COST Action RECROP (CA22157)

Reports on the outcomes of Short-Term Scientific Missions, ITC and Dissemination Grants for the Period 11/2023 – 10/2024

Action number: CA22157

Grantee name: Olha LAKHNEKO

Details of the STSM

Title: Investigation of the impact of drought on photosynthesis-related parameters in bread wheat cultivars contrasting in their drought tolerance

Start and end date: 12/08/2024 to 19/08/2024

Description of the work carried out during the STSM

During my STMS, I was initially trained to perform measurements of photosynthesis-related parameters using the iFL portable photosynthesis system device. These involved evaluation gas exchange (CO_2 assimilation and transpiration rates), chlorophyll fluorescence, linear electron flow in chloroplasts, nonphotochemical chlorophyll fluorescence quenching, dependence of assimilation on CO_2 concentration inside the different leaf types—wheat on various developmental stages, corn, and soybean, in potted plants and on the experimental field. Applying newly acquired skills, we measured the following parameters in flag leaves of two contrasting to drought bread wheat cultivars (drought-tolerant Sofiia Kyivska and drought-sensitive Chyhyrynka) under moderate drought of the ongoing experiment at the host organization. We observed considerable differences in patterns of photosynthesis activity in two cultivars–cultivar Sofiia Kyivska had less downward photosynthesis and recovered effectively in comparison with cultivar Chyhyrynka. The measurements were repeated after recovery of regular watering. Leaf samples were collected and snap-frozen in liquid nitrogen until future proteomics experiments at home facility. The final report was drafted.

Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

This STMS involved two primary goals within 10 days. I assess that I succeeded in reaching both of them—mastering a new technique for measuring photosynthesis-related parameters with the specific portable tool and assessing the contrasting performance of two bread wheat cultivars under moderate drought and subsequent recovery. With the results obtained through this project, we contributed to

fulfilling the RECROP Research Coordination Objective 2, related to collecting data on the response and resilience of crop reproduction to drought as a major stress factor in the era of climate change. Moreover, this STSM promoted the sharing of expertise and facilitated effective partnerships between collaborative research facilities (Research Coordination Objective 5). The results obtained during the training will be included in a joint publication on the contrasting response of wheat cultivars to moderate drought at the reproductive stage, acknowledging the contribution of the mobility STSM grant from COST ACTION ´RECROP' (CA22157). As the experimental leaves were collected, we also agreed on a follow-up

research plan for assessing global and redox proteome dynamics in flag leaves of drought-tolerant cultivar Sofiia Kyivska and drought-sensitive cultivar Chyhyrynka under moderate drought at the flowering stage of ontogenesis and subsequent recovery. Additionally, we discussed the strategy of determining the impact of moderate drought at the flowering stage on the grain quality alteration of the future harvest of experimental samples.

Action number: CA22157

Grantee name: Stela Papa

Details of the STSM

Title: "Investigation of the impact of heat on the early development of wheat cultivars, based on transcriptomic and metabolomic analyses"

Start and end date: 29.04.2024 to 24.05.2024

Description of the work carried out during the STSM

The goal of this STSM was to investigate the sensitivity and tolerance of local wheat cultivars in Albania to high-temperature (HT) treatments, with the aim of understanding the genetic, molecular, and physiological basis of this sensitivity in seedlings of *Triticum aestivum* L. during their early development, and the implications for crop yield.

We investigated different wheat cultivars based on their agronomic performance, identifying those that display tolerance or sensitivity to extreme environmental abiotic conditions. The aim was to enhance resilience in a sustainable manner by pushing the limits of genetically inherited stress tolerance. To achieve this goal, we performed the following transcriptomic, metabolomic, and bioinformatic analyses:

- RNA extraction from plant material grown under stress conditions at the Department of Biotechnology, University of Tirana. RNA extraction was realised with S.N.A.P Total RNA Isolation Kit. Lot: 222387 by Invitrogen
- Plant metabolite extraction for primary metabolites analysis.

Leaves were harvested from the plants after treatment and then stored at −80 °C until extraction. Metabolite extraction was carried out according to Asami *et al., 2003*. Quality control (QC) samples were prepared by pipetting equal volumes of the samples in a designated GC-MS vial for analysis.

- Metabolomics data acquisition for primary metabolites:

Metabolomics data acquisition for primary metabolites was performed in GC-MS, PERKIN ELMER GC-MS. Sample preparation was realised according to Michailidis et al., 2017. The GC–MS analysis was carried with a Thermo Trace Ultra GC equipped with ISQ MS and TriPlus RSH autosampler (Switzerland). Peaks were identified according to the mass spectra of known standards or using the NIST 11 and GOLM databases when standards were not available. The detected metabolites were assessed based on the relative response compared to internal standard adonitol and expressed as relative abundance.

- Plant metabolite extraction for secondary metabolites analysis.

Samples of dried and ground solid residues samples (0.05 g) were extracted with 10 mL 70% methanol for 15 min at 30 ◦C using an ultrasonic bath (frequency 37 kHz, model FB 15051, Thermo Fisher Scientific Inc., Loughborough, England). The clear supernatants were mixed,

filtered through membrane filter with porosity of 0.45 µm, diluted with the standard solution of IS and either subjected directly to HPLC–MS analysis.

- Metabolomics data acquisition for secondary metabolites

Separation and detection of different secondary compounds present in the plant extracts was performed by a Shimadzu Nexera HPLC system (Kyoto, Japan), which consists of two LC-30AD pumps, DGU-20A5 degasser, CTO-20AC column oven, SIL-30AC auto injector, SPD-M40 diode array detector (DAD) and a triple quadrupole mass spectrometer (model LCMS-2020), which was operated with an electrospray ionization (ESI) interface according to Ainalidou et al., 2016. Samples were subjected to MS scanning for compound identification. The main phenolic compounds of samples were identified by comparing their retention time, UV profile and mass spectra of unknown peaks with those of authentic standards or with literature data. The majority of the phenolics detected were quantified using the calibration curves of corresponding standard solutions. When standards were unavailable, compounds with similar structure were used instead to perform quantification of the phenolic compounds.

- Statistical analysis for metabolomics

For physio-chemical data, the statistical analysis was conducted using SPSS (SPSS v21.0., Chicago, USA) by multivariate analysis of variance (MANOVA) statistically significant differences were detected based on post hoc method (Duncan's Multiple Range Test; $P \leq 0.05$) the number of means to be compared.
Meanwhile for secondary metabolites, the values refer to mean ± standard deviations of three parallel measurements. One-way analysis of variance (ANOVA) was used to test for differences among the means for different extracts, according to the Duncan's multiple range test. Possible correlations between different groups of phenolics as measured with LC-MS and spectrophotometric methods and antioxidant activity were evaluated by Pearson's and two-tailed significance coefficients.

Further statistical evaluation are still being carried out using PCA(Principal Component Analysis), a statistical analysis method and graphical Excel analysis Box and whisker and Heatmaps.

The resulting PCA reduced the dimensionality of the data to present summarised indices from the data matrix for better visualisation and interpretation. Box-and-whisker plots showing quantitative differences of metabolites per cultivar expressed as normalised concentration. Heat-maps on the other hand express the distribution of annotated metabolites among wheat cultivars. All the data obtained were introduced and compared previously in NIST and GOLM library metadata.

