Plan Overview

A Data Management Plan created using DMPonline

Title: How plants deal with heat and cold: Molecular mechanisms of auxin transport and signaling in response to temperature stress

Creator: Petra Marhava

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Funder: European Research Council (ERC)

Template: SLU-General

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Project abstract:

Ambient temperature above or below a threshold can adversely affect plant growth and development, and even lead to death. The tightly regulated distribution of the hormone auxin throughout the plant body controls an impressive variety of developmental processes that tailor plant growth and morphology to environmental conditions. Although non-optimal ambient temperature can alter auxin transport, the precise nature of this alteration and the underlying molecular mechanisms remain enigmatic. Hence, the aim of HOT-AND-COLD is to dissect the molecular mechanisms involved in auxin transport and its downstream signaling upon temperature stress, down to the tissue and cell-type-specific level, focusing on the root of the model organism Arabidopsis thaliana. To achieve this aim, I will combine high-resolution imaging techniques integrated with a temperature-controlled stage system, mass-spectrometry-based phosphoproteomics, TRAP-seq and chemical screens in a multifaceted approach that has never been used for such a study in plant root systems. Using this approach, I expect to reveal: (i) the temperature-responsive phosphoproteome of membrane proteins; (ii) the link between changes in membrane fluidity and the dynamics of auxin transport components within the plasma membrane; (iii) cell-type-specific translatomes that orchestrate auxin transport upon temperature shock as well as in the gradual temperature stress response; and (iv) sensors and components of the signaling pathways controlling plant acclimation to temperature stress. Taken together, the fundamental knowledge obtained through this research will contribute to the mechanistic understanding of plant responses to the temperature variability that will accompany climate change. Such understanding is key for anticipating the impacts of climate variability on agricultural and natural ecosystems.

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How plants deal with heat and cold: Molecular mechanisms of auxin transport and signaling in response to temperature stress

1. General Information

1.1 DMP - DMP Title

Marhava-ERC StG-HOT AND COLD-2021-DMP

1.2 DMP - Date of Creation

2023-02-22

1.3 DMP - Date of Modification

2023-02-22

1.4 DMP - Version

v.1.0

1.5 DMP - Contact Person

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1.6 Project - Project Title

How plants deal with heat and cold: Molecular mechanisms of auxin transport and signaling in response to temperature stress

1.7 Project - Project Description

	Analysis	Technique	Format	Depsitory
Objective 1	Phosphoproteomic analysis	Mass-spectroscopy	RAW, PEAK and RESULT file format	ProteomeXchange Consortium via PRIDE
Objective 2	Imaging	Confocal imaging Fluorescent microscopy, scanner	.TIF, .JPEG	BioImage Archive
Objective 3	TRAPseq analysis	RNA-seq	FASTA	Array Express

Temperature stress affects the geographical distribution of plants, limit plant productivity in agriculture, and threaten food security. Plants have an impressive capacity to acclimatize to temperature stress by initiating an intricate pattern of signal transduction, involving several integrated pathways. In particular, the plant hormone auxin has been shown to play a role in regulating plant

growth and development under both high and low temperature stress. However, the molecular mechanisms by which auxin transport is regulated at the cellular level upon temperature stress remains enigmatic. The aim of this research proposal is to dissect the molecular mechanisms involved in auxin transport and its downstream signaling upon temperature stress, at the tissue and cell-type specific level. My central hypothesis is that auxin transport is altered rapidly after exposure to temperature stress by (de)phosphorylation events and later adjusted through plasma membrane nano/microdomain reorganization, regulated by a cell type-specific set of genes. To address this hypothesis, I will start up and lead an independent research group, working towards achieving the following specific objectives (O1-4):
1) To determine how the activity of auxin transporters is controlled upon temperature stress.
2) To decipher how membrane fluidity affects auxin transport in response to temperature stress.
3) To uncover cell type-specific regulons mediating temperature stress control of auxin transport and signaling.
4) To identify and characterize stress sensors and dissect the temperature signaling responsive pathway.

HOT-AND-COLD will provide detailed knowledge about phosphoproteome of membrane proteins, cell type-specific translatome, and discovery of sensors and components of signaling pathways controlling plant acclimation to temperature stress. The project will also provide a novel “layer” of knowledge about how properties of the plasma membrane, which is highly affected by temperature, regulate the auxin transport machinery to establish auxin minima, maxima and gradients. When synthetized, the outcomes of my project will thus break new ground in our understanding of how auxin transport is regulated at the tissue and cell-type-specific level upon temperature stress. The insights into responses at the cellular level provided by my project will benefit both plant hormone and abiotic stress research, in particular that on temperature stress and climate change adaptation. Data will be collected by means of running experiments, analysed by a number of different instruments, assessed and treated by use of computational programs, published openly, and finally archived at SLU

1.8 Project - Project ID

SLU.genfys.2021.4.1-13.

