INTERNSHIP PROPOSAL M2 RESEARCH 2022-2023

TITLE: Cause and consequence of the effect of temperature-dependent regulation of MPK3 and MPK6 on plant immunity

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Number of PhD currently supervised: 0

Possibilty to give rise to a PhD proposal : ■ YES ☐ NO

Direct presentation of the proposal to the students: ■ YES ☐ NO

■ 01/09/20 (IPS2) ☐02/09/20 (IJPB)

NEW : we plan to communicate, for information, the internship proposals to M1 students so that they can get an idea of the research themes of the field. Would you agree to have your proposal distributed to them?

■YES ☐ NO

INTRODUCTION, SCIENTIFIC CONTEXT :

10 000 years ago, the stabilization of earth climate allowed humanity to develop agriculture and settle down. Since then, at the end of XIX Century, human societies started to burn fossil energies at large scale resulting in emissions of Green House Gas (GHG) that trigerrered a rapid and global climat changes. The most important consequences result in an increase in average of local and global temperatures and rainfall changes which are highly detrimental to plant growth and productivity throughout their crop cycle. Projections from the Intergovernmental Panel on Climate Change predict an increase of 1.5 ° C between 2030 and 2052 and of 3°C by at the end of the century (IPCC, 2020). These global changes will also facilitate the shift poleward of plant pathogens, and consequently biotic stresses, which are already a major cause of crop losses, are expected to increase in the future. The combination of biotic and climate-change-related abiotic stresses is therefore a major threat for world food security, and there is an urgent need to understand how plants are able to respond to such combination of stresses. This knowledge will hopefully give us hints on how to adapt crops for the foreseen terrible world of the next century.

Fortunately, plants have evolved ways to cope with biotic stress induced by plant pathogens. The warfare between plants and pathogens has been formalized in the Zig-Zag model (Jones and Dangl, 2006). This model dissects the successive and interconnected layers of signaling pathways such as MAMP-Triggered Immunity (MTI), Effector-Triggered Susceptibility (ETS) and Effector-Triggered Immunity (ETI) giving rise to plant susceptibility and resistance. MTI, ETS and ETI have been reported to be differentially modulated by temperature, although an integrative comprehension has not emerged yet.

The Stress Signaling (IPS2) team aims to clarify the role of Mitogen Activated Protein Kinase (MAPK) modules in the perception of environment. MAPK modules define signaling cascades that are composed of a MAP3K (MAP2K Kinase), a MAP2K (MAPK Kinase) and a MAPK, activating each other in series by phosphorylation to mediate a signal from a receptor to proper responses (*i.e.* gene expressions, enzyme regulations …). The group largely contributed to demonstrate that MAPK modules function in plant adaptation to pathogens as well as the mitigation of abiotic stresses, such as salt, cold and drought (Danquah et al., 2015; Lang et al., 2021). However, nothing is known about the role MAPK modules could play to adapt plant immunity to temperature increases. The available data suggest that MAPK modules are not important actors of high temperature (HT) signalling but could have pivotal roles in the integration of temperature stimulus on biotic stress responses. Our preliminary

results indicate that MPK3, but not MPK6, disappears after a temperature shift, theoretically shutting down important branches of MTI and ETI signalling.

RESEARCH PROPOSAL :

The fact that (i) MPK3 is an important actor of PTI and ETI signalling (Tsuda et al., 2013; Lang et al., 2021), (ii) MPK3 amount decreases at HT and (iii) MPK3 and MPK6 are inactivated at HT suggests a working hypothesis in which MPK3 and MPK6 inactivation at HT could compromise plant immunity. The main objective of M2 training is to **characterize the effect of temperature-dependent regulation of MPK3 and MPK6 on immunity**. Four subgoals (SG) will be addressed.

SG1. Setup a robust and reproductible framework of HT conditions for further experiments

Our preliminary experiments showed that MPK3 disappearance does not occur in the same temperature range in *in vitro* (plantlets on agar plates) and *in vivo* conditions (plants in pots) suggesting a combined effect with other parameters. Using western blot, MPK3 and MPK6 amount will be monitored in plants grown in various conditions. We will have a particular interest for the combination of temperature with air humidity, water availability and plant age. It will also help to setup a robust and reproductible framework of HT conditions for further experiments.

SG2. Identifying the causes of MPK3 desappearance involved in HT

Once growth conditions well setup, inhibitors of transcription/translation/degradation will be used to identify the main mechanism(s) involved in HT-dependent MPK3 disappearance as well as evaluate MPK3 half-lives and synthesis/degradation kinetics at various HT.

SG3. Search for a temperature-dependant phenotype in mutants *mpk6* and *mpk3*

We will wonder why the MAPK6 expression and activity are longer maintained during HT. The use of *mpk6* mutant available in the laboratory will help to understanding the importance of MPK6 *versus* MPK3 in heat stress responses. The search for a temperature-dependant phenotype in mutants *mpk6* and *mpk3* will be privileged. We will concentrate first to integrative parameters such as survival during *in vitro* and *in vivo* conditions and in case of success, we will perform finer phenotyping, such as measurement of stomatal conductance, leaf temperature, photosynthesis efficiency, gene expression.

SG4. Demonstrate that MPK3 decay at high temperature has a major effect on immunity

To show the role of HT-induced MPK3 decay, we will characterize MPK3-dependent immune phenotype at various temperatures. Both MTI and ETI will be assessed using simplify but robust systems. For MTI, the MAMP flg22 will be used to assess by qPCR analysis whether genes published to be induced by in an MPK3-dependent way at 22°C are still responsive at higher temperatures. If successful, we will perform a broader transcriptomic comparison of WT and *mpk3* upon flg22 treatment at various temperatures to validate that the whole MPK3-dependent gene set does not respond to HT. For ETI, an ETI-inducible system, for example DEX::AvrRpt2, will be introgressed by crossing in *mpk3* and this lines will be used to perform transcriptomic experiments. It will allow identifying MPK3-dependent genes in the context of ETI as well as validate that this gene set is not responsive anymore at HT.

METHODOLOGIES :

The project largely relies on the ability to apply precise and reproducible stresses to the model plant *Arabidopsis thaliana*. The candidate will take advantage of available growth cabinets and perform heat and flg22 stresses on soil-grown adult plants, mutants and WT. She/he will also use plantlets grown *in vitro* on agar plates. The laboratory gathered a large number of T-DNA mutants and transgenic lines as well as generated some new CRIPR mutants. The morphology of WT and mutant plants will be compared first by measurement and weighting. The expression of genes of interest (MAP2K, PTI marker genes) will be assessed using RT-qPCR.

This project also largely relies on biochemical approaches. Kinase assay after immunoprecipitation with appropriate antibodies will be used as well as western blots to assess protein amounts and protein phosphorylations.

Overall, techniques necessary for the project are commonly used in the team or easily accessible in collaborator's laboratories in the vicinity.

REFERENCES (maximum 5)

Danquah, A. *et al.* (2015) *Identification and characterization of an ABA-activated MAP kinase cascade in Arabidopsis thaliana.* Plant J. 82:232

IPCC (2020). Climate Change and Land - SPM.

Jones, J.D.G. and Dangl, J.L. (2006) The plant immune system. Nature 444 : 323-329.
Lang, J. et al. (2021). Both transient and sustained MPK3/6 activities positively control expression of NLR genes in PTI and ETI. bioRxiv: 2021.04.01.437866
Tsuda, K. et al. (2013) Dual regulation of gene expression mediated by extended MAPK activation and salicilic acid contributes to robust innate immunity in *Arabidopsis thaliana*. plosS Genet. 9.