

# Abschlussbericht

**Project title: KETCHUP**

**Project number: K287/2019**

## Executive Summary

*Please briefly summarize the most important points of the individual chapters of the interim report here (max. 4500 characters). The executive summary may be similar to the performance review report, but unlike the latter, it will be published.*

The project KETCHUP focusses on understanding how tomato plants sense and respond to heat stress. This is a collaborative project between the Leibniz Institutes in Großbeeren (IGZ), Halle (IPB) and Gatersleben (IPK). We have used high-throughput phenotyping, metabolomics and genome-wide RNA-seq methods to identify key pathways and mechanisms controlling key responses to high temperature, including hypocotyl elongation, trichome differentiation and the expression of the heat shock response.

## 1. Achievement of objectives and milestones

*Please explain briefly the implementation of the most important objectives and milestones of your project compared to the planning presented in the application. If applicable, also explain any objectives/milestones that have not been achieved, or that have been achieved only in part. Please also explain the most important points of the final financial statement and the final financial plan compared to the original financial planning.*

**WP1 Detailed phenotyping (IPK Gatersleben and IGZ Grossbeeren).** We have performed extensive phenotyping by RNA-seq of the reference line money maker as well as the heat resistant wild species *Solanum pennellii*. These reference datasets are highly valuable and are allowing us to determine the underlying transcriptional regulatory networks as proposed. Phenotypic analysis of physiological traits associated with photosynthetic efficiency such as Fv/Fm in the large scale experiments at Gatersleben did not yield large scale differences between the broad range of accessions we chose, so we have modified this WP to include hypocotyl elongation in response to temperature as a key trait. In a large scale effort, researchers from IPK Gatersleben visited the IGZ Grossbeeren and analysed the hypocotyl temperature responsiveness of a large MAGIC population. In parallel, samples were collected for transcriptome analysis. This has produced a very large and valuable dataset providing phenotypic data for the thermomorphogenesis response at both the level of whole plant

responses (hypocotyl elongation) as well as the transcriptome. This data is being used to map QTLs to identify the major determinants of thermomorphogenesis in tomato.

## **WP2. Transcriptomic analyses and network identification (IGZ Grossbeeren and IPK).**

Two major objectives have been completed:

1. Meta-analysis of all heat stress-related RNA-seq experiments in tomato available in public domain, including the overlap with drought and salinity stress (26 publications). In total 338 samples were remapped, including 92 heat, 72 drought, 37 salt, 137 control sample. From the total of 88 contrasts, genes have been networked and classified in respect to the specificity of their regulation in response to heat stress across 8 tissues, 22 genotypes and different time scales. Genes of the core heat stress-response were identified, as well as gene modules of stress- tissue- and time-specific response, providing an unbiased functional annotation of heat-related gene function in tomato. A manuscript compiling the results of the meta-analysis, providing a computational toolkit for similar meta-analyses, and highlighting evolutionary context of observed patterns has been written and published as a pre-print (<https://www.researchsquare.com/article/rs-4337825/v1>). This manuscript has been peer-reviewed and is being revised for resubmission.
2. Generation of unique data resource. The relevant transcriptomes have been collected and are being analyzed to identify key network components. These network components also include phenotypic traits, metabolites and phytohormones. In 2022 we performed a large-scale heat-stress experiment including time series data for heat stress response and recovery in two contrasting genotypes, including *S. lycopersicum* cv. Moneymaker and cv. Saladette. Phenomic data, including 293 phenotypic traits + 3 photosynthetic efficiency parameters have been collected. In parallel, time-series samples have been collected and analyzed using RNA-seq-based transcriptomics (228 samples in total). We have also just completed collecting mass spectrometry-based metabolomics data for 531 polar metabolites (GCMS mass features), as well as targeted quantification data for 10 phytohormones for the same samples. LCMS-based quantification of phenylpropanoids and lipid compounds is currently underway.
3. The third objective, network reconstruction, is underway. Currently we use a set of linear (e.g. regularized regression) and nonlinear (e.g. Random Forest) methods to estimate predictive relationships between gene expression and phenotypes in context of heat-stress response. This work is in progress, and includes systematic integration of the literature legacy data (meta analysis) of the data with the hypocotyl-elongation data from IGZ Grossbeeren.

