerance to extreme environments. To assess its potential in tomato breeding, we compared the resistance of cultivated tomato (*Solanum lycopersicum*) and *S. chilense* to heat, drought, and salinity stress while also investigating reproductive barriers to facilitate hybridization. These strategies, combining orphan crops and wild genetic resources, offer promising avenues for developing climate-resilient agriculture.

O36. Influence of light and increased temperatures on the photosynthetic parameters and grain yield of winter wheat varieties over two growing seasons

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The length and intensity of light and high temperatures influence wheat growth and development, leaf photosynthesis and ultimately grain yield. This study investigated the influence of weather conditions on chlorophyll *a* fluorescence parameters and grain yield of six winter wheat varieties over two growing seasons (2021/2022 and 2022/2023). In the first growing season, average temperatures were higher than the thirty-year average, especially in February during tillering stage when measured maximum quantum yield of primary photochemistry (TR_O/ABS) was significantly positively correlated with final grain yield. Further, only the highest yielding variety remained TR_O/ABS and performance index on absorption basis (PI_{ABS}) at the same significant level between tillering and stem elongation stage. In the second growing season, average temperatures were higher from thirty-year average, especially during December and January, and higher rainfall during April and May favoured faster wheat development and increased virus and disease infections. That was also resulting in shorter daylight period, which had a negative effect on the photosynthesis and decrease in grain yield up to 68%, compared to first growing season. The highest-yielding varieties showed the lowest increase in TR_O/ABS between tillering and stem elongation stage thus showing prolonged photosynthetic activity.

O37. Advancing Tomato Breeding for Heat Tolerance: Development of Resilient Hybrids for Climate Change Adaptation

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The increase in temperatures due to current climate change dramatically affects crop cultivation, resulting in yield losses and altered fruit quality. Tomato is one of the most important horticultural products, and although it can withstand a wide range of climatic conditions, heat stress can affect plant growth and development especially at the reproductive stage. In this context, the constitution of superior hybrids showing heterotic effect is one common strategy adopted in plant breeding to face the stress. In the present work, a breeding program was developed aimed at obtaining new heat tolerant hybrids by using one selected thermotolerant and high-fruit quality genotype (E42) as parental line, which was crossed with three thermotolerant indeterminate genotypes. Field evaluations conducted under high temperatures during two years in two different environments allowed us to select one hybrid showing superior quality and productivity. Moreover, it displayed the best compromise between stability among the environments and performances of the plant according to the Multi-Trait Selection Index, also presenting heterosis respect to the parental lines for most of the assessed traits. Genomic analyses evidenced a high distance of the parental lines (between 40.4% and 67.8%) and allowed us to highlight heterozygous regions and genes with impactful variants on the hybrids; some of these also colocalize with QTLs related to the number of fruits and total soluble solid content. Our results showed how adequate combinations of parental lines can drive phenomena like heterosis and heat tolerance, enhancing the constitution of resilient hybrids that can cope with environmental challenges.

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O38. Evaluation of bioenergy and forage production potential of sweet sorghum (*Sorghum bicolor* L. Moench) inbred lines in a semi-arid Mediterranean environment

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Sweet sorghum possesses considerable potential as a bioenergy or forage crop thanks to its wide adaptability to marginal lands, low input requirement, high drought tolerance, yield potential, resource use efficiency, and sugar accumulation, which can easily be converted to bioethanol and biogas via fermentation. Sweet sorghum is also known as the sugarcane of desert or camel crop. It can be easily ensiled due to its high sugar content and low buffering capacity. Additionally, it can produce a higher biomass yield than maize with a lower nitrogen and water supply. All these desirable agronomic and biochemical characteristics make it one of the most logical options in the Mediterranean, where water scarcity is the major challenge to achieving the sustainable bioenergy and forage production. In this study we aimed to evaluate bioenergy and forage production potential of 12 sweet sorghum inbred lines selected among 300 accessions by comparing them with 7 improved sweet sorghum and 5 forage sorghum varieties for biomass, theoretical ethanol, and methane yields, and the contents of brix, cell wall components, crude fat, and ash. Our future goal is to improve the new varieties with high adaptability to deteriorating climate conditions for the sustainable forage and bioenergy production.

