

Ecole doctorale Environnements-Santé
Dossier de projet de thèse « Contrat doctoral Etablissements »
ANNEE 2021

TITRE DU PROJET:

Understanding how pea manages drought stress while preserving nutritional seed quality: role of vacuolar stored sulfate

FINANCEMENT I-SITE

Il est en phase avec l'axe 2 (Territoires, environnement, aliments)

Ce projet est aussi proposé au titre de la bourse générale FRM oui non
Ce projet est aussi proposé au titre de la bourse Cardiologie FRM oui non

1) Renseignements administratifs sur la direction de thèse :

Directeur de thèse HDR :

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UMR 1347 Agroécologie, Philippe Lemanceau

Co-Directeur de thèse:

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Prénom : Markus

Grade : Akademischer Oberrat. Group leader at the Centre for Organismal Studies Heidelberg

HDR : Qualified to supervise the work of PhD students at the University of Heidelberg

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Heidelberg University, Centre for Organismal Studies, Department of Molecular Biology,
Prof. Rüdiger Hell

Pour faciliter les échanges avec Markus Wirtz, le projet de thèse est rédigé en Anglais (accord du secrétariat de l'Ecole doctorale).

2) Descriptif du projet de thèse :

Nom et label de l'unité de recherche : UMR 1347 Agroécologie, pôle GEAPSI, **team FILEAS**

Localization: Mendel Building, INRAE center of Dijon (17, rue de Sully)

Nom du directeur de thèse : Karine Gallardo

Co-direction avec Markus Wirtz, University of Heidelberg, Allemagne

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Titre du projet : Understanding how pea manages drought stress while preserving nutritional seed quality : role of vacuolar stored sulfate

Description du projet (2 pages maximum) :

• **Context and originality.** This joint French-German project aims to improve food quality and security by enhancing stress tolerance and nutrient remobilization for seed filling in pea (*Pisum sativum*). Pea is of increasing economic importance in Europe since it can contribute to satisfying the growing demand for plant proteins for human consumption and animal feed. Remarkably, pea is also an important crop for sustainable agriculture systems due to the ability of its root system to establish symbiosis with soil bacteria that fix atmospheric nitrogen. Two factors threatening pea yield and seed quality in the field are: 1, abiotic stress-induced growth defects; and 2, stress-induced imbalances of amino acid homeostasis for seed protein production. As a result of anthropogenic global warming, drought was one dominant factor impairing pea yield in France and Germany over the last decade¹. Recent work of the German/Wirtz team has established the role of sulfate as the xylem-borne root-to-shoot signal for soil drying-induced stomatal closure (Batool et al., 2018, *Plant Cell*). In a joint Sino-German research consortium, the Wirtz team established a novel rice variety displaying improved nutritional seed quality and abiotic stress resistance due to optimized sulfur metabolism (Sun et al., 2021, *Nature Commun*). At the same time, the French team uncovered the importance of sulfur nutrition for drought tolerance in pea (Henriet et al., 2019, 2021, *J Exp Bot*). In parallel, genomics information of relevant legumes, including pea, was uncovered (Hufnagel et al., 2020, *Nature Commun*, and Kreplak et al., 2019, *Nature Genet*), which opens new avenues for addressing seed quality and abiotic stress resilience in these crops. Motivated by these novel findings and resources, both teams aim to intensify their existing collaboration on the importance of membrane-localized sulfate transporters for improving seed amino acid biosynthesis and sulfate-mediated stress resilience (Zuber et al., 2010, *BMC Plant Biol*; Zuber et al., 2010, *Plant Physiol*). The PhD project presented here focuses on the tonoplast-localized sulfate transporter-4 (**SULTR4**) that remobilizes the macronutrient sulfur from storage vacuoles. The results are expected to demonstrate the biological significance of stress-induced remobilization of vacuolar sulfate to support stress resilience and seed protein yield and quality in this crop.