- Results obtained from metabolomics:

The application of HPLC-MS and GC-MS to evaluate primary and secondary metabolites in wheat cultivars allowed us to elucidate differential metabolite features and provided various methods to classify metabolite profiles. Both HPLC-MS and GC-MS led to the identification of metabolites belonging to polyphenols, flavonoids, organic acids, amino acids, and sugars.
The stress of temperature in Triticum exposed to high temperature was accompanied with the alternation of several metabolites. There found in total 27 compounds of primary metabolites. The most abundant compounds were glucose, fructose, citric acid, malic acid, cadaverine, L-threonine, aspartic acid and silanol. The less abundant compounds are lactic acid, valine, oxalic acid, glycine and gluconic acid. In some cultivars it was noted the accumulation of several amino acids, including threonine and aspartate while there was a variation in the amounts of various sugars, such as glucose and fructose as well as several alcohols (e.g., inositol, glycerol and mannitol). Still the data are preliminary and the work is ongoing to further results and data processing. The application of LC–MS-based metabolomics in this study allowed for the elucidation of the differential secondary metabolite features of metabolomes of wheat cultivars

and has provided a gateway to classification methods through metabolite profiling. There found approximately 36 compounds, out of which 16 of them have the greatest abundance. The most noted compounds were neochlorogenic acid, catechin, Procyanidin B1, biotin, vanillic acid and Epigallocatechin galate. There is no much variation though among different cultivars about some other compounds such as syringic acid, coumaric, gallic acid and caffeic acid.

The identification of these significant metabolic compounds reveals the effects of biotic and abiotic stress, as well as other strategies, on the growth and development of *Triticum aestivum* L.

- Training on RNASeq.

RNA sequencing (RNA-seq) is one important method nowadays to perform quantitative analysis of whole cell (single-cell) / population (bulk) experiments. RNA- seq belongs to the umbrella term OMICS analysis and is created by NGS sequencers like Illumina Solexa. The main aim of RNA-seq is to:

• Identify differentially expressed genes between treatments to find biomarker (i.e. between two conditions in our example control and disease)
• The discovery of new transcripts, splice variants and fusion genes.

Training was focused on High throughput sequencing RNA-seq. I was well informed on different types of library preparation such as Random Hexamer priming, library preparation for mRNA, library preparation with UMI etc. NGS used in the Institute of ELGO-Dimitra was Illumina and the Genome Analyser used acquires Solexa which yields 8000 Gb in 17-46 hours. As well, the training was focused in different applications of NGS (Next Generation Sequencing) and RNASeq, such as Quantitative genetics, large scale genotyping studies, evolutionary studies, comparative expression analysis and co-expression analysis.

- Training in bioinformatic data analysis.

Training in bioinformatic data analysis was based in FastQ software analysis and NGS Data Sets from Processing to Differential Expression Analysis. Training exercises were focused on 1) database analysis and 2) using tools in Galaxy Framework webserver to process datasets and identify significant differentially expressed genes. Database analysis was performed by downloading NGS datasets from public databases and then learning to use it with the Web-based platform Galaxy.

Description of the STSM main achievements and planned follow-up activities

The research conducted at the Institute of Plant Breeding and Genetic Resources of ELGO-Dimitra was important in achieving the STSM goals. This research significantly enhanced my knowledge, particularly in transcriptomics, metabolomics, and bioinformatics analysis. I am now more confident in applying this knowledge within my institution and in my future career endeavours.
The research contributed to the three main goals of the RECROP project: (1) identifying the genetic, molecular, and physiological makeup of crop sensitivity; (2) creating a roadmap for developing resilient crops; and (3) providing guidelines for exogenous treatments to sustainably increase resilience and push the limits of genetically inherited stress tolerance.
Specifically, the main achievements of this STSM align with the goals of WG1, which focuses on tools to decode stress response and tolerance in crop reproduction, and WG3, which aims to improve crop yield under suboptimal environmental conditions using genetic approaches.

The results from this STSM will be published in peer-reviewed journals, and the collaboration between our institutions will continue through further projects and exchanges.

Action number: CA22157

Grantee name: Srđan Zec

Details of the STSM

Title:Elucidation of thermotolerance mechanisms in tomato

Start and end date: 15/08/2024 to 15/09/2024.

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

The main goal of this training was to acquire knowledge in techniques and methods for functional gene analysis aimed at assessing thermotolerance of different tomato genotypes. In this regard, during the four-week training, I conducted the following experiments:

1. **Assessment of thermotolerance in tomato seedlings:**
   Three-day-old tomato seedlings grown on MS nutrient medium had their hypocotyl length measured initially. The seedlings were then exposed to high temperatures (40°C) for 4 hours. Four days after the heat stress, the hypocotyl length was measured again, and a comparison was made between the growth rate of seedlings kept at optimal temperature (controls) and those exposed to high temperatures.

2. **Detection of heat shock proteins (HSP) using the Western blot method:**
   I was trained to perform the Western blot method, which includes:
   - Isolation of proteins from plant material samples;
   - Separation of proteins by electrophoresis;
   - Transfer of proteins from the gel to the membrane;
   - Addition of antibodies followed by incubation;
   - Signal detection.

3. **Screening for genes involved in heat stress response:**
   I monitored changes in the expression of genes responsible for the synthesis of HSP proteins under optimal conditions and heat stress using the qRT-PCR method, where I was trained to perform the following technical procedures:
   - RNA extraction from plant material;
   - Obtaining cDNA from RNA using reverse transcriptase;
   - Performing PCR to confirm the absence of gDNA and successful cDNA synthesis;
   - Conducting qRT-PCR and analyzing results to quantify the expression of target genes.

4. **Stable transformation of DNA in tomato cells via *Agrobacterium tumefaciens:***
   I became familiar with the process of tomato transformation using *Agrobacterium tumefaciens*. A few days old tomato seedlings were selected for the mutation process, during which their cotyledon leaves were wounded and infected. These leaf segments were subsequently used to generate mutant plants in a suitable nutrient medium.

Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

During this four-week STSM, all the planned goals were achieved. The activities and training sessions undertaken in Dr. Fragkostefanakis's laboratory, in collaboration with his proficient team, have equipped me with valuable knowledge and skills. I deepened my understanding of regulatory mechanisms involved in controlling gene expression and gained practical experience with various tools for gene function analysis and decoding. I became familiar with the protocols and trained to independently perform Western Blot and qRT-PCR analyses, as well as interpret the obtained results. By evaluating

thermotolerance and examining the gene expression linked to heat shock protein synthesis, I was able to assess the level of heat shock resistance in the genotypes relevant to my research. The knowledge and skills I have acquired will be implemented in the laboratory at the Institute of Field and Vegetable Crops, where I work, enhancing both the capacities and the potential of our facilities. Moving forward, I plan to continue evaluating the thermotolerance of the genotypes available in the Institute's tomato genetic collection, which will guide the selection of parental lines for breeding new tomato genotypes with improved resilience to extreme climate conditions. Moreover, I aim to include genotypes exhibiting a strong heat stress response in a repository that will be accessible online to RECROP members.

Action number: CA22157

Grantee name: Rada Šućur

Details of the STSM

Title: Determining the status of yellow rust (*Puccinia striiformis* f. sp*. tritici)* and Septoria leaf bloch (*Septoria tritici)* in historically important Serbian wheat varieties

Start and end date: 02/09/2024 to 23/09/2024

Description of the work carried out during the STSM

Before arriving at the Sivas University of Science and Technology, 64 wheat genotypes were collected. 20 varieties were created in Serbia and the rest originated from different European countries, so due to their characteristics and desirable genes, they are used in wheat breeding programs in Serbia. The original plan included the detection of genes responsible for tolerance to yellow rust (*Puccinia striiformis* f.sp. *tritici)* and septoria leaf blotch (*Septoria tritici*), however, in addition to these two pathogens, the resistance genes status of powdery mildew (*Blumeria graminis* f. sp. *tritici*) was also checked.