O39. Evaluation of Resistance Genes in Wheat Cultivars Against Major Pathogens Under Abiotic Stress Conditions

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Abiotic stress caused by high temperatures and variable weather conditions is becoming more frequent in crop production. A change in weather conditions favors the development of certain pathogens, so biotic stress also occurs as a consequence of abiotic stress. Numerous environmental events such as relative humidity, temperature, CO_2 concentration, wind and a combination of these factors, affect epidemics of wheat pathogens.
Various *Yr*, *Pm* and *Stb* resistance genes in wheat germplasm were identified so far, such as *Yr5, Yr10, Yr15, Yr24/Yr26, Yr32, YrSp* for yellow rust and *Pm1a, Pm2, Pm3/Pm8/Pm17, Pm5e, Pm21/Pm12, Pm24, Pm33, Pm41, Pm51, Pm60, Pm64, Pm69, MlZec1* and *MlAB10* against *B. graminis*. For resistance to Septoria tritici blotch, 23 genes were confirmed: from *Stb1* to *Stb20, StbSm3*, *StbWW*, and *TmStb1*. The resistance potential evaluation of 60 bread wheat cultivars against *Puccinia striiformis* f. sp. *tritici* (yellow rust), *Blumeria graminis* f. sp. *tritici* (powdery mildew,) and *Zymoseptoria tritici* (septoria tritici blotch) was carried out at Sivas University of Science and Technology. Screenig of *Yr15* and *Yr5* gene is amplified in majority of samples with frequency of 98.55% and 95%, respectively. *Pm24, Pm38* and *Pm60* genes was present in diferent ratio, videlicet 19, 5, and 44 out of 60 wheat genotypes carried a desirable gene. Only *Pm61* was not detected in any of the wheat samples. Regarding *Z. tritici* resistance, we screened 15 *Stb* genes (*Stb1, Stb2, Stb3, Stb4, Stb5, Stb6, Stb7, Stb8, Stb9, Stb10, Stb11, Stb12, Stb13*, and *Stb15*) and confirm presents of all genes in tested material.

O40. Metabolomic profiling provides insights into pistil adaptation to heat stress in durum wheat lines carrying segmental introgressions from the wild grass *Thinopyrum ponticum*

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The foreseen progressive temperature increases are expected to harm the mainly rain-fed durum wheat (DW) production in the Mediterranean Basin. Heat stress particularly affects the reproductive potential, for which modern DW needs novel genetic variability conferring efficient stress-responsive mechanisms. Chromosome introgressions from the wheat wild relative *Thinopyrum ponticum* into DW showed to increase yield-contributing traits, including grain number, under heat stress (HS). Hence, to identify differentially modulated metabolites, a metabolomic profile was developed of female reproductive organs (pistils) sampled from plants subjected to HS at anthesis. Three DW-*Th. ponticum* introgression lines, having different amounts of alien chromatin transferred on DW chromosome arm 7AL, were used. Their pistils, expected to be directly involved in seed set and development and ultimately grain yield, were collected from spikes of stressed (3-day heat-shock) and control plants and analysed by an untargeted metabolomics approach via UHPLC/MS and successive statistical and pathway analyses. The results showed the presence of defined portions of *Th. ponticum* introgressions to enhance DW heat tolerance through the maintenance of sugar and energy production (Calvin and TCA cycle), thus contributing to the observed higher spike fertility. Highly accumulated biomarkers under the stress in pistils, such as allantoin, UDP, glutathione and ascorbic acid, were identified as indicators of higher heat tolerance in the introgression lines and a heat-tolerant control. The present study confirmed wheat wild relatives as a valid source of useful genes for crop improvement and validated metabolomic phenotyping as a powerful tool to integrate in breeding for climate- resilient crops.

O41. Molecular Pathways to Resilience: A Dive into Sunflower Drought Tolerance

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Drought poses a significant challenge to crop production, even for sunflower, which is often regarded as a moderately drought-tolerant crop. Under severe drought conditions, sunflower yield losses can reach up to 60%, with the germination and reproduction phases being particularly vulnerable to such stressful conditions. Through testing *in vitro* conditions and rhizotrones, we have identified drought tolerant and sensitive sunflower genotypes which are a part of Institute of Field and Vegetable Crops breeding program. The most drought tolerant and sensitive genotypes were grown in pots and, at the beginning of bud phase, drought stress was introduced to a subset of plants by withholding water. Morphological and physiological parameters, such as plant height, leaf area, stomatal conductance etc., were monitored throughout the experiment, and leaf samples were collected for transcriptome analysis at the end of the experiment. Key molecular pathways linked to drought tolerance in sunflower were revealed as well as specific genes with potential applications in marker-assisted breeding programs aimed at improving sunflower drought resilience.