**WP3 Secondary metabolites and trichome responses (IPB Halle).** The original observation that was the basis for our contribution to this project was that heat stress (37°C) induces a developmental switch leading to the formation of type IV trichomes on tomato leaves. Type IV trichomes produce acylsugars, that are known to be involved in the defense against herbivores. We could demonstrate a strong increase in acylsugar production under heat treatment. In addition, we also observed changes in the production of terpenes by type VI trichomes, with an increase in monoterpenes and a decrease in sesquiterpenes. Interestingly, the type IV trichome phenotype is dependent on a functional jasmonate signaling pathway, because tomato *jai* mutants do not show this response. Transcriptome data over a time course of hours to days were generated and are being analyzed to identify transcription factors involved in these processes. A manuscript compiling the results on the developmental and metabolic changes upon heat stress and their dependence on jasmonate signaling has been published as a pre-print (<https://www.researchsquare.com/article/rs-4337825/v1>) and this work is being peer-reviewed.

## **WP4 Detailed screening of diverse tomato lines (IPK Gatersleben)**

Three screening experiments have been performed.

1. In 2020 *S. lycopersicum* cv. Moneymaker and cv. Saladette as well as 72 introgression lines + parents (*S. lycopersicum* M82 & *S. pennellii*; 768 plants in total), were analyzed

in terms of heat stress response and recovery in an automated phenotyping facility. 262 phenotypic traits were measured before, during and after heat treatment as well as in the parallel untreated control (24 time points)

2. In 2022 we performed a heat-stress high-throughput phenotyping of 22 selected genotypes (768 plants in total). 293 phenotypic traits + 3 photosynthetic efficiency parameters were measured daily in heat-stress and recovery phase, as well as in control conditions. Two of these genotypes (*S. lycopersicum* cv. Moneymaker and cv. Saladette) were sampled for the transcriptomic and metabolomic analyses (228 samples; WP2).
3. The same 22 genotypes were analyzed for the heat-induced hypocotyl elongation and accompanying changes in gene expression. The phenotyping was performed on 277 plants. RNA-seq analysis in 6 time points (covering the whole day-night cycle) was performed on seedlings of *S. lycopersicum* cv. Moneymaker and cv. Saladette in during heat response and in control conditions.

Results of these experiments are currently being compiled into a manuscript where we characterize dynamics and phenomic complexity of heat response and its variation between tomato genotypes. Marker traits for stress response, sensitivity and tolerance are identified and their predictive power for stress recovery is determined. Traits are linked with gene candidates exhibiting correlative and functional associations.

The last fourth screening experiment of the tomato GWAS population targeted at identification of genetic associations of the heat-induced hypocotyl elongation process, as well as accompanying gene expression changes (eQTL) has been initiated and is planned to be completed until end of 2024 (task linked to WP5).

WP5 Association mapping and QTL analysis to identify key stress genes (IGZ). We have identified hypocotyl elongation in response to temperature as a key trait for QTL analysis, and we have performed phenotyping of key accessions for variation in this response. We will then perform QTL mapping on the relevant lines.

WP6 Validation of heat-stress regulatory networks (IGZ, IPK, IPB). We have identified two pathways as playing major roles in mediating the heat stress response in tomato. Firstly, a large set of heat shock proteins (HSPs) are directly up-regulated by the heat shock factor (HSF) class of transcription factors. This is apparent from clustering analyses, GO-term enrichment and bioinformatic analysis of heat shock elements (HSEs). Secondly, we have identified a key role for the evening complex (EC), which comprises EARLY FLOWERING 3 (ELF3), ELF4 and LUX ARRYPATHO (LUX). We have discovered in tomato that the EC binds key temperature induced targets including TOC1 and PRR7, and the binding of ELF3 is temperature dependent, suggesting a direct molecular mechanism. We are now planning experiments to gene-edit these key candidates.

## 2. Activities and obstacles

*Please outline the work and activities of the project partners during the funding period. Please also explain any obstacles or failures you may have encountered that led to delays in the schedule, to deviations from the original proposal or work plan or to goals not being met. Explain any advances in the research area that have become known during the project's implementation and their impact on the project. Briefly comment on the necessity and appropriateness of the work performed.*

We have maintained excellent communication between the three groups, with regular meetings hosted at the respective institutes in rotation. During the project, a graduate student from IPK, Dennis Psaroudakis, also visited IGZ for several months to phenotype and perform RNA-seq analyses of the tomato seedlings. There were of course some delays during the Corona epidemic, but it has been very helpful to have the support of the Leibniz society in the form of a project extension. There were some delays in hiring at the start of the project, which

lead to some members starting several months before others, but we have sought to overcome these obstacles.