At the beginning, the samples were subjected to germination for a period of 14 days in a greenhouse. When the wheat developed enough leaf mass, we started preparing the samples. The process of determining the presence of resistant genes was carried out at the Department of Plant Protection in the molecular genetics laboratory, and consisted of the following steps:

1. DNA was isolated from fresh green wheat leaves using the cetyltrimethylammonium bromide (CTAB) protocol.
2. In order to determine both quality and quantity of extracted DNA semples, the standard 1% agarose gel electrophoresis method and Nanodrop (DS11 FX, DeNovix, Wilmington, DE, USA) was applied.
3. Training on molecular characterization was carried out using Retrotransposons markers and gene specific primers for diseases
4. Detection of the *Pm, Yr* and *Stb* genes were performed using Polymerase chain reaction (PCR). DNA molecular markers specific to these loci were *Yr 15* for yellow rust, and 16 primers for septoria leaf bloch.
5. Product of PCR reaction were tested using 2% agarose gel electrophoresis, and resultes were visualized using UV imager

Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

During the visit to the Sivas University of Science and Tecnology, Turkey, all the goals foreseen in the plan were achieved. In addition to 2 significant diseases (yellow rust and septoria leaf bloch) that were analyzed, there was an opportunity to determine the presence of a powdery mildew tolerance gene. The results showed that most of the tested samples have resistance genes, which is an important data that can be used in breeding programs. More detailed results and discussions about this research will be

presented and published in journals. In the future, it is planned to expand the germplasm collection, where, in addition to the already conducted analyzes of resistance to some biotic factors, resistance to abiotic factors will also be investigated (which is in line with the goals of the Action). This visit made it possible to prepare a part of the PhD thesis, that deals with intercrops, specifically in this case a mixture of wheat and peas. In 6 wheat varieties that were part of the collection that was tested for the mentioned pathogens, the presence of resistant genes was confirmed. It is planned to continue cooperation with the Department of Plant Protection, where in the future the mentioned germplasm collection will be studied more extensively and some recommendations will be given to plant breeders which genotypes are more suitable as parental components depending on the goal of breeding.

Action number: CA22157, Reproductive Enhancement of CROP resilience to extreme climate (RECROP)

Grantee name: Manuel Buendia Monreal

Details of the STSM

Title: Isolation of single cells from ovules of Arabidopsis thaliana

Start and end date: 05/09/2024 to 14/09/2024

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

First, we revised the fundaments of the protocol for isolating single cells from ovules. I took note of all the reagents and materials that we need to buy in Milan to do the experiment; this includes a special combination of enzymes, small glass bottom dishes, DNA LoBind 0.5 mL tubes, 0,58 x 1,00 x 80 mm capillaries, and the cell injector/collector Cell Tram 4r Air. We also had a look at the plants growing from the Arabidopsis seeds that I sent weeks in advance (the fluorescent reporter line of synergid cells “MYB98-GFP”, the reporter line of synergid, egg and central cells “FGR7.0”, and the crosses MYB98-GFP x vdd and FGR7.0 x vdd. Unfortunately, the plants were still juvenile so they were not producing flowers yet. However, Marta Flores had some other flowering plants with fluorescent reporters of the ovule cells with the purpose of teaching me the protocol.

Then, I learned how to collect the ovules into the glass bottom dish containing the enzyme solution. First, I have to select unpollinated flowers, cut them and paste them on a slide with double side tape, isolate the pistils with a couple of tweezers, open the valves with a needle, and scrap off the ovules from the placenta and put them into the drop of enzyme solution. I had to do this with 3 or 4 flowers for each sample in order to get around 50 ovules. Finally, I had to take the floating ovules to the bottom of the dish, and close the dish adding wet paper to keep the humidity avoiding the formation of mannitol crystals. The dishes were slightly shaken for 2-3 hours and then we could see the protoplasts, with cells of diverse sizes released from the ovules. We prepared the microcapillaries by extending glass bars applying 69 C in a vertical capillary puller. I learned how to prepare them and also brought those capillaries to Milan to start using them. The next days, I collected ovules into the enzyme solution, incubate them for 2-3 hours and then isolate single cells using the Cell Tram. First, I had to get familiar with the morphology of the synergid and egg cells. Then, I learned how to differentiate those cells with fluorescence, and how to manipulate the Cell Tram collector. After three days of using it, I was able to collect 12 -15 cells per 4-5 h session, which is a good amount of cells for sending to sequence. In addition, we took confocal images of the synergid, egg and central cells of protoplasted ovules in order to have a morphological reference for the upcoming isolations.

Description of the STSM main achievements and planned follow-up activities

The main goal of the STSM was to learn the technique of isolating single cells from ovules using the cell collector Cell Tram. I learned all the steps of the protocol thanks to the experience and help of Maria Flores at ITQB in Portugal. By the end of the STSM, I was able to collect a good amount of cells per session, so I am ready to start using the Cell Tram at the University of Milan. After having collected single synergid cells, we will send them to sequence and analyse their transcriptome with the aim of identifying the genes responsible for the synergids cell death.

The transcriptomic results will be publicly available and will be the basis of a publication. We will keep in contact with Maria Flores and Jorg Becker for collaborating in the following steps of the single cells isolation and sequence.

The experimental setup for protoplasting ovules and isolating single synergid, egg and central cells was defined and can also be used to isolate single cells from other species.

SCIENTIFIC REPORT	
Reference	Short Term Scientific Mission COST CA22157
Grant recipient	**Esteban Burbano Erazo** Instituto de Biología molecular y celular de plantas, IBMCP (Spain) eburbanoe@gmail.com
Host Researcher	Maria Manuela Rigano University of Naples Federico II, Portici (Naples, Italy)mrigano@unina.it
Period	from 06/09/2024 to 25/09/2024
STSM Reference Code	**COST-STSM - CA22157-e1243bac**
STSM Title	Tomato lines adapted to shade, drought and temperature stress