Keywords: *Helianthus annuus L., abiotic stress, transcriptomics*

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O42. Image Analysis-Driven High-Throughput Phenotyping and Genome-Wide Association Studies for Mapping some Agronomic Traits in Buckwheat

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Despite its exceptional nutritional profile and adaptability to marginal environments, buckwheat (*Fagopyrum* spp.) remains an underutilized crop, receiving limited attention in global agriculture. As an orphan crop, it lacks extensive breeding programs, policy support, and commercial investments, constraining its yield potential and food security and functional foods under climate change. This study utilizes high-throughput phenotyping and genome-wide association studies (GWAS) to explore agronomic traits in 94 buckwheat accessions grown in a greenhouse taken through image analysis. Spatial and temporal data were collected using a multispectral camera every five days, starting one week after seedling emergence. At 50 days post-emergence, 3D plant data were recorded using a Microsoft Azure Kinect Development Kit, enabling phenotyping of traits such as leaf count, branch count, flower count, plant height, number of branches per plant, and spatial distribution. At 90 days post-emergence, mature seeds were harvested for RGB image analysis of seed morphology. Vegetation indices derived from multispectral and RGB data provided insights into plant health, biomass, chlorophyll content, and phenological transitions. High-resolution seed imagery enabled precise phenotyping of seed count, size, pigmentation, and shape. A total of 120,000 SNP markers revealed substantial genetic variability in buckwheat accessions from Korea. Population structure analysis categorized accessions into distinct clusters. Whole genome DArTseq-generated SNP markers are being used for GWAS analysis for various agronomic and morphological traits derived from image analysis, offering new insights into genome mapping, thus enhancing the understanding of buckwheat growth and development through Precise genomics.

Keywords: *Image analysis, high throughput phenotyping, machine learning, precision agriculture, climate change*

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O43. Approaches in modern corn breeding with reducing anthesis-silking interval (ASI) and ClimaPannonia presentation

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The main goal for developing novel organisms is to follow growth of human population. However, selection pressures in different breeding programs might lead to a decrease in genetic variety. Breeding objectives can be divided into groups according to where they come from, with those from the business sector and those from research institutions being distinguished. Effectively evaluating genotypes' reactions to a shorter anthesis-silking interval (ASI) and making the most of available resources to create more productive hybrids are two crucial tactics in this sector. Results from an experiment carried out in partnership with KWS, a commercial seed house, at nine sites across four countries—Romania, Hungary, Croatia, and Serbia—will be presented. During the ASI period, maize hybrids were subjected to abiotic stressors such as drought and high heat in eight of environments. Second part of the presentation will be dedicated to the ClimaPannonia - Building climate resilience via large scale uptake of systemic solutions in agricultural ecosystems in the Pannonian region. Horizon Europe project which will start at the 1st of March 2025 and will offer many solutions to the climate changes in Pannonia region through four agricultural sectors: (*i*) agroforestry, (*ii*) water-food nexus, (*iii*) crop and (*iv*) catle production.

Keywords: *ASI, breeding, corn, ClimaPannonia*

P57. Hormone-mediated regulation of stress responses during wheat reproductive stage

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Agricultural production is particularly vulnerable to climate change especially as a result of rapid global warming. Changes in weather patterns often result in decreased wheat grain yields. During reproductive phase of wheat, drought can delay or completely inhibit flowering resulting in reduced grain number and yield. Further, climate change alters plant physiology and immune response to pathogens. Phytohormones have specific roles in helping the plants to cope with stressful conditions. Abscisic acid (ABA) is a phytohormone that participates in tolerance mechanism to drought by regulating stomatal aperture, plant transpiration, and water absorption by roots but on the other hand in biotic stress has a role in signalling as it is known that can promote the growth of pathogens. Salicylic acid (SA) is functioning as an antioxidant by decreasing oxidative stress during stress. The objectives of this study were to compare the performances of wheat varieties in reproductive stage under drought and Fusarium head blight (FHB) in terms of ABA and SA concentrations. Drought caused significant changes of most important yield-related traits that were significantly reduced in drought susceptible varieties, but ABA and SA from flag leaves were not significantly changed during reproductive phase. During pathogen attack in reproductive phase, it was shown that increased ABA concentration from spikes participated in increased FHB susceptibility in wheat. Future work to more specifically define the role of ABA and SA during stress will require functional tests with tissue specific localisation of different metabolites in a time-course manner at several measurement points.