1.9 Project - Project Leader

Petra Marhava; petra marhava@slu.se; Department of Forest Genetics and Plant Physiology; Swedish University of Agricultural Sciences; https://orcid.org/0000-0001-5904-1657

1.10 Project - SLU Focus Area

- SLU Research

1.11 Project - Start Date

2022-09-01

1.12 Project - End Date

2027-08-31

13. Project - Funding

- Yes

1.14 Project - Funder Name

- Other

European Research Council (ERC; 501100000781)

1.15 Project - Funder Grant ID

101042198

1.16 Project - Contributor

Postdoc	Manvi Sharma	manvi.sharma@slu.se	Department of Forest Genetics and Plant Physiology	Swedish University of Agricultural Sciences
Postdoc	Ling Cheng	ling.cheng@slu.se	Department of Forest Genetics and Plant Physiology	Swedish University of Agricultural Sciences
PhD	?	Department of Forest Genetics and Plant Physiology	Department of Forest Genetics and Plant Physiology	Swedish University of Agricultural Sciences

2. Data Description and Collection or Reuse of Existing Data

2.1 Will newly collected/produced data, already existing data, both, or neither be used in the project? (multiple answers are allowed)

- Collecting/producing new data

Only new data will be collected/produced as part of the project. Reusing already existing data was considered but no existing data could be found that is in line with the project's aim and goal.

2.2 What type of data will be newly collected/produced and/or reused and how will new data be collected/produced and/or already existing data reused?

Identification of the root phosphoproteome of membranes, protein behaviour within plasma membrane, cell type-specific translatomes of the root and identification of key components in pathways that play a role in temperature stress responses, will be newly collected data. Samples will be collected in the laboratory or at the growth facility and subsequently analysed in our laboratory or in the laboratory of our collaborators or facility/company. The following table provides an overview of the types of data collected and how. The provenance of the data used in the project will be documented in a "readme" text file.

Type of Data	***New/Reused Data***	***Collection of Data***
Root phosphoproteome in response to temperature stress	*Newly collected data within the project.*	Protein extraction will be performed according to ShortPhos protocol in the laboratory and samples will be sent to our collaborators to perform mass-spectrometry analysis and basic bioinformatics. Bioinformatic analysis to generate target/substrate network will be performed with guidance of our (UPSC) Bioinformatic facility
Protein behaviour within plasma membrane upon temperature stress	*Newly collected data within the project.*	High-resolution imaging by confocal microscopy combined with temperature controlled stage system. In addition, we will also use FCCS spectroscopy technique.
Root translatome in response to temperature stress	*Newly collected data within the project.*	Samples will be collected in the laboratory and isolation of translating mRNAs will be performed according to TRAPseq protocol. Samples will be send to RNA-seq analysis and bioinformatic analysis will be performed with guidance of UPSC bioinformatics.
Identification of small compounds and components involved in temperature responsive pathway	*Newly collected data within the project.*	Chemical genomic screen and forward genetic screen will be performed in the laboratory and growth facility. Samples from our top candidates will be sent for RNA-seq. For target identification, we will use biochemical methods DARTS and CETSA, which will be performed by our future collaborators.

2.3 What kind of data will be collected/produced and/or reused? (multiple answers are allowed)

- Qualitative data
- Quantitative data
- Experimental data
- Derived/compiled data
- Reference/canonical data
- Numerical data
- Visual data (images)
- Mixed media data (video)

The following table provides an overview of the kind of data that is collected and reused in the project.

Type of Data	Data Category	Data Type	Data Nature
Root phosphoproteome in response to temperature stress	Quantitative, Qualitative	Experimental Derived/compiled Reference/canonical	Numerical
Protein behaviour within plasma membrane upon temperature stress	Quantitative, Qualitative	Experimental	Numerical Visual Video
Root translatome in response to temperature stress	Quantitative, Qualitative	Experimental Derived/compiled Reference/canonical	Numerical
Identification of small compounds and components involved in temperature responsive pathway	Quantitative, Qualitative	Experimental Derived/compiled Reference/canonical	Numerical Visual

2.4 In what format will the data collected/produced and/or reused come in?

The following table provides an overview of the various types of formats the data collected and reused comes in. Preference is given to commonly used and open data formats.

Type of Data	Data Format	Open/Proprietary Format
Root phosphoproteome in response to temperature stress	.csv (.xlsx), .raw	Open
Protein behaviour within plasma membrane upon temperature stress	.csv (.xlsx) .png, .tiff, .avi, .mp4	Open
Root translatome in response to temperature stress	.csv (.xlsx), fastq	Open
Identification of small compounds and components involved in temperature responsive pathway	.csv (.xlsx), .png	Open

2.5 What volume of data will be collected/produced and/or reused throughout the project's lifetime?

- 1 - 10 TB

I dont know yet, but the estimated total volume of data will present no problems with regards to sotrage and backup, access, and preservation.