Summary

Plants detect the presence of other plants by detecting reduced ratios of red to far-red light (R/FR). This proximity shade (low R/FR) signal prompts elongation and reduces photosynthetic pigment levels, which are undesired responses for plant cultivation. This mission was conducted in the laboratory of Prof. M. Manuela Rigano (UNINA), which has made many important contributions to elucidate the physiological mechanisms trough light and temperature control thermo-morphogenesis in Tomato plants, with great recognition worldwide. The principal objective of this mission was to gain skills and theorical knowledge about the application of abiotic stress, such as proximity shade, drought, temperature in tomato plant growth and development and photosynthesis. The training involved hands-on exposure to specific methodologies to quantify including gas exchange, chlorophyll fluorescence and plant adaptation to abiotic stress. During this mission were grown wild type, CRISPR cas9 edited lines for the gene *HY5*, a *Solanum pennellii* introgression line putatively tolerant to proximity shade and in the cultivated line M82, under W light (W, ca. 150 µmol photons m−2 s−1; R:FR ratio of 3 to 5) and simulated proximity shade (light W+FR, ca. 150 µmol photons m−2 s−1; R:FR ratio 0.17 to 0.19). The tolerance of this lines to abiotic stress is currently under way. Finally statistical scrips in R were design to analyze the information that will be obtained with these experiments. In conclusion this experience funded by COST RECROP ACTION CA22157, allowed an important training to developed skills in both experimental design and advanced statistical analysis techniques in plant physiology research. This mission was very enriching for my training as a PhD, learning about different experiments carried out by our collaborators to search for tomato genotypes tolerant to abiotic stresses. In addition, it strengthened alliances for future research

work and collaborations

Report

During the STSM experiments were set up in order to evaluate shade response in tomato plants under abiotic stress. Analyses were carried out in wild type, mutants tomato plants, a *Solanum pennellii* introgression line putatively tolerant to proximity shade and in the cultivated line M82. CRISPR cas9 edited lines for the gene *HY5* in the MicroTom background were grown under W light (W, ca. 150 μmol photons m^{-2} s^{-1}; R:FR ratio of 3 to 5) and simulated proximity shade (light W+FR, ca. 150 μmol photons m^{-2} s^{-1}; R:FR ratio 0.17 to 0.19) for 11 days. A clear phenotype of shade tolerance with lower hypocotyl and epicotyl elongation, and reduced leaf area under W + FR was observed in the *hy5* mutant. The tolerance of this line to abiotic stress is currently under way. Additionally, during this period scripts in R were designed to analyze data coming from previous experiments. These scripts were used to analyze the difference in final yield and fruit quality in tomato plants (a *Solanum pennellii* introgression lines and the cultivated line M82) grown under simulated proximity shade. Using these statistical analyses, a higher fruit quality was evidenced in both genotypes under W+FR conditions. This script will be also used to analyze data coming from subsequent experiments. RNA samples were also extracted from fruit coming from these tomato lines and will be used for RNA sequencing experiments to be performed in the future. The mission was important to strengthen the network between the participant institutes. This work will continue in collaboration with the host institute.

We validate the proximity shade tolerance in a CRISPR cas9 edited lines with *HY5* gain of function and in a *S. pennellii* introgression line. The phenotype of these lines showed a promising tolerance response under proximity shade, contributing to the creation of a promising collection of hyposensitive tomato lines to shade. Transcriptomic analyses on these lines are planned and will be carried out in collaboration with the host institution in future collaborations. Additionally, the characterization under another abiotic stress considering the role of the master transcription factor HY5 in the tolerance to drought and high temperature is in process. Those results will contribute to the characterization of new resilient genotypes of tomato under abiotic stress; objective aligned with the WORKING GROUP 2 “Description of the effects of abiotic stresses on reproductive tissues and their relevance for resilience and yield” and TASK 2.1: “Determine the effects of abiotic stresses on crop reproduction.” And WORKING GROUP 3 “Improvement of crop yield under suboptimal environmental conditions using genetic approaches.”, and specifically the task TASK 3.3, generating a strategy to promote multiple tolerance to abiotic stress in tomato.

Additionally, if the results under drought and heat stress generate promising results the collaboration between institutes will allow to continue with possible transcriptomic analysis under abiotic stresses to identify the molecular mechanisms associated to the stress response in the tomato edited lines. Finally, this mission contributed to my formation like a PhD and allowed me learn methodologies to use in future experiments.

Reference: Short Term Scientific Mission COST CA22157
Grant recipient: **Dr Esma Ulusoy** USKUDAR UNIVERSITY esma.ulusoy@uskudar.edu.tr
Host Researcher: Dr Cristina Barrero Sicilia THE UNIVERSITY OF HERTFORDSHIRE c.barrero-sicilia@herts.ac.uk
Period: 01/08/2024 to 30/09/2024
STSM Reference Code: **COST-STSM - CA22157-13933fa8**
STSM Title Decoding Heat Stress Responses and Nutritional Changes in Barley Seed Development Using Next-Generation Sequencing and Spectroscopy

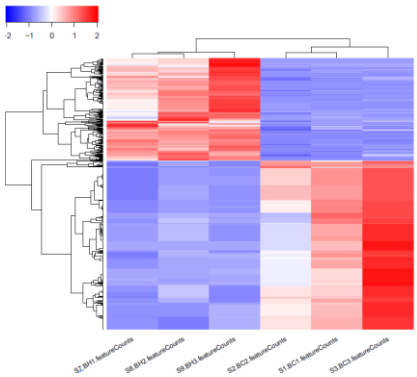
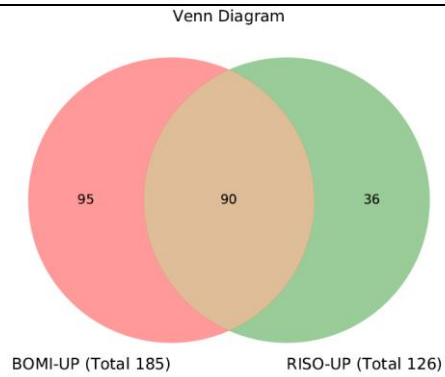
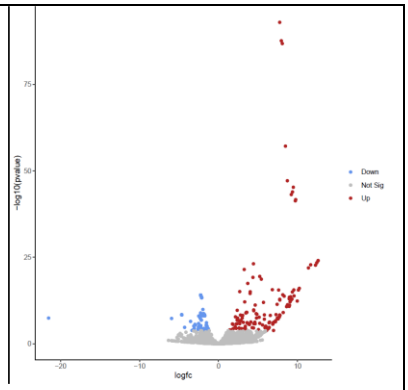
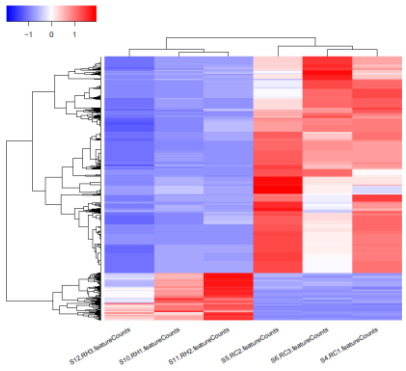
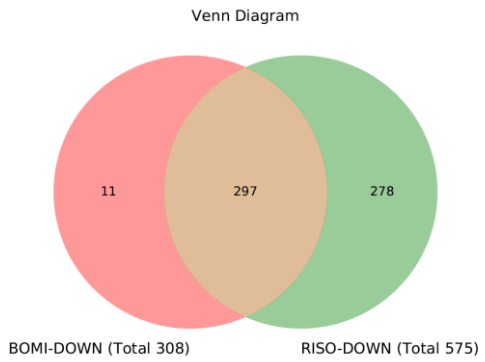
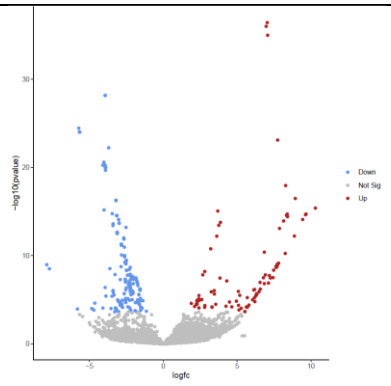
Since starting my studies in August, I have gained incredible experiences in terms of my career. I have had the opportunity to work primarily on molecular biology, nanoparticles, and histology. However, genetics has always been an area I wanted to develop but could not find the opportunity to do so. For the first time, I started to see myself as a complete molecular biology and genetics expert with my studies here. First of all, I would like to express my gratitude to the RECROP team for helping me achieve this important goal.