P58. Assessment of Wild Tomato Relatives (*Solanum pennellii*) for Enhancing Yield and Fruit Quality Under Low-Input and Saline Cultivation Conditions

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Salinity is a critical abiotic stressor that significantly limits tomato (*Solanum lycopersicum* L.) productivity, particularly in arid and semi-arid regions. Leveraging the genetic diversity of wild relatives such as *Solanum pennellii,* through the use of introgression lines (ILs), represents a promising strategy to improve salt tolerance. In this study, the performance of eight ILs and a commercial hybrid (Formula F1) was evaluated under three soil salinity levels (1.88, 6.44, and 8.63 mS/cm), with the aim of identifying salt-tolerant genotypes capable of sustaining yield and fruit

P59. **Development of Molecular Tools for Breeding Purposes – Identification of SNPs Associated with Tomato Tolerance to Broomrape**

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Tomato (*Solanum lycopersicum*) is one of the most important cultivated plant species in terms of both economic value and nutritional significance. Despite considerable scientific research, the investigation into the parasitism of broomrape (*Orobanche spp.* & *Phelipanche spp.*) and the genetic mechanisms underlying resistance to it remains limited. In the present study, nine tomato introgression lines (*Solanum lycopersicum* × *Solanum pennellii*) and one commercial hybrid (*Solanum lycopersicum*) were evaluated to investigate the presence of single nucleotide polymorphisms (SNPs) in the coding region of three genes previously identified as significantly differentially expressed during early broomrape parasitism, indicating a potential role in resistance. Whole genome sequencing (WGS) was conducted on a commercial hybrid and an introgression line—previously identified as susceptible and tolerant, respectively, based on early-stage broomrape interaction studies—uncovering polymorphisms in the target genes between the two genotypes. Specific primers were designed in the exon regions of these genes, and the presence of SNPs was assessed using High Resolution Melting (HRM) analysis testing ten genotypes in total. The results demonstrated that whole genome sequencing enables the identification of SNP polymorphisms among different tomato genotypes, which may influence gene expression and regulation, potentially affecting plant tolerance to broomrape parasitism. The exploitation of such polymorphisms could lead to the development of molecular and functional markers, allowing for the rapid and reliable selection of broomrape-resistant tomato genotypes. This would facilitate the implementation of targeted breeding programs aimed at improving broomrape tolerance.

P60. Assessing Commercial Tomato Hybrids in Low-Input Systems: Agronomic Performance and Nutritional Evaluation Toward Climate-Resilient Farming

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Commercial tomato hybrids are known for their high productivity in intensive, high-input agricultural systems, yet their adaptability to low-input and organic farming remains understudied. With the European Green Deal aiming to transition 25% of EU agricultural land to organic production by 2030, this study evaluates whether commercially available greek tomato (*Solanum lycopersicum* L.) hybrids can perform effectively under low-input conditions. Furthermore, the potential of plant growth-promoting rhizobacteria (PGPR) to enhance crop performance in such systems is examined. A tailored microbial consortium—containing Azotobacter chroococcum, Clostridium pasteurianum, Lactobacillus plantarum, Bacillus subtilis, and Acetobacter diazotrophicus—was applied as a root inoculant to stimulate plant growth. Seven tomato hybrids popular in the Greek market were tested across three cultivation systems: (1) high-input hydroponics, (2) low-input farming without PGPR, and (3) low-input farming with PGPR supplementation. Key metrics, including yield, fruit quality, nutritional content, morphological characteristics, and leaf mineral composition, were analyzed. A techno-economic assessment was also performed to determine the financial feasibility of utilizing high-input-bred hybrids in low-input systems. The findings indicate that existing commercial hybrids are poorly adapted to low-input cultivation, exhibiting suboptimal agronomic efficiency and economic returns. This underscores the necessity for dedicated breeding initiatives to develop tomato varieties tailored for sustainable, resource-efficient agriculture. Notably, this research provides a holistic evaluations of commercial hybrid performance under low-input regimes, filling a significant gap in current agronomic knowledge.

