Sorbonne Université/ China Scholarship Council program 2020

Thesis proposal

Title of the research project: ***Analysis of MPK8/TCP module functions in regulating gene expression during seed germination in Arabidopsis thaliana***

Keywords: plants, seeds, germination, signaling, protein kinases, transcription factors.

Joint supervision: yes (Dr Puyaubert Juliette) /~~no~~

Joint PhD (cotutelle): ~~yes (name/surname)~~ /no

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Subject description:

Seed germination is among the most important events of plant lifespan, as it determines the survival and efficient development of the emerging plantlet. To trigger germination only when environmental conditions guarantee optimal seedling growth, mature seeds are naturally dormant and dormancy has to be released before germination occurs [1]. In this scheme, two hormones, gibberellins (GA) and abscisic acid (ABA) regulate germination antagonistically, with GA stimulating dormancy release and germination, when ABA inhibits germination and triggers dormancy [2]. The molecular mechanisms underlying ABA/GA balance and controlling dormancy, dormancy release and germination are complex and far from fully unraveled [3]. A set of transcriptional regulators controlled by ABA/GA balance have been identified, e.g. ABI5, DELLAs or DOF6 [2]. These regulators undergo multiple post-translational modifications that affect their stability, and are at the root of their interplay to promote or restrict germination [4].

In this context, we recently identified a member of the MAP kinase family, MPK8, as a regulator of seed dormancy in *Arabidopsis thaliana* [5]. *Mpk8*-KO seeds exhibit enhanced dormancy at harvest compared to wild-type seeds, associated with a lower sensitivity to GA. At the molecular level, the expression of germination- and GA-responsive marker genes is impaired in *mpk8* seeds. We further evidenced that MPK8 interacts *in vivo* with TCP14, one of the 23 members of the TB1/CYC/PCF1 (TCP) family of transcription factors. TCP14 is a well-known regulator of germination involved in GA-controlled responses [6]. The comparison of WT, *tcp14* and *mpk8* dry and imbibed seed transcriptomes by RNAseq highlights that i) dry seed transcriptomes are similar for all genotypes, ii) *tcpα* and *mpk8* transcriptome strongly diverge from that of WT seeds after imbibition and iii) that more than 80% of the genes de-regulated in *tcp14* are similarly de-regulated in *mpk8* seeds. Taken together these data suggest that *tcp14* and *mpk8* are functionally linked to regulate germination. We recently identified other TCPs operating in the control of seed dormancy. Interestingly, one of these transcription factor designated TCPβ also interacts with MPK8. Nevertheless, *tcpβ* seeds behave differently from tcp14 seeds, being less dormant. A range of molecular markers is also found deregulated in mutant seeds, suggesting a function for TCPβ contrasting with that of TCP14. At this stage, it is suggested that TCP14 and TCPβ could function in an opposite manner and that MPK8 might constitute a common upstream signal, regulating directly or indirectly both TFs.

The PhD project will aim ii) at deciphering if and how the regulation of TCP14 and TCPβ by MPK8 is responsible for their opposite function during germination and ii) at getting a holistic picture of the interplay between TCP14 and TCPβ in reprogramming gene expression during seed germination.

As a first step, double mutants (*tcp14tcpβ*, *tcp14mpk8* and *tcpβmpk8*), available in the group will be phenotyped at the physiological (temperature, hormone responses) and molecular level during germination. In relation to the GA-related phenotype of single mutants, the expression of marker genes of the GA pathway will be analyzed. As already demonstrated for TCP14, recombinant TCPβ phosphorylation will be assessed using immuno-precipitated MPK8-GFP from plants. The residues phosphorylated in TCPβ will be identified and compared to those of TCP14, in collaboration with the proteomic platform of our Institute. The relevance of TCPβ phosphorylation for the regulation of TF activity will be addressed by transactivation assays. We benefit of a reporter vector where Luciferase gene is under the control of a specific TCP-responsive promoter. This reporter construct will be transfected in tobacco leaves together with combinations of TCPβ and/or MPK8 expression vectors. Additional experiments will be performed with TCPβ mutated on phosphorylated residues. Finally, as TCP14/TCPβ interact with additional transcriptional regulators, the effect of phosphorylation on the interaction of both TCPs with such partners will be analyzed, so as its possible effect on TCP-driven gene expression. In this purpose, transactivation experiments will be performed in the presence of these regulators together with the combinations mentioned above will be carried out.

To get a holistic view of TCP14/TCPβ dependent gene expression during germination, a large-scale transcriptome analysis will be performed by RNAseq using WT, *tcp14* and *tcpβ* seeds, in collaboration with the transcriptomic platform of IPS2 (Orsay). This analysis will highlight the genes commonly or specifically regulated by both TCPs in seeds, together with possible interplays, and will be compared with our available RNAseq data on *mpk8* mutant seeds. Selected markers identified by this approach will be further analysed in *tcp14tcpβ* seeds. The data obtained will be correlated with the profiles of TCP14 and TCPβ protein abundance during seed imbibition, determined on *pTCP14-TCP14-GFP* and *pTCPβ-TCPβ-GFP* seed lines available in our team. To identify genes directly targeted by TCP14 and/or TCPβ in seeds, ChIP-seq experiments will be performed using the *pTCP14-TCP14-GFP* and *pTCPβ-TCPβ-GFP* seed lines The relevance of TCP binding for transcriptional regulation will be demonstrated on selected genes.

As a whole, this project will bring new insights on the different functions of TCPs in seeds and on the role of phosphorylation mediated by MPK8 in this process.

References

[1] Finch-Savage WE and Leubner-Metzger G (2006) *New Phytol*, **171**, 501-523. [2] Shu K.*et al* . (2016) *Mol. Plant,* **9**, 34-45. [3] Diaz-Vicandos P *et al*. (2013) *Plant Cell Rep*., **32**, 1491-1502 [4] Yu F *et al*. (2015) *Trends Plant Sci*., **20**, 569-575. [5] Zhang W. et al. (2019) *Plant J*., **100**, 677-692. [6] Tatematsu K. et al. (2008) Plant J., 53, 42-52.

Profile of the applicant

We are seeking an outstanding young scientist who has recently received a M.Sc. degree in Plant Biology, or possibly in Biochemistry, Molecular biology or equivalent. Applicants should have a demonstrated background and interest in biochemistry, cell signaling and/or molecular physiology. Previous experience on Arabidopsis seed biology would be an asset. **Communication skills, aptitude for collaborative work and fluent English are mandatory**. Applicants should fullfil requirements for being eligible for CSC fellowships.

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