Purpose of the Visit: Our research project focused on studying how plants respond to ABA (abscisic acid) sensitivity under heat stress conditions.

Description of the work carried out: Our work involved conducting transcriptomic analysis, which allowed us to examine the activity of genes in response to these specific conditions. We successfully extracted high-quality RNA, constructed a library of genetic material, and conducted sequencing at the University of Hertfordshire. These tasks, including RNA extraction, cDNA synthesis, and sequencing, represented my initial foray into molecular analysis and working with barley, a significant learning experience for me.

Description of the main results obtained: Our ongoing collaboration includes using bioinformatics to analyze the data and identify potential candidate genes that play a role in plant stress responses. Unfortunately, due to time constraints, we could not expand the study to include the development of seeds and recovery stages. However, we are actively seeking funding to allow me to return and continue this important work.

Description of how the STSM was embedded in research project(s): We have identified genes upregulated and downregulated by heat stress in the BOMI and RISO groups. We have illustrated the overlapping genes in the Venn diagram, heatmap, and volcano plot, but our research is still ongoing.

		
BOMI Up&Down genes Heatmap2	BOMI/RISO Upregulated genes Venn Diagram	BOMI Up&Down genes Volcano Plot
		
RISO Up&Down genes Heatmap2	BOMI/RISO Downregulated genes Venn Diagram	RISO Up&Down genes Volcano Plot

Possible future collaboration with Host Institution: This collaboration has not only significantly advanced our research but has also provided me with valuable hands-on experience in the fields of molecular biology and genetics, particularly in the study of how plants respond to stressful conditions during the critical stages of seedling establishment and seed development.

Photo 1. With my collaborator Cristina Barrero Sicilia and me.

Photo 2. We also participated in various events at the university #Red4Research 2024

Photo 3. CE room Barley growth cabinet

Photos 4&5. Glasshouse and CE room workings.

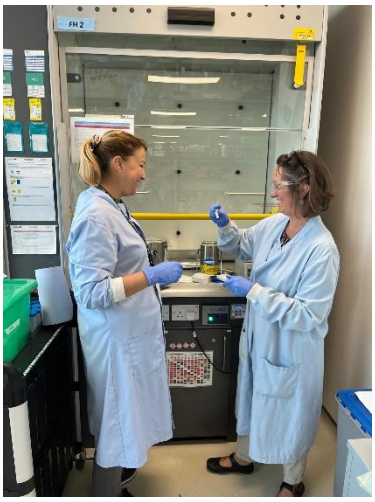


Photo 6. RNA isolation exp.

Photo 7. Root and leaves measure exp.

Photo 8. cDNA synthesis and barcoding.

Photo 9. RNA quality checking with agarose gel electrophoresis.

Photo 10. cDNA synthesis and barcoding.

Photo 11. MINION priming and cDNA library loading.

Photo 12. 4-month-old BOMI/RISO heat stress exp.

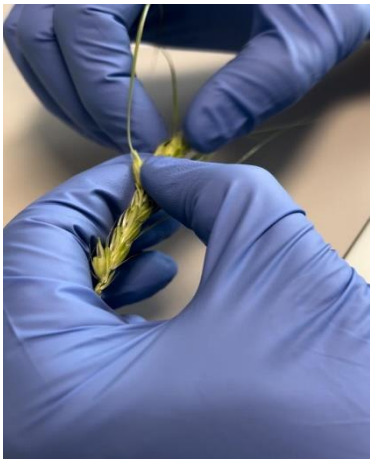


Photo 13. 4-month-old BOMI/RISO seeds prep.

Photo 14. 4-month-old BOMI/RISO seeds prep.

Action number: CA22157-COST ACTION "RECROP"

Grantee name: Consuelo Penella Casañ

Details of the STSM

Title: Identifying Reproductive Phase Resilience in Crops through gas exchange and A/Ci curve analysis: an eco-physiological approach

Start and end date: 01/09/2024 to 07/09/2024

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

(max. 500 words)

During the Short-Term Scientific Mission (STSM), the main goal was to study the resilience of bean crops during their reproductive phase when subjected to environmental stress, particularly poor water quality and high boron concentrations. This STSM involved eco-physiological analysis, with a strong focus on gas exchange measurements and the A/Ci curve analysis, both crucial tools for understanding the plants' photosynthetic efficiency under stress.

The work began with an introductory meeting where I was introduced to the research team and briefed on the overall goals of the project, safety protocols, and the methodological approaches to be employed. The team provided an overview of the facilities and the specific techniques that would be used, laying the foundation for the experimental work that followed.

In the initial days, I participated in both theoretical and practical sessions focused on gas exchange and A/Ci curve analysis. Theoretical discussions covered the significance of these measurements in plant eco-physiology, particularly in terms of how they relate to photosynthesis, transpiration, and stomatal conductance. The practical component involved hands-on experience in collecting data from the bean plants under study, which had been subjected to high levels of boron in water. The gas exchange measurements were taken to assess the plants' ability to manage water use efficiency and carbon assimilation under these adverse conditions.

Over the course of the week, we successfully conducted A/Ci curve analyses, which are essential for understanding the limitations of photosynthesis at the biochemical level, particularly under stress. This involved measuring parameters such as the maximum rate of carboxylation (Vcmax) and electron transport rate (Jmax), which provided insights into how boron stress affects the photosynthetic apparatus of the beans. We observed changes in the photosynthetic rate, as well as in stomatal conductance and transpiration, indicating how boron toxicity impacts gas exchange and water regulation.

The final phase of the work involved discussing the preliminary findings with the research team. We compared the results obtained during the STSM with the existing literature on plant responses to boron stress and environmental challenges. The discussions were crucial for contextualizing our findings and generating hypotheses for future research. Based on the data collected, we formulated initial recommendations for improving the resilience of bean crops to boron toxicity, particularly during their reproductive phase, which is a critical period for ensuring crop yield.

Description of the STSM main achievements and planned follow-up activities

One of the key achievements of this STSM was the successful completion of the A/Ci curve analysis, which provided valuable insights into how bean plants respond to elevated boron levels, particularly during their reproductive phase. The analysis highlighted the limitations imposed by boron toxicity on the photosynthetic machinery, especially in terms of carboxylation efficiency and electron transport rate. This information is crucial for understanding how environmental stressors affect photosynthesis, which in turn impacts plant growth and productivity.

Additionally, we identified non-destructive physiological markers that could serve as indicators of stress tolerance in beans. These markers, derived from gas exchange measurements, offer practical tools for monitoring the health of crops without the need for invasive methods. By detecting early signs of stress, farmers and researchers can take timely action to mitigate the effects of poor water quality and boron toxicity, ultimately improving crop management and resilience.

The STSM also contributed to a deeper understanding of how bean crops manage water use efficiency and carbon assimilation under stress. By analyzing transpiration rates and stomatal conductance, we gained insights into how these plants regulate water loss and carbon uptake in response to boron stress. This knowledge is essential for developing strategies to enhance the resilience of beans, particularly in regions where water quality is a major concern.

The next step following this STSM is to conduct a more in-depth analysis of the A/Ci curves generated during the experiments. This will involve further examination of the physiological thresholds at which boron toxicity begins to significantly impact photosynthesis. A more detailed understanding of these thresholds will be crucial for developing crop management strategies that can mitigate the negative effects of boron on plant growth.