• **Framework and preliminary data.** In contrast to the reference plant *Arabidopsis*, the pea genome encodes for only one *SULTR4* protein. We applied a TILLING (Targeting Induced Local Lesions in Genomes) approach to identify two mutations impairing *SULTR4* function in the cultivar "Caméor". The first point mutation led to the formation of a stop codon (W/-) at position W⁷⁸, while the second converts glutamate⁵⁶⁸ into lysine (E/K). Glutamate⁵⁶⁸ is located in a C-terminal domain that is essential for the transport activity of *SULTR4*. The two *sultr4* mutants, back-crossed twice with the wild-type, displayed lower amounts of sulfur-

¹ <https://www.terresinovia.fr/>; <https://ourworldindata.org/grapher/pea-yields>

rich storage seed proteins. This impairment was evident irrespective of the sulfur supply. Upon sulfur deficiency, the lower leaves (source of nutrients) of *sultr4* mutants displayed a stay-green phenotype, strongly suggesting that sulfur limitation-induced senescence had been suppressed. This exciting finding implied that SULTR4-mediated remobilization of vacuolar sulfur is critical for the stress-induced definition of source-sink relationships. We confirmed this hypothesis by determining the sulfur fluxes from source to sink tissues, feeding stable isotope-labeled sulfate followed by IRMS. The definition of the seed as the dominant sink for resources is the most critical trigger for efficient seed filling at the end of the crops' life cycle. Thus, our findings allow hypothesizing that regulation of vacuolar sulfate remobilization by SULTR4 is not only required for drought stress resistance, see Batool et al. (2018, *Plant Cell*), but also contributes to developmentally-triggered seed filling by defining source-sink relationships.

- **Objectives.** In this project, we will exploit the recently identified *sultr4* mutants to address the following questions: **(Q1)** What is the role of vacuolar sulfate remobilization in nutrient recycling and senescence-induced autophagy? **(Q2)** What is the biological significance of vacuolar sulfate remobilization from leaves for the establishment of seed protein composition? **(Q3)** How do these processes or pathways interact with the pea response to drought, combined or not with sulfur deficiency? The thesis will provide insights into the role of sulfate in the trade-off between seed quality establishment, which relies on both leaf nutrient remobilization and seed storage activities, and drought tolerance. The expected results will provide a framework for improving and stabilizing pea seed yield and quality through the breeding of pea varieties with higher sulfate remobilization efficiency.

- **Working hypotheses and research strategies**

- (Q1) Role of vacuolar sulfate remobilization in nutrient recycling and autophagy**

[M1-M8; one manuscript to be prepared for publication of Q1 data].

Here we aim to identify the role of SULTR4-mediated remobilization of vacuolar sulfate for controlling the remobilization/recycling of nutrients in pea leaves. A global transcriptome profiling of leaves from sulfur-deprived wild type and *sultr4* mutants exhibiting the stay-green phenotype is already available (FILEAS team). The quantitative analysis of these large data sets in the Gallardo lab will identify SULTR4-mediated molecular processes that will be further characterized. Among the processes that will be the subject of targeted molecular studies is autophagy, which contributes to nutrient remobilization during senescence. Previous work by the Wirtz team revealed that sulfur deficiency promotes autophagy by decreasing the activity of the sensor kinase Target of Rapamycin (TOR, Dong *et al.*, 2017). We hypothesize that the lack of remobilization of sulfur from source leaves to sink tissues in the *sultr4* mutants maintains TOR activity and, consequently, suppresses autophagy in source leaves of *sultr4*. TOR and autophagy quantification will be performed in the Wirtz lab.

- (Q2) Role of vacuolar sulfate in the establishment of nutritional seed quality**

[M8-M20; one manuscript to be prepared on Q2]

Mature *sultr4* seeds contain lower levels of sulfur-rich storage proteins than wild-type seeds. Remarkably, the depletion of sulfur-containing amino acids is associated with the accumulation of sulfate in mature *sultr4* seeds, suggesting vacuolar sulfate is not used for sulfur amino acid production within the seed itself. To understand the impact of vacuolar stored sulfate on seed metabolism, we will profile the transcriptome and metabolome of *sultr4* and wild-type lines seeds at different developmental stages. Next, we will dissect the impact of sulfur limitation with the previously established omics approaches (RNA-seq & metabolomics) on this process in the wild type and the *sultr4* mutants. Based on these data, we will compute a multi-omics network to study the coordinate regulation of these processes with genes encoding sulfur-rich storage proteins. Specific metabolic pathways can

then be characterized (e.g. enzyme activities, cysteine levels) to confirm the relevance of omics-identified processes.