P61. Evaluation of Local Tomato Varieties for Yield and Fruit Quality under Elevated Soil Salinity Conditions

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Tomato (*Solanum lycopersicum*) is one of the most widely consumed horticultural crops worldwide. Introduced to Europe in the 16th century, the species rapidly diversified, giving rise to numerous local varieties through farmer-led selection and adaptation to distinct microclimates, soil types, and culinary traditions. These local tomato landraces constitute a valuable genetic reservoir, offering traits related to sensory quality, resilience to environmental stresses, and adaptation to low-input farming systems. However, in recent decades, the expansion of intensive agriculture and the dominance of commercial hybrids have led to a dramatic erosion of this genetic diversity, placing many traditional varieties at risk of extinction. Preserving and re-evaluating these landraces is essential not only for biodiversity conservation but also for the development of resilient cropping systems in the face of climate change. Soil salinity, in particular, is becoming a major constraint for tomato cultivation in many Mediterranean areas. Identifying and promoting local varieties that can maintain productivity and fruit quality under saline conditions can contribute significantly to sustainable agriculture and food security. The present study was conducted at the experimental farm of the Alexandria Campus in 2023. A total of 35 traditional tomato landraces for fresh consumption were cultivated in a randomized complete block design (3 replications with 10 plants per replication), inside an unheated greenhouse under low-input cultivation practices, in saline soil conditions (10.39 mS/cm). The varieties were evaluated and described based on morphological traits (plant, flower, and fruit characteristics), yield parameters (early and total yield), and fruit quality attributes. Genetic materials exhibiting superior performance can be further integrated into breeding programs aimed at developing Greek salt-tolerant cultivars or hybrids with excellent fruit quality.

P62. Assessment of Traditional Pepper Varieties for Yield Performance and Nutritional Quality under Low-Input Conditions

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Traditional pepper (*Capsicum annuum* L.) germplasm constitutes a critical reservoir for the preservation of agrobiodiversity and the enhancement of sustainable agricultural systems. These landraces often exhibit superior adaptation to local edaphoclimatic conditions and express inherent resistance to biotic and abiotic stresses, attributes that are not always prevalent in commercial hybrids. Furthermore, traditional varieties are strongly associated with enhanced organoleptic properties and nutritional value, as their selection has historically aligned with the cultural and dietary preferences of specific agroecosystems. In the face of climate change and escalating resource constraints, the deployment of genotypes capable of maintaining agronomic and nutritional performance under low-input conditions is of paramount importance. The present study aimed to assess the agronomic behavior and postharvest fruit quality of traditional pepper varieties cultivated under low-input systems. The experiment was conducted in 2024 at the experimental facilities of the Hellenic Agricultural Organization – DIMITRA in Thermi, utilizing a RCBD (three replications of ten plants per accession) in an unheated, open greenhouse. A total of twenty-five traditional accessions were evaluated for morphological descriptors, yield components (early and total yield), and fruit biochemical attributes including nutritional and antioxidant profiles. Specific genotypes demonstrated superior performance either in productivity (e.g., 'Kampanoula') or in nutritional value parameters (e.g., 'Filuria', characterized by elevated phenolic content and ascorbic acid concentration at commercial maturity). These accessions represent promising candidates for direct utilization in low-input cultivation systems or as parental material in breeding programs targeting the development of resilient Greek cultivars or hybrids combining stress tolerance with high nutritional quality.

P63. Genotypic variation in tomato under drought and heat stress: implications for breeding

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Drought limits the growth and development of crops, causing significant yield losses that impact global food security. It can affect morphological, physiological, and biochemical processes in plants. As a non-destructive method, chlorophyll fluorescence can be used to study the effects of abiotic stress factors on plant growth and development. The aim of the present study is to determine the effects of drought and high temperatures on agronomic and physiological traits in 20 tomato accessions from the collection of the Maritsa Vegetable Crops Research Institute, including breeding lines, varieties, and local populations. The plants were grown under field conditions with two irrigation regimes – optimal and reduced. Drought stress was applied during the reproductive phase of tomato development by reducing the irrigation rate by 50%. Changes were observed in productivity-related traits and key parameters of chlorophyll fluorescence. Cherry-type genotypes demonstrated good tolerance to the applied stress, in contrast to large-fruited tomato varieties and populations. A genotype-specific differentiation in total chlorophyll content in the leaves of the tested accessions was identified.