In collaboration with the host institution, we plan to integrate the findings from this STSM into ongoing breeding programs aimed at developing bean varieties with improved resilience to environmental stress. The non-destructive markers identified during the study could serve as valuable tools in these programs, helping to select and develop crops that are better suited to withstand poor water conditions and other stressors.

We also plan to prepare a research paper summarizing the findings of this STSM. This paper will detail the effects of boron toxicity on gas exchange and photosynthesis, as well as the practical implications for crop management. The publication of this paper will help disseminate our findings to the wider scientific community and contribute to the broader goals of improving agricultural sustainability.

Finally, we are exploring the possibility of expanding this research to include other crops that are also affected by poor water quality and boron toxicity. By broadening the scope of the research initiated during this STSM, we hope to develop more comprehensive strategies for enhancing crop resilience to environmental stressors, ultimately contributing to global food security.

Action number: **CA22157**

Grantee name: **Boushra Shalha**

Details of the STSM

Title: **Cross talking of heat and light stress and its importance for tomato development**

Start and end date: **22/08/2023 – 10/09/2023**

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

During this Short-Term Scientific Mission (STSM), I focused on three primary objectives, which involved training in various methodologies, equipments, and software techniques. The three main tasks completed are as follows:

Task 1. Effect of light on the nucleocytoplasmic shuttling of HSFA1a

To achieve this task, transgenic tomato seedlings from the PCaMV35S::GFP-HSFA1a line were subjected to different continuous light quality and intensity regimes. Following this, the seedlings were exposed to heat stress at 40°C for 1 hour. The analysis was conducted using a confocal laser scanning microscope, focusing on two organs: the cotyledons and hypocotyls, to assess GFP expression. Results from two replicates showed that seedlings treated with blue and red light, combined with heat stress, exhibited increased nuclear GFP expression in both organs compared to the control groups, which were kept in darkness and at room temperature.

Task 2. Involvement of SR46a splicing factor in phototropism of tomato seedlings

Prior to this experiment, the growth rate of wild-type and SR46a mutant seedlings was optimized by measuring hypocotyl length under blue light and dark conditions to prevent interference with phototropic behaviour. After optimization, the phototropic response was tested using a setup that applied unilateral 0.5 µE blue light for 4 hours, with live camera scanning every 5 minutes to track the seedlings' light-induced bending and the result analysis were obtained using image j. This study needs to be repeated using different light intensities to gain a clearer understanding of the phototropic behaviour of SR46a mutant seedlings compared to the wild type.

Task 3. Light and temperature-dependent formation of nuclear condensates by SR46a

The electroporation protocol for Agrobacterium transformation with the GFP-SR46a construct was followed, and the infiltrated leaves of *Nicotiana benthamiana* were subjected to 1 hour of heat stress at 40°C, along with control treatments. Positive transformants expressing GFP-SR46a were analyzed using a confocal laser scanning microscope. Heat-stressed leaves exhibited increased nuclear GFP expression along with the formation of nuclear aggregates, while control leaves showed both nuclear and cytoplasmic expression. Additionally, immunoblotting analysis was performed on protein extracts from the treated leaves to confirm the presence of the GFP-tagged protein.

The final outcomes of the STSM will be presented at COST RECROP conferences focusing on stress biology, allowing for the sharing of findings with RECROP members including the host institution to promote discussions on future research directions and potential collaborations.

Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

The training received from the host laboratory on light response methodologies, along with access to advanced tools for detecting biomolecular condensates during this Short-Term Scientific Mission (STSM), has been invaluable. It provided hands-on experience with research techniques and equipment, guided by experts in the field, fostering innovative approaches in research. Furthermore, this opportunity has laid the foundation for future collaborative research initiatives by addressing challenges in stress resilience. Understanding the interplay between temperature and light is crucial for developing strategies to combat the effects of climate change on food security. All relevant publications and presentations will appropriately acknowledge RECROP for its support in this training.

Action number: CA22157

Grantee name: Nataša Lukić

Details of the STSM

Title: The molecular mechanism of flooding in wheat plants

Start and end date: from 01/04/2024 to 12/04/2024

Description of the work carried out during the STSM

During my STSM within the COST Action CA22157, at the Institute of Molecular Genetics and Genetic Engineering (IMGGE), I conducted research on the molecular mechanisms underlying hypoxia caused by partial submergence and reoxygenation in two wheat genotypes: "Julia" and "Nova Bosanka". While the literature on this topic is limited, I aimed to close the gap by investigating the role of reactive oxygen species (ROS) and antioxidative genes in hypoxia.

To achieve this, I performed experiments to analyse the gene expression of key antioxidative genes, including SOD, CAT, POX, and APX, as well as genes involved in anaerobic metabolism (such as alcohol dehydrogenase and lactate dehydrogenase), and ethylene-responsive transcription factors 1 and

2. I focused on the effects of partial submergence and reoxygenation periods on gene expression in the two wheat genotypes.

During STSM, I did homogenisation, RNA isolation, and reverse transcription into cDNA and gene expression in two exposed to control, waterlogging and recovery conditions for 1, 3, 8 and 6 days.

Firstly, all samples were homogenised to a fine powder and then used for RNA extraction. From genotype "Nova Bosanka", RNA was extracted using RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and stored at – 70˚C before being used for qRT-PCR. RNA from "Julia" samples was extracted using a GeneJET RNA purification kit (Thermo Fisher Scientific) according to the manufacturer's instructions and stored at – 70˚C.

Another step for RNA samples from genotype "Julia" was DNase treatment to remove potentially contaminating genomic DNA from RNA samples before cDNA synthesis (used Invitrogen DNA removal Kit, Thermo Fisher Scientific). For this, 10µg of RNA were mixed with 0.1 volume 10X DNase I Buffer and 1 µL rDNase and incubated at 37˚C for 30 min. Further. 0.1 volumes of DNase Inactivation Reagent was added, additionally incubated at room temperature for 2 min and centrifugated at 10.000 g for 1.5 min. The concentration of RNA was measured using BioSpec-nano, Shimadzu. Secondly, we did cDNA reverse transcriptase using RevertAid First Strand cDNA Synthesis Kit, according to the Thermo Fisher Scientific protocol using Random Hexamer Primer and the RevertAid™ Reverse Transcriptase. Total cDNAs were diluted 1:4 with nuclease-free water. The reverse transcription PCR steps were amplified as follows: after cDNA synthesis, PCR with an amplification profile of 10 min at 25˚C, then 60 min at 42˚C and 10 min at 70˚C, was performed. Horizontal electrophoresis was used to check the cleanliness of cDNA and RNA.

The last step done during my STSM was gene expression using Real-time q-PCR. Prior to the SYBR Green assay, total cDNAs were diluted 1:4 with nuclease-free water. Reactions were performed in a volume of 12,5 µL contained 0,5 µL of each primer and 1X Power SYBR Green PCR Master Mix (Thermo Scientific). Real-time PCR was performed on the Mic Real Time PCR Cycler (Bio Molecular Systems) with the following cycles: 2 min at 50 ◦C, 10 min at 95 ◦C and 40 cycles of 95 ◦C for 15 s, 58◦C for 1 min. Amplification of PCR products was detected in real time and results were analysed using micPCR software (Bio Molecular Systems) and calculated as 2−dCt. The values of the relative gene expression changes were calculated by applying actin gene reference. Primer for alcohol

dehydrogenases did not work properly because of that I could not completed gene expression using this primer. However, host institution supervisor will complete that part soon after my STSM.