(Q3) Role of SULTR4 and external sulfur supply for drought tolerance in pea

[M20-, at least one manuscript to be prepared on Q3]

The *sultr4* mutants and wild-type lines will be subjected to drought stress, sulfur deficiency, and a combination of both stresses. The stresses will be applied during the reproductive period using the 4PMI high-throughput phenotyping platform. A subset of *sultr4* and wild-type plants will be harvested for quantitative analysis of the metabolome, the transcriptome, and the proteome at the vegetative stage. Another subset of plants will be kept until maturity for determining seed yield and quality, including protein content and composition. Motivated by the published finding that xylem-borne sulfate triggers stomatal closure by facilitating abscisic acid production (Batool *et al.*, 2018), the drought stress-induced stomatal closure responses of the *sultr4* and the wild type will be quantified with established methods in the Wirtz lab. This part of the work will provide knowledge about the contribution of sulfate to key processes triggering the drought response and about the trade-off between seed quality establishment and water stress tolerance in pea.

In addition to provide fundamental knowledge on the role of vacuolar sulfate for stress tolerance and seed quality, the results will serve to reveal favorable alleles of relevant genes (e.g. encoding sulfate transporters or regulators of sulfur transport systems from Q1, Q2 et Q3) in a collection of pea ecotypes hosted in UMR Agroecology, which would pave the way for breeding pea varieties with higher stress resilience and seed nutritional value.

References cited (the two supervisors of the thesis are in blue):

- Batool S, Uslu VV, Rajab H, Ahmad N, Waadt R, Geiger D, Malagoli M, Xiang CB, Hedrich R, Rennenberg H, Herschbach C, Hell R & [Wirtz M](#) (2018), Sulfate is Incorporated into Cysteine to Trigger ABA Production and Stomatal Closure. *Plant Cell*: 30, 2973-2987.
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- Henriet C, Balliau T, Aimé D, Le Signor C, Kreplak J, Zivy M, [Gallardo K*](#) and Vernoud V*. 2021. Proteomics of developing pea seeds reveals a complex antioxidant network underlying the response to sulfur deficiency and water stress. *J Exp Bot*, doi: 10.1093/jxb/eraa571.
- Hufnagel B, Marques A, Soriano A, Marquès L, Divol F, Doumas P, Sallet E, Mancinotti D, Carrere S, Marande W, Arribat S, Keller J, Huneau C, Blein T, Aimé D, Laguerre M, Taylor J, Schubert V, Nelson M, Geu-Flores F, Crespi M, [Gallardo K](#), Delaux PM, Salse J, Bergès H, Guyot R, Gouzy J, Péret B. 2020. High-quality genome sequence of white lupin provides insight into soil exploration and seed quality. *Nat Commun*. 11: 492.
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- Sun S-K, Xu X, Tang Z, Tang Z, Huang X-Y, [Wirtz M](#), Hell R & Zhao F-J (2021), A molecular switch in sulfur metabolism to reduce arsenic and enrich selenium in rice grain. *Nature Commun*, doi: 10.1038/s41467-41021-21282-41465.
- Zuber H, Davidian JC, [Wirtz M](#), Hell R, Belghazi M, Thompson R, [Gallardo K](#). *Sultr4;1* mutant seeds of *Arabidopsis* have an enhanced sulphate content and modified proteome suggesting metabolic adaptations to altered sulphate compartmentalization. *BMC Plant Biol*: 2010 Apr 28;10:78. doi: 10.1186/1471-2229-10-78. PMID: 20426829; PMCID: PMC3095352.
- Zuber H, Davidian JC, Aubert G, Aimé D, Belghazi M, Lugan R, Heintz D, [Wirtz M](#), Hell R, Thompson R, [Gallardo K](#). (2010) The seed composition of *Arabidopsis* mutants for the group 3 sulfate transporters indicates a role in sulfate translocation within developing seeds. *Plant Physiol*: 154: 913-26.

Financement du projet – partie Recherche (montants acquis, type de contrat)

The ANR project PEAValue (2020-2024, 563 k€ acquis, Coord. Karine Gallardo), which aims at improving the protein value of pea seeds, will provide funds to characterize the mutants in UMR Agroecology. A COST Action (R3PO, Coord. Melisande Blein-Nicolas) on Research Network for Protein Production in Photosynthetic Organisms, with Karine Gallardo as participant, has been submitted in November 2020. If accepted, the project will complement funds for short term missions. In Markus Wirtz's lab, the following and future funding will secure research activities at the University of Heidelberg: 2020 - 2024: WI 3560/6-1 (~ 34 k€ supplies for Ph.D. student funded in collaboration with HE1848/19/1) ; 2018-2022: WI 3560/1-2 (~ 300 k€, 1 Ph. D. student + supplies); 2018 - 2021: WI 3560/4-1 (~ 300 k€, 1 Ph. D. student + supplies); 2016 to 2020: INST 35/1102-2 (~300 k€, 1 Ph. D. student + supplies)

Connaissances et compétences requises

Biologie moléculaire, bio-informatique et statistiques, physiologie végétale.