P64. Evaluation of Drought Tolerance in Bulgarian Tomato Landraces

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Drought is a major abiotic stress factor that significantly reduces crop productivity by negatively impacting plant growth and development. It poses a serious threat to global food security, necessitating the implementation of various adaptation and mitigation strategies. One promising approach is the use of local germplasm to identify drought-tolerant genotypes for integration into tomato breeding programs. In this context, we investigated the effects of a 50% reduction in irrigation on the morphological and physiological parameters of tomato landraces to identify those with drought tolerance. The study evaluated five landraces and two controls under both optimal and reduced irrigation conditions. Key traits such as yield, yield-related attributes, color, firmness, and chlorophyll fluorescence were assessed. Results revealed significant variation in the drought responses of the landraces. Yield was the most severely affected trait, with reductions ranging from 7% to over 70% in susceptible accessions. Physiological parameters also exhibited considerable changes under water stress, with reductions in the Fv/Fm ratio (maximum quantum yield of PSII) and the Fv/Fo ratio (maximum primary yield of photochemistry) by 8.2% and 35.5%, respectively, after 14 days of drought exposure. Landrace 1352 showed a significantly lower reduction in yield and physiological parameters under drought conditions and could be utilized in breeding programs to develop new tomato lines combining drought resistance with high fruit quality.

P65. Agro-Biological Evaluation Of Local Small-Fruited Pepper Collections

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Conservation of genetic diversity of cultivated crops, grown for food is a priority worldwide and this is a part of the activity of the Institute of Plant Genetic Resources (IPGR) related to the implementation of the National Programme for Plant Genetic Resources (PGR). Expeditions for old local varieties in isolated parts of Bulgaria where the current trends to introduce modern cultivars have not been attained and where biodiversity is preserved were conducted. During the period 2018-2022, a survey of local small pepper accessions (C*apsicum annum subsp. microcarpum var. shipka*) of different geographical origin of Bulgaria were organized. As a standard Dzhulyunska shipka variety were used. Important biochemical parameters responsible for the quality and nutritional value of the fruit were evaluated. The study aims to establish the rate of genetic similarity and genetic remoteness of the specimens stored in the national gene bank.The classifications will increase the objectivity in the evaluation of accessions and the opportunity for application of different directions in the selection of small–fruited/pepper.

Keywords: *local accessions, small -fruited pepper, biochemical indicesgenetic similarity, genetic remoteness,*

P66. Genetic Characterization of Putative Cretan Chestnut Cultivars

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The Cretan chestnut belongs to the European or Sweet chestnut species and has long been intertwined with the traditional lifestyle holding significant economic value. However, the genetic resources of the Cretan chestnut have been understudied despite the threats posed by various activities. This study sets out to evaluate the Cretan Sweet chestnut. A total of 83 trees representing four of the most well-known local cultivars—Strovliani, Rogdiani, Koutsakera, and Katharokastania—were sampled from the Chania region. These samples were analyzed using 15 quantitative leaf morphological traits and seven simple sequence repeat (SSR) markers. The phylogenetic analysis showed high levels of heterozygosity for most of the markers and high diversity between the 83 samples. The cluster analysis for the total samples showed differentiation of three big clusters. The first cluster grouped Katharokastania and Strovliani. The second cluster contained Rogdiani and the third mostly Koutsokera samples. The morphological analysis of leaves indicated significant variability among the Cretan chestnut samples. The most distinguishing traits were identified using principal component analysis including leaf area, perimeter, height, width, circularity, aspect ratio and leaf roundness. The cluster analysis highlighted a clear clustering into three main groups: the first group of Rogdiani, is distinguished by smaller leaves. The second group, Strovliani, is characterized by higher values of leaf circularity. The third group, Koutsakera, has the largest leaf perimeter and area. This variability reflects the diverse adaptations of the local cultivars to their environment, offering valuable insights for further research and conservation efforts.