Description of the STSM main achievements and planned follow-up activities

During my STSM, I had a highly productive collaboration that yielded significant progress in just two weeks. I am proud to say that I achieved the primary objective of the STSM: mastering a new qPCR method. With nearly 55 samples to work with, I am confident that I have successfully applied the techniques of homogenisation, RNA isolation, DNA-free treatment, and cDNA synthesis and qPCR to a high standard. I feel that my STSM was a resounding success that allowed me to hone my skills and make valuable contributions to my field of research. I owe much of this success to the excellent guidance and communication from my supervisor, Dr. Marija Vidović and Dr. Jelena Samardžić. I'm also grateful for the invaluable support and assistance provided by PhD student Ana Pantelić, who made my experience even more rewarding. Also, I believe this project and its results fit the scientific objectives of the RECROP Action as I aimed to detect the mechanism of hypoxia occurring during partial submersion, like hypoxia-related events to detect and select genotypes more tolerant to this type of stress. The results obtained through this project resonates strongly with the objectives outlined by RECROP Working Group 2, specifically Task 2.4. This Task of the RECROP Action seeks to identify critical metabolic pathways, cellular processes, and molecules, spanning from metabolites and hormones to DNA, RNA, and proteins, that are essential for stress response and tolerance in plants. This STSM also encompass Task 3.1 of Working Group 3, which aims to identify hybrids resilient to various stresses. By elucidating the antioxidative capacity and changes in the concentration of phenolic compounds, amino acids, and organic acids in response to water stress, the study seeks to correlate these factors with plant tolerance mechanisms. The last step will be data acquisition and manuscript writing. The research results that will be carried out through this STSM project will be presented at international conferences. This work will be followed by a collaborative beamtime in Summer this year. Also, after this project, it is much more likely that this collaboration will continue further in the future. Overall, my STSM was an enriching experience that allowed me to acquire new skills, deepen my understanding of my field, and forge valuable connections with my colleagues. I am excited to see what new opportunities lie ahead, and I'm grateful for the chance to participate in such a valuable program.

Action number: CA22157

Grantee name: Ayat Bakery

Details of the STSM

Title: Bioinformatics and Computational Techniques in Molecular Biology

Start and end date: 05/08/2024 to 23/08/2024

Description of the work carried out during the STSM.

During this Short-Term Scientific Mission (STMS) I have learned the use of essential bioinformatic tools. At the first week I successfully learned how to use these essential tools, establishing a good basis for the following more advanced use. During the second week, I checked more advanced subjects, including integrating various biological datasets such as genomics, transcriptomics, and proteomics. I also touched some predictive modeling techniques aimed at forecasting biological outcomes. This phase of the program deepened my understanding of how to combine and analyze diverse data types to draw meaningful biological conclusions. During the final phase of the STSM, I put the knowledge gained in the first two weeks into practice. This practical application included hands-on experience in data integration and modeling, which strengthened my theoretical understanding and significantly enhanced my bioinformatics skills. Additionally, I received training in advanced data visualization

techniques, which significantly improved my ability to interpret and present complex biological data effectively. The STMS concluded with a comprehensive review of the skills and knowledge I had gained, coupled with discussions on potential future research applications. This thorough review combined my learning and provided valuable insights into how the skills developed during the STMS could be applied to my ongoing and future research goals.

1 This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

Description of the STSM main achievements and planned follow-up activities

Through this STSM, I learned the fundamentals of the R and Python programs and the evaluation methods for sequence analysis, the visualization of structures, the use of molecular biology databases, and the application of statistical methods with R / R Studio. I gained hands-on experience in writing small analysis scripts using Python. Also, I learned the essential basis of employing the R code to perform downstream RNA-Seq analysis with R., and also the analysis of the Upstream RNA with a Linux shell. Finally, I learned the basics of how to perform and present a complete RNA-Seq analysis. Additionally, I gained knowledge in using essential bioinformatics tools, such as protein structure prediction methods, genome annotation tools, and sequence alignment software. These programs are crucial for analyzing my PhD results. One significant result was the combination of biology and computational science, which improved my capacity for productive multidisciplinary teamwork, a critical skill for my PhD research and professional goals in the future. My research skills have greatly improved due to the training I received through this Short-Term Scientific Mission (STSM), especially in understanding the resilience of crops like the tomato used in this study. In the future, the results of this STSM will advance the understanding of the scientific community in this field by contributing to at least one publication. Furthermore, this program's promotion of collaboration has created opportunities for upcoming research initiatives. All relevant papers and presentations will appropriately credit RECROP for supporting this training.

Action number: CA22157

Grantee name: Álvaro Ignacio Vidal Valenzuela

Details of the STSM

Title: Harnessing transcriptomics and precision protoplast Transformation for Climate-Resilient Grapevine

Start and end date: 20/05/2024 to 20/06/2024

Description of the work carried out during the STSM

In the initial week, I optimised vectors using historical data derived from a systems biology framework. This involved analysing public SRA datasets, specifically RNA-sequencing data under water deficit conditions. The chosen sgRNA served as a foundational element for the subsequent preparations of either ribonucleoprotein complexes or transformation vectors, for the RNP complexes, I synthesised the sgRNA during this week. The second week was dedicated to the preparation of protoplasts, utilising somatic embryos extracted from the anthers of 110 Richter grapevine rootstock, and non-embryogenic Ramsay cell line. Protoplast isolation proceeded via cell wall digestion. Transitioning from the latter part of the second week into the early days of the third, I initiated the transformation of protoplasts. This process employed either ribonucleoprotein-mediated or vector-based methods. The transformation’s success was monitored through the epifluorescence of an integrated protein marker, by using a fluoroscan equipment. The final week involved close monitoring of the transformed embryos, focusing

on the regeneration of the cell wall and the proliferation of protoplasts. The culture medium was systematically altered to facilitate these developments, with the presence of Calcofluor White Stain fluorescence serving as a key indicator. The main point of deviation was to try to cell-sorting the protoplast after transformation, but by now, that part was not achieved because of the capacities of the cytometer used.

Report on the outcomes of a presentation and participation in a

Dissemination Conference1

Action number: CA22157

Grantee name: **Sotirios Fragkostefanakis**

Conference Details

Conference title: SEB 2024 Prague

Conference web-page: https://www.sebiology.org/events/seb-conference-prague-2024.html Conference venue2: Clarion Congress Prague

Conference start and end date: 02/07/2024 to 05/07/2024

Accepted oral contribution details

Title of the presentation: Heat stress transcription factors and splicing regulators: brothers in arms to survive heat

Co-authors:

Other details of the presentation: P2 - FROM SENSING TO REMEMBERING: PLANTS' RESPONSES TO TEMPERATURE FLUCTUATIONS

Outcome of the conference participation

Description of the outcome of the conference presentation, including contacts made and potential for future collaborations.

Networking and visibility: My talk attracted more than 80 attendees from various fields of plant biology. I had the chance to talk to many about current projects and results. Comments for my talk have been posted on different professional platforms including X and LinkedIn and have received many views.

Collaborations: New collaborations have been planned with two colleagues working on thermotolerance and particularly thermomemory.

RECROP: The end of my talk included a promotional video of RECROP and presentation of our COST Action. This attracted the attention of many people who approaches me to ask for details for activities, and process of application for membership. Already from that day several new applicants have registered to become WP members. Importantly, a colleague from Norway expressed interest to become MC member.