### 3. Results and successes

*Please present the key results and successes in research (publications, completed theses and dissertations, acquisition of third-party funds, scientific events, etc.) and transfer (consultancy, technology transfer, public relations work). What activities are planned for further exploitation of the project results? Please use the Excel template to register all basic information for this and briefly explain this basic information here.*

The project is yielding excellent results. The PhD students Dennis Psaroudakis and Robert Säbel are both on track to write-up and submit their theses on time. We have not yet submitted the manuscripts for the main projects since they are still underway.

Dennis Psaroudakis - manuscripts underway:

1. Understanding Tomato Heat Stress Response: A large-scale Transcriptomic Meta-Analysis – submitted as a pre-print and under review
2. Phenomic dynamics and genetic diversity of tomato heat response (preliminary title) - in preparation
3. Phenomic and expression GWAS analysis of the interaction between heat response and development in tomato plants (preliminary title) - planned

### 4. Equal opportunities, career development and internationalisation

*Please describe briefly your initiatives and measures to ensure equal opportunities concerning gender and internationalisation, especially in staff development and recruitment. Please also describe any measures taken to promote the careers of young researchers. Please use the tables in the Excel template to register all basic information for this and briefly explain this basic information here.*

The positions for the project were all openly advertised, and we selected excellent candidates based on their scientific strength. We have a strong and international representation of scientists on the project. At the beginning of the project, the lead PI from Gatersleben was Dr. Astrid Junker. Dr. Junker was however recruited away from the institute as the project was starting, and so we were lucky to have the recruitment of Dr J Jakub Szymanski in her place. This did however lead to a gender disparity in our team, but this was unforeseeable and unavoidable.

The early career researchers have benefited from extensive training in communicating and sharing their scientific results. Specifically, Mr Robert Säbel gave a talk at the international Solanaceae meeting in 2022 in Thessaloniki, Greece. Mr Säbel has also been invited to speak at the 2023 Jasmonate conference later this year. He has presented a poster during PSSC 2021 and gave a talk in 2022. Mr Dennis Psaroudakis presented a talk at the international Solanaceae meeting in Thessaloniki, Greece in 2022 on understanding the tomato heat response. Mr Psaroudakis also presented a poster in 2023 for a meeting on Translational Research in Crops, Ghent, Belgium.

### 5. Structures and collaboration

*Please describe the structure of collaborations during the funding period, including any obstacles and challenges. Where relevant, describe changes to the governance of your project and/or the addition of new partners or collaborative relationships. If new collaborative relationships were established with institutional partners during the funding period, please briefly describe the scientific benefit for your project. If the changes resulted in additional agreements or changes to the existing collaboration agreement, please attach copies to this report. Please use the tables in the Excel template to register all basic information for this and briefly explain this basic information here.*

The major change in the project was caused by the transition of Dr. Astrid Junker away from the IPK Gatersleben. We were fortunate to hire Dr. J. Jakub Szymanski in the same role. The collaborations between the three institutes have proceeded well. It was disruptive during the Coronavirus epidemic, but we were able to substitute online Zoom meetings when travel and in-person meetings were not possible.

## 6. Quality assurance

*Please describe briefly your measures for quality assurance, in particular in terms of complying with good scientific practice and making your research results available (open access). Please also state whether or not animal testing has been conducted. If this is the case, please give a short description of your measures ensuring animal welfare.*

No animal testing was required in this project. We are aiming to publish our research findings in open-access journals where possible. In all cases, large scale datasets are being submitted to the respective online repositories. For example all the RNA-seq data is being submitted to the NCBI GEO database.

## 7. Additional resources

*Please estimate the value of in-kind resources generated within the project at your institute, the participating Leibniz institutes and/or the university partners. Please differentiate between personnel cost (for scientific and non-scientific staff) and consumables.*

*For funding programmes with co-funding (i.e. Leibniz Programme for Women Professors, Leibniz Junior Research Groups, Leibniz ScienceCampi, Leibniz Research Alliances), please also indicate the amount of co-funding by the Leibniz institution(s) and (if applicable) the cooperation partners.*

Not applicable.

## 8. Outlook

*Please describe the most important future research questions related to the work performed.*

Key tasks prior to project completion include concluding key projects describing (1) trichome development in response to temperature (2) the heat stress network in tomato (3) thermomorphogenesis pathways in tomato and the identification of candidate genes controlling these processes. In all cases we are seeking to have manuscript drafts of these key projects at the time of project completion.