3. Documentation and Data Quality

3.1 What metadata (i.e., contextual information describing the data) and documentation will accompany the data?

We will provide rich metada to the data in form of separate "readme" text files and/or incorporated into the files themselfs. We intend to make data publicly available according to best practise, e.g. BioImage Archive, Array Express and respective metadata standards will be followed.

3.2 How will the data be organised during the project? (multiple answers are allowed)

- By use of file/folder naming convention
- By use of folder structure

3.3 How will the data be managed during the project? Will data be managed with the help of technical equipment/systems?

- No

3.4 What data quality control measures will be used?

All experiments will be repeated at least three times. Use of negative and positive controls is a standard in these kinds of projects. In addition, all the analyses will follow standardized protocols including quality control measures and instruments calibration.

4. Storage and Backup during the Research Process

4.1 How will data, metadata, and other documentation be stored and backed up during the project?

Data will be stored by making use of SLU's centralised file storage system. Thus, data is protected against loss and access to data is secured (i.e., via authorisation). Data is, furthermore, backed up daily (including a back-up at another location; i.e., data is backed up in two separate locations).

Finally, all personell included in the project are responsible for storing data in the provided system. However, the PI is responsible for informing about said file storage system and ensuring that all involved comply.

4.2 How will data be secured/protected during the project?

As data, metadata, and other documentation is stored and backed-up via SLU-IT’s central storage system, SLU-IT ensures data recovery in the event of an incident (i.e., data will be able to be recovered through previously made back-ups). Access to data during the project is controlled via authorisation as well as as read and/or write permissions. With regard to protection policies and security measures, SLU's management system on information security (LIS) will be complied with (including compliance with ISO 27001) and SLU's data protection handbook will be followed. However, as neither personal nor otherwise sensitive data will be managed in the project, no additional protection and security policies as well as measures need be followed or implied, respectively.

4.3 Have SLU's IT department (support@slu.se) or your institution's IT support, Data Management Support (DMS; dms@slu.se), Data Protection Unit (dataskydd@slu.se), and/or Security (sakerhet@slu.se) Unit been contacted with regard to data storage and backup as well as protection? (multiple answers are allowed)

- SLU DMS
- SLU IT/Institutional IT Support

5. Legal and Ethical Requirements, Codes of Conduct

5.1 Do you intend to process sensitive data (e.g., personal information, politically sensitive information, trade secrets, etc)?

- No

5.2 How will compliance with legislation on personal data and on security be ensured? (multiple answers are allowed)

No personal data.

5.3 How will other legal issues, such as intellectual property rights and ownership, be managed? What legislation is applicable?

The PI is currently establishing a membership of Woodheads AB which is a minority owner of SweTree Technologies (STT). STT has a first right of refusal to IP produced in the project.

5.4 What ethical issues and codes of conduct are there, and how will they be taken into account?

Transgenic technology is utilised according to the legislation and the routines establised at Umeå Plant Science Centre. the GMO routines are regularly inspected
by the Board of Agriculture.

6. Data Sharing and Long-Term Preservation

6.1 How and when will data (or metadata) be shared (i.e., made publicly available)? Are there possible restrictions to data sharing and embargo reasons?

All data will be publicaly available after manuscript will be published.
The Phosphoproteomic, imaging and RNAseq data will be deposited as explained above.
Several journals nowadays require inclusion of the raw data in databases or as supplemental information.

6.2 How will data for preservation be selected, and where will data be preserved long-term (e.g., a data repository or archive)?

Selection of documents and data will be done according to Swedish law and with the hlep of SLU's AIR unit. All data will be preserved in repository (descirbed above) as well as archived at SLU (in SLU's e-Archive).

6.3 What methods/systems, software tools, source code or other types of services are needed to understand, access, and use the data?

All data will be made publicly available in an open format. Thus, no special methods/systems, software etc are needed to access, understand, and use the data.

6.4 Will a unique and persistent identifier (such as a Digital Object Identifier [DOI]) to each data set be pursued?

- Yes

DOIs will be gained through deposition of data in BioImage Archive, ExpressArray, etc.

7. Data Management Responsibilities and Resources

7.1 Who (e.g., role, position, and institution) will be responsible for data management?

Petra Marhava, PI, Assistent professor; Department of Forest genetics and plant Physiology, SLU. https://orcid.org/0000-0001-5904-1657

The UPSC bioinformatics platform is in charge of RNAseq data reposition.

UPSC Bioinformatics Facility - Umeå Plant Science Centre

7.2 Do agreements/contracts exist?

- Yes

GRANT AGREEMENT
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7.3 What resources (e.g., costs and time) will be dedicated to data management?

The time that is needed. PI will cover all costs of repository charges and if needed, will purchase hardware for data back up.

7.4 What resources (e.g., costs and time) will be dedicated to ensuring that data will be FAIR (Findable, Accessible, Interoperable, Reusable)?

We will use the time and costs cover by PI's ERC StG as needed to ensure that data will be FAIR.