1 This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

2 For an online conference, specify virtual participation; for hybrid conferences, specify whether it is an in-person or virtual participation.

I confirm that the following documents have been uploaded on e-COST as an integral part of this report:

- the certificate of conference attendance.

- the programme of the conference or book of abstracts / proceedings indicating the oral presentation of the grantee.

- copy of the given presentation.

Report on the outcomes of a presentation and participation in a

ITC Conference

Action number: CA22157

Grantee name: **Jesamine Rikisahedew**

Conference Details

Conference title: ePS2: Second European Congress on Photosynthesis Research

Conference web-page: https://www.eps2.org/

Conference venue : COMPLESSO DIDATTICO DI BIOMEDICINA "IL FIORE DI BOTTA"

Università degli Studi di Padova

Via del Pescarotto, 8,

35131 Padova

Conference start and end date: 24/06/2024 to 28/06/2024

Accepted contribution details

Title of the presentation: Light Acclimation in C3 and C4 Crops: Assessing Ultrastructural Traits in Triticum aestivum and Zea mays Across Different Light Intensities

Type of the presentation: (oral/poster): Poster

Co-authors: Riccardo Scodeller, Tiina Tosens, Ülo Niinemets

Other details of the presentation: specify here any additional details related to the contribution (e.g. title of the session / track of the conference programme in which the contribution is accepted): epS2 Young Session, tailored for PhD students

Outcome of the conference participation

Description of the outcome of the presentation of the accepted contribution, in terms of grantee's visibility, including the establishment of new contacts for future collaborations

The goal of attending and presenting at the ePS2 conference was to share research in the field of anatomical and physiological changes in crop species when grown under various stress conditions. A poster was prepared, printed, and presented at the ePS2 conference, ensuring that it was visible to all who attended. During the stipulated poster sessions, researchers from Europe and around the world were given the opportunity to read and discuss the contents of the poster. The poster covered the effects of light intensity on the growth and development of the crop species Zea mays and Triticum aestivum. Results showed that the C4 plant (Z. mays) exhibited a higher range of anatomical plasticity, allowing this species to better cope with changes in abiotic stressors. The vital aspects of anatomical differences between the C3 and C4 anatomy were developed, analysed, and discussed as well, which was a novel component of this study. Many researchers present at the conference specialised in biophysics and molecular biology, allowing for opportunities for collaboration in terms of using their expertise to further delve into the inner workings of crop species with an emphasis on the effects on agriculture. Additionally, discussions were had with computational biologists working on the Farqhuar method of modelling photosynthesis to work on physiology data collected on this project. Some scientists present have employed different methods of viewing ultrastructural tissues, including 3D microscopy in the form of SBF- or FIB-SEM, which is of great interest for developing this project. Contact was made for the possibility of future collaborations as these specialised microscopes are rare and very few are available for use around Europe. Contact was established with researchers from the following groups: Jan Ingenhousz Institute (microscopy), University of the Balearic Islands (anatomical models for calculating mesophyll conductance) and the University of Cambridge (computational methods for producing photosynthesis models).

Acknowledgement of inclusion of necessary supporting documents to claim the grant

I confirm that the following documents have been uploaded on e-COST as an integral part of this report:

- the certificate of conference attendance.

- the programme of the conference or book of abstracts / proceedings indicating the presentation (oral or poster) of the grantee – submitted as a screenshot and link to website from which individual abstracts can be procured: https://www.eps2.org/program.

- copy of the given presentation (oral or poster).

Report on the outcomes of a presentation and participation in a ITC Conference[1]

Action number: CA22157

Grantee name: Olha LAKHNEKO

Conference Details

Conference title: Botanik-Tagung 2024. International Conference of the German Society for Plant Sciences

Conference web-page: https://botanik-tagung.de/

Conference venue[2]: Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

Conference start and end date: 15/09/2024 to 19/09/2024

Accepted contribution details

Title of the presentation: Proteome alterations in bread wheat cultivars differing in their drought tolerance

Type of the presentation: poster

Co-authors: Oleg Stasik, Ludovit Skultety, Dmytro Kiriziy, Oksana Sokolovska-Sergiienko, Mariia Kovalenko, Maksym Danchenko

Other details of the presentation: Session—Navigating abiotic challenges

Outcome of the conference participation

I discussed my research at the conference with colleagues from German facilities– Martin Luther University Halle-Wittenberg, IPK Leibniz, and Institute of Plant Nutrition, Justus Liebig University. I attended two workshops on "Assay Design Guidelines for qPCR and dPCR" by Dr. Martin Becker and "Writing and Publishing" by Dr. Mary Williams. Afterward, I discussed digital PCR with Dr. Becker, who showed the peculiarities of its operation at the Stilla Technologies stand. Dr. Williams presented a new call for recruiting Assistant Features Editors, which is an excellent opportunity for Early Career Researchers to be involved in the editor processes of Plant Physiology journal. Moreover, we briefly discussed publishing Teaching Tools by Working Group 2 of RECROP COST Action.

Additionally, during the conference, I had an opportunity to visit different companies' booths. I discussed the functionality and tested devices from Hansatech, PP systems, and PSI for measuring photosynthesis efficiency. Making contacts with companies was very useful for familiarizing myself with solutions that are available on the market.

Acknowledgement of inclusion of necessary supporting documents to claim the grant

I confirm that the following documents have been uploaded on e-COST as an integral part of this report:

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- the programme of the conference or book of abstracts indicating the presentation (poster) of the grantee.

- copy of the given poster.

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Report on the outcomes of a presentation and participation in a

ITC Conference

Action number: CA22157

Grantee name: Jurica Duvnjak

Conference Details

Conference title: 3rd International Wheat Congress

Conference web-page: https://www.iwc2024.com/

Conference venue : Perth (Australia)

Conference start and end date: 22/09/2024 to 27/09/2024

Accepted contribution details

Title of the presentation: Drought stress effect on wheat physiology and genes' expression after anthesis

Type of the presentation: poster

Co-authors: Jurica Duvnjak, Hrvoje Šarčević, Rosemary Vuković, Valentina Španić

Other details of the presentation: theme- 004. Improving abiotic stress tolerance in wheat

Outcome of the conference participation

Daily participation in plenary presentation resulted in new knowledge and skills, new ideas for future research, as well as familiarization with problems faced by scientists around the world. New friendships were also made at the conference, which are very important for future research and cooperation with other institutions from other parts of the world. The recipient of the grant presented the results of the work through a poster (Drought stress effect on wheat physiology and genes' expression after anthesis). The poster was displayed at the assigned place during all days of the conference and was available to all participants, and it attracted the interest of many conference participants. During the allotted time for the presentation, the results were explained in more detail and discussed with the interested participants. Poster at conference had an impact on the scientific community, the private sector, and the general public. At this conference the recipient promoted the production of wheat varieties that are more stress resilient. Also, he showed how different environmental stresses such as drought can affect physiology and gene expression in wheat, where targeted participants were got better answers about drought stress tolerance and how to cope with drought stress. Results of this research increased the interest of other scientists who will conduct similar research.

Poster presentation also fulfilled the RECROP aim which is to foster the career development of Early Career Investigators (ECIs).

Acknowledgement of inclusion of necessary supporting documents to claim the grant

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- the programme of the conference or book of abstracts / proceedings indicating the presentation (oral or poster) of the grantee.

- copy of the given presentation (oral or poster).