Préciser le domaine de compétence dans la liste ci-dessous (2 choix possibles maximum – ne pas modifier les intitulés : ils sont imposés par certains sites web) :

Non défini

Agronomie

Biologie, médecine, santé

Chimie

Résumé en français et anglais (limité chacun à 1800 caractères)

Résumé : La thèse, co-dirigée par Karine Gallardo (UMR Agroécologie) et Markus Wirtz (Univ. Heidelberg, Allemagne), sera centrée sur le pois protéagineux. Cette légumineuse produit des graines riches en protéines même en l'absence d'engrais azotés. Son utilisation comme source de protéines en alimentation humaine représente donc un excellent levier pour accélérer la double transition nutritionnelle et agroécologique. Toutefois, le rendement du pois est grandement affecté par le réchauffement climatique. L'équipe de l'Université de Heidelberg a récemment découvert que le sulfate est le premier signal transmis par le xylème qui active la transpiration foliaire en réponse à la sécheresse, tandis que l'équipe locale a montré que le chargement des graines en sulfate provenant de la vacuole et son métabolisme contribuent au remplissage des graines. Sur la base de ces résultats, nous proposons que la remobilisation du sulfate puisse être un point de contrôle de la tolérance à la sécheresse et de la biosynthèse des aminés soufrés (Cys, Met) dans les graines. Ces hypothèses seront étudiées dans le cadre de la thèse en utilisant deux mutants TILLING de pois (*sultr4*) incapables d'utiliser la réserve de sulfate vacuolaire et en appliquant différents régimes de soufre et d'eau. Les génotypes seront caractérisés au moyen d'approches de phénotypage et omiques dans l'UMR Agroécologie. Des approches moléculaires ciblées seront utilisées dans l'Université de Heidelberg, en mettant l'accent sur l'étude de la contribution du sulfate à la biosynthèse d'acide abscissique et à l'autophagie, notamment *via* TOR (Target of Rapamycin). Les résultats apporteront des connaissances nouvelles pour manipuler le métabolisme du sulfate afin d'améliorer la qualité nutritionnelle des graines et la résistance aux stress.

Summary: The thesis project will be carried out under the joint supervision of Karine Gallardo (UMR Agroécologie) and Markus Wirtz (Université de Heidelberg, Germany). It is centered on the pea crop, which accumulates large amounts of seed proteins even in the absence of nitrogen fertilizer, thus representing an excellent source of proteins in support of both nutritional and agroecological transitions. However, pea suffers from global warming, which in recent decades has led to considerable yield losses. The University of Heidelberg's team has recently uncovered that the macronutrient sulfate is the first drought stress-induced xylem-borne signal priming the leaves' transpiration rate to soil drying. The local team provided evidence that seed loading with sulfate from the vacuole and seed sulfate metabolization are relevant triggers for seed filling activities. Based on these findings, we propose that fundamental research on sulfate remobilization can contribute to drought stress resistance and promote sulfur-amino acid accumulation, including methionine, in pea seeds. This will be investigated in the frame of the thesis using two TILLING pea mutants (*sultr4*) that are unable to release sulfate from the vacuole. The impact of different sulfur regimes and drought stress on the wild type and *sultr4* mutants will be characterized in UMR Agroecology by means of phenotyping, metabolomics and transcriptomics approaches, and of targeted approaches at the University of Heidelberg. In particular, the impact of sulfate on abscisic acid biosynthesis, autophagy, and on the sensor kinase Target-of-Rapamycin (TOR) will be quantified. The results will provide the knowledge for the engineering of seed sulfur metabolism to improve nutritional seed quality and abiotic stress resilience in pea.

Préciser le domaine de compétence dans la liste ci-dessous (2 choix possibles maximum – ne pas modifier les intitulés : ils sont imposés par certains sites web) :

Non défini

Agronomie

Biologie, médecine, santé